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Antibacterial Activity of Avicennia Germinans L. Extract and Its Green Synthesized-Gold Nanoparticles Against Drug-Resistant Uropathogenic Escherichia Coli

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ABSTRACT:

The emergence of antimicrobial resistance in Uropathogenic Escherichia coli (UPEC) is a major public health concern in the management of urinary tract infections. This study assessed the antibacterial activity of the crude aqueous extract and green-synthesized gold nanoparticles (AuNPs) of Avicennia germinans L. as potential alternative antibacterial strategies against multidrug-resistant (MDR) UPECs. This involved phytochemical analysis, green synthesis and characterization of AuNPs, and comparative antimicrobial analysis of crude extract and AuNPs. The plant extract contained major phytochemicals, and the successful synthesis of the nanoparticles were confirmed by evidence provided by the spectroscopic and microscopic analysis. The crude aqueous extract showed a dose-dependent antibacterial effect against the test isolates, with isolate E8 showing the strongest susceptibility (15.7 ± 1.0 mm) and E16 exhibiting the lowest (12.7 ± 1.2 mm) at a concentration of 100 mg/mL. In contrast, AuNPs exhibited variable responses across different test concentrations and isolates, with E3 showing the strongest susceptibility (15.7±0.8 mm) at 25 µg/mL. There was statistical significance between antibacterial agents and their concentrations ($p < 0.05$), with the lowest MIC and MBC at 30 mg/mL for the crude aqueous extract and 60 µg/mL for the AuNPs. Considering the difference in concentration units, these findings revealed that the AuNPs were more potent against the MDR UPECs at significantly lower concentrations.

KEYWORDS:

Avicennia germinans; Escherichia coli; Phytochemicals; Urinary tract infection; Multidrug-resistant bacteria; Antibacterial activity; Gold nanoparticle

1. Introduction

Urinary Tract Infections (UTIs) remain one of the commonest bacterial infections, posing severe health and economic burdens. This infection, affecting over 150 million individuals yearly, often causes short-term morbidity, including lower abdominal pain, dysuria, fever, and even permanent kidney scarring [1–3]. Studies have highlighted uropathogenic Escherichia coli (UPEC) as a major aetiological agent associated with community-acquired urinary tract infections (UTIs) [4]. This bacterial species has been extensively studied for its virulence factors, which are responsible for evading the host's immune system and exacerbating infections [5]. Antibiotics, including fluoroquinolone, aminoglycosides, and beta-lactams, are the primary therapeutic options for treating UTIs, but the emergence and spread of multidrug-resistant (MDR) organisms, including MDR UPECs, pose a major challenge. Thus, this has resulted in a growing need for alternative therapeutic approaches, including the application of nanotechnologies in the development of drugs from medicinal plants for the management of drug-resistant infections [6,7].

Nanoparticles of noble metals like gold nanoparticles (AuNPs) have been of significant interest due to their biocompatibility [8,9]. Their predominant antimicrobial activity is often attributed to their cytotoxicity effects against various microbes, resulting from several interactions with the functional groups exposed on the surface of bacterial cells [10]. While physical and chemical synthesis of nanoparticles is possible, green synthesis using medicinal plants is an efficient, less toxic, and more affordable

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approach [1,11,12]. Unlike traditional physical and chemical synthesis methods, green synthesis of nanoparticles is an eco-friendly, efficient, and cost-effective approach [13]. Studies have demonstrated the successful application of plant extracts, such as Syzygium cumini, Benincasa hispida, and Zingiber officinale, as reducing agents for the synthesis of gold nanoparticles and the optimization of their reaction parameters. The nanoparticles synthesized using these plants were effective against Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, and *Escherichia coli* [7,14,15].
Several phytoextracts from plants such as Hibiscus pedunculatus, Hibiscus pusillus, Helichrysum patulum, and Avicennia marina have been found as potential cures for UTIs [16–18]. *Avicennia germinans* L. (black mangrove) has also been highlighted for its antibacterial potential against *E. coli*, S. aureus, and Bacillus sp. [19]. Thus, this plant and its synthesized gold nanoparticle may serve as a promising therapeutic agent against multidrug-resistant uropathogens, including S. aureus, K. pneumoniae, and *E. coli*. Although a recent report indicates that *A. germinans* contains alkaloids, flavonoids, phenols, and tannins [20], there are no reports on its antibacterial activity specifically against MDR uropathogens. Hence, this study assessed the antibacterial activity of crude aqueous extract and green-synthesized gold nanoparticles of *Avicennia germinans* L. against drug-resistant uropathogenic *Escherichia coli* isolates from selected hospitals in Kaduna, Nigeria.

2. Materials and Methods

Collection, Identification, and Preparation of the Plant Sample

The leaves of *Avicennia germinans* were collected in December 2024 from Ogbogoro River in Obio/Akpor LGA (4° 50' 48"N and 6° 55' 50"E), Rivers State, Nigeria. The plant was identified using standard vegetative morphological identification (voucher code: UPH/P/481). This was done at the herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The identified plant leaves were rinsed and dried in a shaded area at 25°C for 45 days, after which they were pulverized into a fine powder using a clean blender [7]. To prepare the crude aqueous extract, 100 g of the plant powder was extracted in 1,000 mL of distilled water and kept at 25°C for 24 hours. The plant mixture was filtered and centrifuged at 5,500 rpm for 20 minutes. The supernatant was collected and filtered twice, using an 11 µm pore size Whatman filter paper (Grade 1). The pH of the plant aqueous extract was measured using a pH meter, and the extract was stored at 4°C for further use [21,22].

Phytochemical Analysis

A qualitative screening was performed using standard procedures for the identification of major phytochemicals present. The tests included Dragendorff's (alkaloids), Ferric chloride (phenols), Shinoda (flavonoids), Liebermann–Burchard (terpenoids), Salkowski (steroids), and the Foam test (saponins). Then, major phytochemicals (alkaloids, saponins, terpenoids, flavonoid, tannins, phenols, and coumarins) were further quantified using standard colorimetric procedures and reported as equivalents (mg/g dry weight). Phytochemical screening and quantification were conducted in triplicate at the Biochemistry Laboratory of Bayero University, Kano [23–27].

Identification of Plant Bioactive Compounds Using Gas Chromatography-Mass Spectrometry (GC-MS)

The crude aqueous extract of A. germinans leaves was analysed using GC-MS (Agilent GC, USA) at the multi-user laboratory of Ahmadu Bello University, Zaria, Nigeria. The aqueous extract was lyophilized and later dissolved in ethyl acetate (at 1 mg/mL). Then, the concentrated extract was filtered using a syringe (0.45 µm). Afterwards, 2 µL of the filtered extract was injected into the GC-MS system using an auto-injector. The GC-MS was equipped with an Rtx-5 capillary column (30 m × 250 µm, with 0.25 µm film thickness), and helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. The oven temperature was set to rise from 60 °C to 280 °C at a rate of 10°C every 2 minutes. A post-run at 50°C (6 min hold), and the MS transfer line was maintained at 300°C. Scans were done at m/z 50 to 450. The MS fragmentation patterns of the compounds were compared and identified using the National Institute of Standards and Technology library, with the mass spectrometer functioning at 70 eV [28].

Green synthesis and Characterization of A. germinans Gold Nanoparticles

The plant extract and a 3 mM gold (III) chloride trihydrate ($AuCl_3 \cdot 3H_2O$) solution were mixed in a

1:3 ratio to achieve a final volume of 400 mL. The mixture exhibited a colour change from yellow to ruby red and was incubated at 40°C for a 24-hour period, monitored for a colour change (ruby red colour) indicating nanoparticle synthesis. Afterwards, the nanoparticles were purified using distilled water and dried at 25°C for 2 minutes [7,14]. AuNPs were characterized using UV-visible spectroscopy (UV-Vis) (T70 UV-Vis Spectrophotometer, PG Instruments Ltd, UK). The morphology was evaluated using scanning electron microscopy (SEM) (Phenom ProX Desktop SEM, Thermo Fisher Scientific), coupled with Energy-Dispersive X-ray (EDX) analysis for elemental characterization. Then, the crystalline nature was accessed through X-ray diffraction (XRD) patterns using a Rigaku X-ray diffractometer (Tokyo, Japan). The average crystallite size of the nanoparticles was estimated using the Debye–Scherrer equation ($D=k\lambda/\beta\cos\theta$). Finally, Fourier transform infrared spectroscopy (FTIR) (Shimadzu IRTracer-100, Kyoto, JA) with a wavenumber range of 400-4000 cm-1 was used to detect the functional groups on the surface of the AuNPs [29–31]. These analyses were conducted at the National Research Institute for Chemical Technology and the National Steel Raw Materials Exploration Agency, Kaduna, Nigeria.

Antibacterial Activity of Crude Aqueous Extract and Green-Synthesized AuNPs of A. germinans

The antibacterial effect of the crude aqueous extract and green-synthesized AuNPs of *A. germinans* was assessed against five UPEC isolates. These isolates were obtained from Barau Dikko Teaching Hospital (E1 and E3), Kawo General Hospital (E8), Jowako Specialist Hospital (E14), and Dan Musa Diagnostic Centre (E16), Kaduna, Nigeria. Their identity was confirmed through Gram staining, biochemical (IMViC) tests, and their MDR status was confirmed through antimicrobial susceptibility testing [32–34]. For the antibacterial activity, concentrations of 10 μg/mL, 25 μg/mL, 50 μg/mL, and 100 μg/mL were prepared for the green-synthesized AuNPs using 3% dimethyl sulfoxide (DMSO), as well as for the aqueous *A. germinans* leaf extract. Ciprofloxacin (10 mg/mL) was the positive control. 0.5 McFarland standardized suspensions of UPEC inoculum (1.5 x 108 CFU/ml) were streaked on Mueller-Hinton (MH) agar in triplicate, and 9 mm wells were created [35]. Then, 50 μL of each solution and concentration were introduced into the designated wells. The plates were incubated at 37°C for 24 hours, and clear inhibition zones were measured (millimetres) [36,37].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

The MIC of the crude aqueous extract and green-synthesized AuNPs of *A. germinans* was evaluated using the macro-broth diluted method using bacterial culture in tryptone soy broth adjusted to 0.5 McFarland standard. Subsequently, 50 μL of bacterial suspension was introduced to each test tube containing MH broth. Then, 50 μL (each) of the green synthesized gold nanoparticles and the aqueous plant extract were introduced separately into test tubes, with final concentrations ranging from 0.1 to 120 μg/mL and 0.1 to 120 μg/mL, respectively. The positive control for both the green-synthesized AuNPs and the plant extract was ciprofloxacin, while the negative control was sterile 3% DMSO and sterile distilled water, respectively. The tubes were incubated at 37°C for 20 hours [14,38]. For the MBC, 50 μL from MIC tubes without growth were subcultured onto Mueller-Hinton (MH) agar plates and incubated at 37°C for 24 hours, after which they were observed for growth [39].

Statistical Analysis

All tests were conducted in triplicate and expressed as mean and standard deviation. The efficacy of the aqueous leaf extract and AuNPs against the UPEC isolates was analyzed for statistical significance ($p < 0.05$) using ANOVA and Tukey's post hoc test with the Statistical Package for the Social Sciences (SPSS) software version 23 (SPSS Inc., Chicago, IL, USA).

3. Results

Phytochemical Composition of A. germinans Leaf Aqueous Extract

The major phytochemicals present in the leaves of *A. germinans* included phenols (7.24 ± 1.32 mg/g), flavonoids (3.14 ± 1.20 mg/g), tannins (2.68 ± 0.94 mg/g), saponins (2.56 ± 1.14 mg/g), and alkaloids (2.42 ± 1.12 mg/g). While coumarins and terpenoids were 1.14 ± 0.89 mg/g and 1.03 ± 0.12 mg/g, respectively (Table 1).

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Table 1: Phytochemical Composition of the Crude Aqueous Extract of *Avicennia germinans* Leaves

S/N	Phytochemicals	Leaf Extract (mg/g dry wt)	
		Qualitative	Quantitative
1	Alkaloids	+	2.42 ± 1.12
2	Saponins	+	2.56 ± 1.14
3	Terpenoids	+	1.03 ± 0.12
4	Flavonoids	+	3.14 ± 1.20
5	Tannins	+	2.68 ± 0.94
6	Phenols	+	7.24 ± 1.32
7	Coumarins	+	1.14 ± 0.89
8	Titerpenoids	-	ND
9	Phytosterols	-	ND
10	Cardiac Glycoside	-	ND
11	Anthraquinones	-	ND
12	Anthocyanins	-	ND

"+" = present, "-" = absent, "ND" = Not Detected. Values are presented as means ± Standard deviation.

Bioactive Constituents Avicenna germinans Leaf Aqueous Extract.

The GC-MS analysis revealed the presence of thirty-four compounds in the aqueous extract of Avicennia germinans leaf (Fig. 1) and (Table 2). These include phenolic compounds, fatty acids (and their esters), hydrocarbons/terpenoids, as well as halogen and nitrogen-containing molecules with Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6- (4-methyl-3-pentenyl) as the most abundant compound (10.93%).

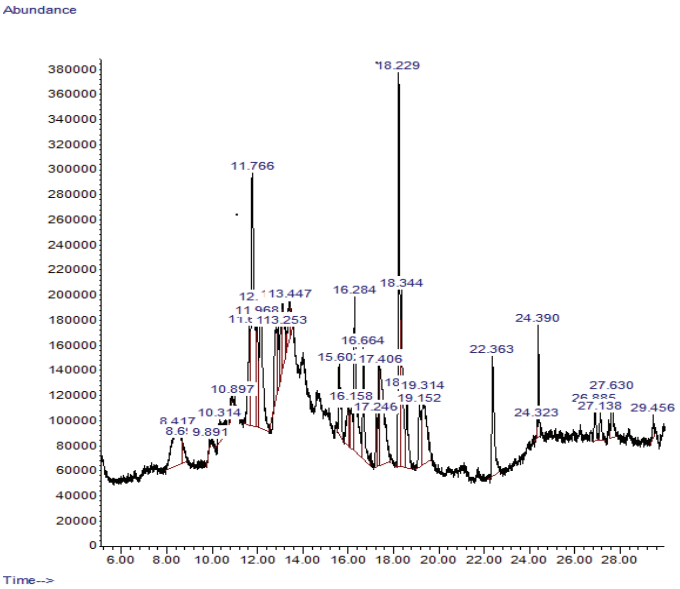

Figure 1: GC-MS Chromatogram of the Crude Aqueous Extract of *Avicennia germinans* Leaves

Table 2: GC-MS Analysis of *A. germinans* Aqueous Leaf Extract

S/N	Compound Name	Area (%)	RT	M.W (g/mol)	M.F
1	Benzenemethanol, 4-hydroxy-.alpha.-[1- (methylamino)ethyl]-, (R*,S*)-	4.8057	8.417	181.23	$C_{10}H_{15}NO_2$
2	1-Hydroxysulfonyl-3,4,4-trimethyl-2-azetidinone	0.6863	8.6954	193.22	$C_6H_{11}NO_4S$
3	Benzenamine, N- (1-methylethyl)-	0.1583	9.8906	135.21	$C_9H_{13}N$
4	4-Hydroxy-3-methylacetophenone	0.7589	10.3144	150.17	$C_9H_{10}O_2$
5	Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-	0.5814	10.8586	313.4	$C_{20}H_{27}NO_2$
6	Benzene, 1- (1,5-dimethyl-4-hexenyl)-4-methyl-	3.3109	11.6558	202.33	$C_{15}H_{22}$
7	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6- (4-methyl-3-pentenyl)-	10.932	11.7663	204.35	$C_{15}H_{24}$
8	Caryophyllene	3.4835	11.9683	204.35	$C_{15}H_{24}$
9	Cyclohexene, 3- (1,5-dimethyl-4-hexenyl)-6-methylene-, [S- (R*,S*)]-	6.0523	12.1559	204.35	$C_{15}H_{24}$
10	5-Undecanol, 2-methyl-	2.7007	12.8381	186.33	$C_{12}H_{26}O$
11	1-Octene, 6-methyl-	0.9354	12.9068	126.24	C_9H_{18}
12	2-Octenoic acid, 4,5,7-trihydroxy	2.3805	13.0844	190.19	$C_8H_{14}O_5$
13	3-Cyclohexen-1-ol, 3-methyl-	1.8003	13.1172	112.17	$C_7H_{12}O$
14	1-Hexyl-2-nitrocyclohexane	0.4752	13.2528	213.32	$C_{12}H_{23}NO_2$

15	Cyclohexane, (2,2-dimethylcyclopentyl)-	0.9966	13.3966	180.33	$C_{13}H_{24}$
16	Tetradecanoic acid	2.308	15.6018	228.37	$C_{14}H_{28}O_2$
17	Phenol, 2,6-diamino-	1.1451	15.981	124.14	$C_6H_8N_2O$
18	Trimethylsilylpyrazole	0.9149	16.0261	140.26	$C_6H_{12}N_2Si$
19	1,4-Benzenediol, 2-methyl-	1.3989	16.1576	124.14	$C_7H_8O_2$
20	Cyclohexadecane	6.115	16.2836	224.42	$C_{16}H_{32}$
21	Pentadecanoic acid, 14-methyl-, methyl ester	3.0711	16.6641	270.5	$C_{17}H_{34}O_2$
22	Didodecyl phthalate	1.0983	17.246	502.8	$C_{32}H_{54}O_4$
23	n-Hexadecanoic acid	2.258	17.3745	256.42	$C_{16}H_{32}O_2$
24	1-Octadecene	9.5566	18.229	350.5	$C_{22}H_{38}O_3$
25	Heptadecanoic acid, 16-methyl-, methyl ester	2.5109	18.5887	298.5	$C_{19}H_{38}O_2$
26	cis-11-Hexadecenal	2.4328	19.1517	238.41	$C_{16}H_{30}O$
27	Undec-10-ynoic acid, dodecyl ester	4.7637	19.3138	350.6	$C_{23}H_{42}O_2$
28	Bis (2-ethylhexyl) phthalate	4.2297	22.3629	390.6	$C_{24}H_{38}O_4$
29	Oxirane, 2,2-dimethyl-3- (3,7,12,16,20-pentamethyl-3,7,11,15,19-henicosapentaenyl)-, (all-E)-	0.1926	24.3226	426.7	$C_{30}H_{50}O$
30	1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)-	1.8139	24.3905	192.34	$C_{14}H_{24}$
31	N-Methyl-1-adamantaneacetamide	1	26.8848	207.31	$C_{13}H_{21}NO$
32	6-Chloro-1-ethyl-4-oxoquinoline-3-carboxylic acid	0.7226	27.1378	251.66	$C_{12}H_{10}ClNO_3$
33	dl-.alpha.-Tocopherol	1.467	27.6299	430.7	$C_{29}H_{50}O2$
34	i-Propyl 9-tetradecenoate	0.4994	29.4562	268.4	$C_{17}H_{32}O_2$

Characterization of A. germinans AuNPs

The synthesized *A. germinans* AuNPs produced a characteristic peak at 590 nm from the UV-Vis spectra (Fig. 2a) and the presence of differently shaped nanoparticles, with nanoprisms and nanotriangles being predominant in the SEM micrograph (Fig. 2b). The AuNPs exhibited a significant degree of aggregation and agglomeration, characterized by irregular spaces between them. EDX spectra for the AuNPs revealed gold molecule signals at 2.12–2.22 keV (Fig. 2c), and an atomic concentration of 97.69% (Table 3). Additionally, there were weak signals, indicating the presence of stromeyerite (zirconium, sulfur, iron, silver, and aluminum). The XRD analysis (Fig. 2d) depicts the crystalline nature of *A. germinans* AuNPs, with well-defined diffraction peaks at 2θ = 38.08°, 44.29°, and 64.56°, corresponding to the 111, 200, and 220 crystallographic planes of face-centered cubic (FCC) gold (Au), respectively. Two weak reflections of the stromeyerite (Ag, Cu, and S) were also revealed at 2θ = 34.12° and 44.29°. The calculated size of the AuNPs was 29.23nm, and the average lattice spacing was 4.075Å. Then, the FTIR spectrum (Fig. 2e) revealed three major peaks at 1635.69 cm^{-1}, 2139.13 cm^{-1}, and 3342.75 cm^{-1}.

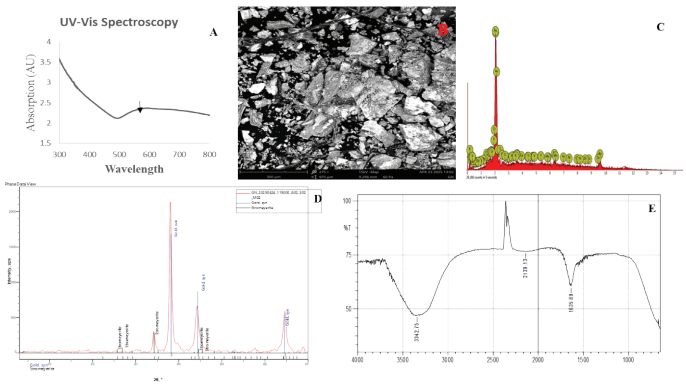

Figure 2: UV-Vis (A); SEM (B); EDX (C); XRD (D); FTIR (E) Characterization of *A. germinans* AuNPs

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Table 3: EDX Elemental Microanalysis of the Biosynthesized Gold Nanoparticles

Element	Atomic Concentration (%)	Weight Concentration (%)
Gold (Au)	97.69	99.16
Zirconium (Zr)	1.19	0.56
Sulfur (S)	0.35	0.06
Iron (Fe)	0.31	0.09
Aluminum (Al)	0.12	0.02
Calcium (Ca)	0.08	0.02
Copper (Cu)	0.07	0.02
Tin (Sn)	0.05	0.03
Silver (Ag)	0.04	0.02
Chromium (Cr)	0.04	0.01
Potassium (K)	0.03	0.01
Magnesium (Mg)	0.03	0.00
Total	**100**	**100**

Antibiotic Susceptibility Pattern of MDR Uropathogenic E. coli Isolates

The antibiogram of the uropathogenic E. coli isolates showed a MAR index ranging from 0.7 to 1, and these isolates were confirmed to be multidrug-resistant to different classes of antibiotics, such as aminoglycosides, cephalosporins, penicillins, and fluoroquinolones (Table 4).

Table 4: MAR Index and MDR profile of the Uropathogenic *E. coli* Isolates

Isolates	MAR Index	MDR status
E1	1	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides
E3	0.9	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides
E8	0.7	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides
E14	1	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides
E16	0.8	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides

Antibacterial Activity of Crude Aqueous Extract and Green-synthesized AuNPs of A. germinans

The antibacterial activity of the crude aqueous extract and green-synthesized AuNPs of *A.germinans* indicated significant inhibitory effects against MDR UPECs (Table 5). At the highest concentration of the plant extract (100mg/mL), isolate E8 showed the strongest susceptibility (15.7 ± 1.0 mm), while isolate E16 had the lowest susceptibility (12.7 ± 1.2 mm) recorded. However, the AuNPs showed an inconsistent dose-dependent result, with isolate E3 exhibiting a high ZOI (15.7 ± 0.8 mm) at 25 µg/mL. Then, the control antibacterial drug (ciprofloxacin) exhibited limited antibacterial activity, with a zone of inhibition (ZOI) of 10.0 ± 0.0 mm recorded for only isolates E1 and E8. Statistical analysis showed a significant difference between the concentrations and isolates ($P < 0.05$). Tukey HSD revealed that treatments with varying superscripts (a vs b) were significantly different from each other. Considering the difference in concentration units, the nanoparticles indicated greater potency, based on their lower unit concentration. The least MIC and MBC values for the plant extract were recorded at 30 mg/mL and 60 µg/mL for the AuNPs (Table 6).

Table 5: Average Zone of Inhibition of *A. germinans* Crude Aqueous Extract, Gold Nanoparticle, and Control (Ciprofloxacin)

Antibacterial Agent	Concentrations	Mean Zone of Inhibition (mm) of the UPEC Samples				
		E1	E3	E8	E14	E16
Plant Extract	100 (mg/mL)[a]	13.7±1.3	13.7±1.6	15.7±1.0	13.7±1.5	12.7±1.2
	50 (mg/mL)[a]	13.7±1.5	12.2±1.2	12.0±0.0	12.5±1.3	12.5±0.5
	25 (mg/mL)[a]	12.8±1.4	0.0±0.0	10.2±0.3	12.0±1.7	12.3±1.2
	10 (mg/mL)[b]	11.8±1.1	0.0±0.0	0.0±0.0	0.0±0.0	9.7±0.3
Gold Nanoparticle	100 (μg/mL)[a]	11.0±1.0	12.0±0.9	13.7±0.3	0.0±0.0	0.0±0.0
	50 (μg/mL)[b]	10.5±0.5	11.0±0.9	14.7±1.2	0.0±0.0	0.0±0.0
	25 (μg/mL)[b]	11.0±0.5	15.7±0.8	11.0±0.9	0.0±0.0	0.0±0.0
	10 (μg/mL)[b]	0.0±0.0	11.0±0.0	10.0±0.9	0.0±0.0	0.0±0.0
Control	10 (mg/mL)[b]	10.0±0.0	0.0±0.0	10.0±0.0	0.0±0.0	0.0±0.0

According to Tukey's HSD, concentrations with the same alphabet indicate no significant statistical difference ($P > 0.05$), while those with different alphabets indicate a significant statistical difference ($P < 0.05$).

Table 6: MIC and MBC of *A. germinans* Crude Aqueous Extract and Green Synthesized Gold Nanoparticles

Antibacterial Agent	MIC					MBC				
	E1	E3	E8	E14	E16	E1	E3	E8	E14	E16
Plant Extract (mg/mL)	-	30	120	120	-	-	30	120	120	-
Gold Nanoparticle (μg/mL)	60	60	60	120	-	120	60	-	-	-
Ciprofloxacin (mg/mL)	-	-	-	-	-	-	-	-	-	-
Distilled Water	-	-	-	-	-	-	-	-	-	-
3% DMSO	-	-	-	-	-	-	-	-	-	-

Values are triplicate, represented as the mean. "-" = no inhibitory/no bactericidal activity

4. Discussion

Urinary tract infections remain one of the common bacterial infections, and the spread of MDR uropathogens further exacerbates the burden of this infection. As conventional antibiotics are gradually waning, there is a need for more effective and safer alternative treatment options. Plant extracts and green-synthesized nanoparticles have been a major focus for researchers as alternatives to conventional antibiotics [7,17]. In this study, both the crude aqueous extract and green synthesized AuNPs of *A. germinans* were tested for their antibacterial properties against UPECs. This study revealed the presence of major phytochemicals, such as alkaloids, flavonoids, tannins, and phenols. Also, the AuNPs synthesis was successful with the presence of heterogenous nanoparticles. Furthermore, the study highlighted a dose-dependent antibacterial activity for the plant extract and an inconsistent dose-dependent antibacterial activity for the AuNPs against selected UPECs.

The presence of the phytochemicals in *A. germinans* aligns with the findings of Rajeswari et al. [19], conducted on the methanolic, hexane, ethyl acetate, petroleum ether, and dichloromethanolic extracts of this plant. Several studies have highlighted the antibacterial potential of a majority of the bioactive compounds found in *A. germinans* leaves, especially phenolic compounds, fatty acids

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(and their esters), and hydrocarbon/terpenoid molecules of plant-derived compounds [40–42]. The most abundant compound, Bicyclo[3.1.1] hept-2-ene, 2,6-dimethyl-6- (4-methyl-3-pentenyl) (10.93%), belongs to a group of compounds known as sesquiterpenoids. These have been known for their antibacterial activities, even against *E. coli* [43]. Other compounds, such as methyl 14-methylpentadecanoate (3.0711%), 4-Hydroxy-3-methylacetophenone (0.7589%), n-Hexadecanoic acid (2.258%), and Oxirane (0.1926%) derivatives have also been found present in the leaf extract of *A. alba* [44]. The formation of *A. germinans* AuNPs was further confirmed by the UV-Vis peak at 590nm. Based on previous studies, AuNPs exhibit characteristic peaks within the range of 500nm to 600nm [14,15,45]. The SEM micrograph showed heterogeneous nanoparticles with evidence of aggregates and agglomerates. Geometrical shapes are prevalent among nanoparticles and have been described as capable of influencing their biological activities [46–48]gold nanoparticles (AuNPs. The gold molecule signals (2.12–2.22 keV) and atomic concentration of (97.69%) from the EDX spectra highlight the purity and yield of gold present in the nanoparticles [49]. The XRD peaks at 2θ = 38.08°, 44.29°, and 64.56° closely align with those recorded by Clarance et al. [50].
Also, the AuNPs measured 29.23nm in size; Fouda et al. [15] reported a calculated size of 18nm. The lattice value 4.075Å, aligns with the finding Karuppiah et al. [51]. From the FTIR spectra, the peak observed at 1635.69 cm^{-1} corresponds to the N-H vibrations of the primary amines in proteins. Then, the absorption band at 2139.13 cm^{-1} reveals the presence of C≡C internal alkyne stretching. Finally, the peak at 3342.75 cm^{-1} suggests the presence of the hydroxyl (OH) group [52–54]. This implies that the AuNPs contain important metabolites and proteins, featuring amines, alkynes, and alcohols, which are initially present in the *A. germinans* leaves [19].

While the preliminary quantitative analysis highlighted phenols to be the most abundant phytochemicals, the GC-MS analysis revealed a sesquiterpenoid to be the most abundant compound. This may be attributed to the selective extraction of mostly volatile, non-polar constituents by ethyl acetate used in the GC-MS analysis, whereas aqueous extraction retains more polar phenolics [61,62]human beings have traditionally employed many folkloric herbal resources as complementary and alternative remedies, and these remedies have played a pivotal role in modern medicines for many decades, as scientists have used them to develop drugs. We studied the effects of employing solvents with varying polarity on the yields of phytochemical components extracted from the plant Rhazya stricta. We used chloroform–methanol (1:1. The colour change to ruby red, indicating the synthesis of AuNPs, is attributed to surface plasmon resonance [14]. Aggregation and agglomeration of AuNPs occur due to electrostatic forces and are influenced by higher metal salt concentrations, longer reaction or incubation times, and post-synthesis drying [63,64]. The presence of stromeyerite can be attributed to the natural elements present in *A. germinans* [65–67]. Then, the functional groups highlighted by the FTIR spectra are responsible for the gold salt reduction (i.e., Au^{3+} to Au^{0}) [52].
Now, the high MAR index and MDR of the obtained UPEC isolates align with the findings of Nkene et al. [55] on *E. coli* isolated from suspected UTI patients in Keffi, Nigeria, and other reports on the MDR status of UPEC isolates [56]this cross-sectional study was conducted to predict the association of phenotypic and genotypic resistance traits in uropathogenic *Escherichia coli* (UPEC. The dose (concentration) dependent activity of the aqueous plant extract agrees with the findings in previous studies, reported for *Piper betle* and *Solanum khasianum* [57,58]. The least MIC and MBC recorded were at 30 mg/mL for the plant aqueous extract and 60 µg/mL for the nanoparticles are different from the report of Arsene et al. [59], who highlighted lower MICs and MBCs for methanolic extracts of the leaves of *Amaranthus tricolor, Mentha piperita,* and *Anthocephalus cadamba,* with MICs ranging from 0.36 mg/mL to 125 mg/mL, and MBCs of 21.67 mg/mL when tested against UPECs.

Compared to the plant extract, the inconsistent dose-dependent (non-linear) antibacterial activity recorded for the gold nanoparticles may be due to the formation of aggregates or agglomerates and particle heterogenity in such nanoparticle solutions. The presence of aggregates could prevent the AuNPs from interacting with the bacterial cells [60]. Nevertheless, Diksha et al. [7] recorded a higher MIC value of 450 µg/mL for an MDR UPEC using *Syzygium cumini* gold nanoparticles.

This is the first report on the antibacterial activity of the crude aqueous extracts and green-synthesized AuNPs of *A. germinans* against MDR UPEC. It also provides evidence that supports the pharmacological potentials of *A. germinans*

leaves and their suitability as reducing and capping agents for the synthesis of antibacterial AuNPs. However, this study did not include the formation of AuNPs with controlled shapes and sizes, nor did it assess their cytotoxic effects. In addition to this, future studies should also involve dynamic light scattering (DLS) and zeta potential measurements for the nanoparticles, time–kill kinetics, growth curve inhibition studies, anti-biofilm or quorum-sensing inhibition assays, and mechanistic investigations.

5. Conclusion

The crude aqueous extract of *A. germinans* leaves was confirmed to contain significant amounts of alkaloids, saponins, terpenoids, flavonoids, tannins, phenols, and coumarins. The plant extract contained 34 bioactive compounds, with Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl) (10.93%) being the most abundant. The green synthesis of AuNPs using *A. germinans* leaf extract was successful, as further confirmed by standard spectrophotometric and microscopic analysis. This study confirms the antibacterial activities of both the crude aqueous extract and green-synthesized AuNPs of *A. germinans* against MDR UPECs. Further studies should investigate the antibacterial activity of different bioactive compounds in the leaves and other toxic effects.

Conflicts of Interest

The authors declares no conflict of interest.

Author Contribution

Ewofere, E.E.: Conceptualisation, Methodology, Investigation, Data Analysis, Original Draft Writing, and Curation.
Oaikhena, E.E: Supervision, Validation, Review and Editing.
Egbe, N.E.: Review and Editing.
Afegbua, S.L. : Conceptualisation, Review and Editing, Data Curation and Visualisation, Validation, and Supervision.

Data Availability

Further data supporting this study are available at zenodo.org

References

1. Marouf R, Mbarga J, Ermolaev A, Podoprigora I, Smirnova I, Yashina N, et al. Antibacterial activity of medicinal plants against uropathogenic *Escherichia coli.* Vol. 14, Journal of Pharmacy and Bioallied Sciences. 2022.

2. Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C. Urinary Tract Infections: The Current Scenario and Future Prospects. Pathogens. 2023 Apr 20;12(4):623.

3. Kumwenda P, Semu P. Characterization of urinary tract infections among females at a tertiary hospital in Malawi: a retrospective study. BMC Infect Dis. 2025 Apr 5;25(1):466.

4. Shrestha BK, Tumbahangphe M, Shakya J, Chauhan S. Uropathogenic *Escherichia coli* in urinary tract infections: A review on epidemiology, pathogenesis, clinical manifestation, diagnosis, treatments and prevention. Vol. 6, Novel Research in Microbiology Journal. 2022.

5. Whelan S, Lucey B, Finn K. Uropathogenic *Escherichia coli* (UPEC)-Associated Urinary Tract Infections: The Molecular Basis for Challenges to Effective Treatment. Microorganisms. 2023 Aug 28;11(9):2169.

6. Crintea A, Carpa R, Mitre AO, Petho RI, Chelaru VF, Nădășan SM, et al. Nanotechnology Involved in Treating Urinary Tract Infections: An Overview. Vol. 13, Nanomaterials. 2023.

7. Diksha D, Gupta SK, Gupta P, Banerjee UC, Kalita D. Antibacterial Potential of Gold Nanoparticles Synthesized From Leaf Extract of *Syzygium cumini* Against Multidrug-Resistant Urinary Tract Pathogens. Cureus. 2023 Feb 10;

8. Küünal S, Rauwel P, Rauwel E. Plant extract mediated synthesis of nanoparticles. In: Emerging Applications of Nanoparticles and Architecture Nanostructures. Elsevier; 2018. p. 411–46.

9. Gecer EN. Phytochemistry of *Centaurea saligna*: silver nanoparticle synthesis, quantification of natural compounds, antioxidant and antibacterial activity. Sci Rep. 2025;15(1).

10. Aljabali A, Akkam Y, Al Zoubi M, Al-Batayneh K, Al-Trad B, Abo Alrob O, et al. Synthesis of Gold Nanoparticles Using Leaf Extract of Ziziphus zizyphus and their Antimicrobial Activity. Nanomaterials. 2018 Mar 19;8(3):174.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1820

11. Álvarez-Martínez FJ, Barrajón-Catalán E, Encinar JA, Rodríguez-Díaz JC, Micol V. Antimicrobial Capacity of Plant Polyphenols against Gram-positive Bacteria: A Comprehensive Review. Curr Med Chem. 2020 Jun 2;27(15):2576–606.

12. Sharma A, Yadav MK. Nanomaterials and nanotechnology : A comprehensive study of synthesis, characterization and multifaceted applications. Biochem Cell Arch. 2023 Dec;23(S1).

13. Qindeel M, Barani M, Rahdar A, Arshad R, Cucchiarini M. Nanomaterials for the diagnosis and treatment of urinary tract infections. Vol. 11, Nanomaterials. 2021.

14. Al Saqr A, Khafagy ES, Alalaiwe A, Aldawsari MF, Alshahrani SM, Anwer MdK, et al. Synthesis of Gold Nanoparticles by Using Green Machinery: Characterization and In Vitro Toxicity. Nanomaterials. 2021 Mar 22;11(3):808.

15. Fouda A, Eid AM, Guibal E, Hamza MF, Hassan SED, Alkhalifah DHM, et al. Green Synthesis of Gold Nanoparticles by Aqueous Extract of *Zingiber officinale*: Characterization and Insight into Antimicrobial, Antioxidant, and In Vitro Cytotoxic Activities. Applied Sciences. 2022 Dec 15;12(24):12879.

16. Devi AS, Rajkumar J. In vitro Antibacterial Activity and Stability of *Avicennia marina* against Urinary Tract Infection Pathogens at Different Parameters. Pakistan Journal of Biological Sciences. 2013 Jun 15;16(19):1034–9.

17. Awuchi CG. Medicinal plants: the medical, food, and nutritional biochemistry and uses. International Journal of Advanced Academic Research | Sciences. 2019;5(11).

18. Cock I, Mavuso N, Van Vuuren S. A Review of Plant-Based Therapies for the Treatment of Urinary Tract Infections in Traditional Southern African Medicine. Evidence-Based Complementary and Alternative Medicine. 2021 Jul 29;2021:1–20.

19. Rajeswari G, Prakash B, Gowrisanker J, et al. Study of preliminary analysis and antibacterial activity of *Avicennia germinans.* . Int J Adv Interdiscip Res . 2013;(1):28–31.

20. Bonilla-Loor M, Solórzano-Zambrano L, Pazmiño A, Segovia-Cedeño D, Delgado-Mera J, Tuárez-Párraga M, et al. Phytochemical characterization and antioxidant potential of black mangrove (Avicennia germinans L.) from the northern coast of Ecuador using aqueous and ethanolic extraction. S Afr J Chem Eng. 2025 Oct;54:451–60.

21. Evbuomwan L, Chukwuka EP, Obazenu EI, Ilevbare L. Antibacterial activity of <i>Vernonia amygdalina</i> leaf extracts against multidrug resistant bacterial isolates. Journal of Applied Sciences and Environmental Management. 2018 Feb 7;22(1):17.

22. Salgado P, Bustamante L, Carmona DJ, Meléndrez MF, Rubilar O, Salazar C, et al. Green synthesis of Ag/Ag2O nanoparticles on cellulose paper and cotton fabric using Eucalyptus globulus leaf extracts: Toward the clarification of formation mechanism. Surfaces and Interfaces. 2023 Aug;40:102928.

23. Akinseye OR, Morayo AE, Olawumi AS. Qualitative and Quantitave Evaluation of the Phytochemicals in Dry , Wet and Oil Extracts of the Leaf of *Morinda lucida*. J Biol Agric Healthc. 2017;7(7).

24. Ameen OA, Hamid AA, Yusuf Q, Njoku OG, Oseni TO, Jamiu W. Quantitative and Qualitative Assessment of Phytochemicals in Methanolic Extracts of Hurricane Weed (*Phyllanthus amarus* Schumach. &Thonn) Plant. Journal of Applied Sciences and Environmental Management. 2021 Apr 14;25(2):159–65.

25. Das S, Goswami M, Yadav R, Bandyopadhyay T. Quantitative Estimation of Terpenoid Content in Some Tea Cultivars of North East India and Their *in vitro* cell cultures using an optimized spectrophotometric method. J Adv Sci Res. 2022 Apr 30;13(03):112–7.

26. Rajkumar G, Panambara PAHR, Sanmugarajah V. Comparative Analysis of Qualitative and Quantitative Phytochemical Evaluation of Selected Leaves of Medicinal Plants in Jaffna, Sri Lanka. Borneo Journal of Pharmacy. 2022 May 31;5(2):93–103.

27. Bakir Çilesizoğlu N, Yalçin E, Çavuşoğlu K, Sipahi Kuloğlu S. Qualitative and quantitative phytochemical screening of *Nerium oleander* L. extracts associated with toxicity profile. Sci Rep. 2022 Dec 11;12(1):21421.

28. Savanth S, Fernandes J, D Souza NG. Phytochemical Profiling and Quantitative Analysis of Bioactive Compounds in *Sansevieria trifasciata* Laurenti Leaf Extracts. Res J Pharm Technol. 2025 Jun 12;2567–74.

29. Dzimitrowicz A, Jamróz P, diCenzo GC, Sergiel I, Kozlecki T, Pohl P. Preparation and characterization of gold nanoparticles prepared with aqueous extracts of Lamiaceae plants and the effect of follow-up treatment with atmospheric pressure glow microdischarge. Arabian Journal of Chemistry. 2019 Dec;12(8):4118–30.

30. Sathiyaraj S, Suriyakala G, Dhanesh Gandhi A, Babujanarthanam R, Almaary KS, Chen TW, et al. Biosynthesis, characterization, and antibacterial activity of gold nanoparticles. J Infect Public Health. 2021 Dec;14(12):1842–7.

31. Giri AK, Jena B, Biswal B, Pradhan AK, Arakha M, Acharya S, et al. Green synthesis and characterization of silver nanoparticles using *Eugenia roxburghii* DC. extract and activity against biofilm-producing bacteria. Sci Rep. 2022 May 19;12(1):8383.

32. Daoud N, Hamdoun M, Hannachi H, Gharsallah C, Mallekh W, Bahri O. Antimicrobial Susceptibility Patterns of *Escherichia coli* among Tunisian Outpatients with Community-Acquired Urinary Tract Infection (2012-2018). Curr Urol. 2020 Dec;14(4):200–5.

33. Nwokolo CJ, Ugwu MC, Ejikeugwu CP, R. Iroha I, Esimone CO. Incidence and antibiotic susceptibility profile of uropathogenic *Escherichia coli* positive for extended spectrum β-lactamase among HIV/ AIDS patients in Awka metropolis, Nigeria. Iran J Microbiol. 2022 Jun 20;

34. Igbonezu VN, Eruteya OC, Uguomore EO, Umezurike KC, Okonkwo PO. Proximate Analysis and Occurrence of Antibiotic Resistant *Escherichia coli* in Street Vended Ready to Eat Edible Larvae of Rhynchophorus phoenicis in Port Harcourt, Nigeria. International Journal of Pathogen Research. 2024 Aug 12;13(4):105–18.

35. Oyama MO, Egbebi AO, Akharaiyi FC. Phytochemical analysis and antibacterial activities of some plant extracts on Staphylococcus aureus isolates from patients receiving hospital treatments in Ekiti State, Nigeria. Journal of Herbmed Pharmacology. 2019 Jan 2;8(1):14–20.

36. Khuda F, Ul Haq Z, Ilahi I, Ullah R, Khan A, Fouad H, et al. Synthesis of gold nanoparticles using *Sambucus wightiana* extract and investigation of its antimicrobial, anti-inflammatory, antioxidant and analgesic activities. Arabian Journal of Chemistry. 2021 Oct;14(10):103343.

37. Zeleke B, Mekonnen Z, Bireda M, Yitbarek M, Dendir A. Phytochemical screening and antimicrobial activity of *Polygala sadebeckiana* Gürke extracts on bacterial isolates from Wound samples of patients with "Shimetere." BMC Complement Med Ther. 2024 Feb 1;24(1):72.

38. Ahmed SS, Shariq A, Alsalloom AA, Babikir IH, Alhomoud BN. Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. Int J Health Sci (Qassim) . 2019;13(2):48–55.

39. Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against *Staphylococcus aureus*. Biomater Investig Dent. 2020 Jan 1;7(1):105–9.

40. Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Endringer DC, Fronza M, et al. Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. Molecules. 2019 Jul 5;24(13):2471.

41. Song W, Xin J, Yu C, Xia C, Pan Y. Alkyl ferulic acid esters: Evaluating their structure and antibacterial properties. Front Microbiol. 2023 Feb 10;14.

42. Kauffmann AC, Castro VS. Phenolic Compounds in Bacterial Inactivation: A Perspective from Brazil. Antibiotics. 2023 Mar 24;12(4):645.

43. Li HY, Yang WQ, Zhou XZ, Shao F, Shen T, Guan HY, et al. Antibacterial and Antifungal Sesquiterpenoids: Chemistry, Resource, and Activity. Biomolecules. 2022 Sep 9;12(9):1271.

44. Eswaraiah G, Peele KA, Krupanidhi S, Kumar RB, Venkateswarulu TC. Identification of bioactive compounds in leaf extract of *Avicennia alba* by GC-MS analysis and evaluation of its in-vitro anticancer potential against MCF7 and HeLa cell lines.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1820

J King Saud Univ Sci. 2020 Jan;32(1):740–4.

45. Oliveira M, Sousa A, Sá S, Soares S, Pereira AC, Rocha AC, et al. Harvesting the Power of Green Synthesis: Gold Nanoparticles Tailored for Prostate Cancer Therapy. Int J Mol Sci. 2024 Feb 14;25(4):2277.

46. Santhosh PB, Genova J, Chamati H. Green Synthesis of Gold Nanoparticles: An Eco-Friendly Approach. Chemistry (Easton). 2022 Apr 25;4(2):345–69.

47. Barnawi N, Allehyani S, Seoudi R. Biosynthesis and characterization of gold nanoparticles and its application in eliminating nickel from water. Journal of Materials Research and Technology. 2022 Mar;17:537–45.

48. Timoszyk A, Grochowalska R. Mechanism and Antibacterial Activity of Gold Nanoparticles (AuNPs) Functionalized with Natural Compounds from Plants. Pharmaceutics. 2022 Nov 25;14(12):2599.

49. Suriyakala G, Sathiyaraj S, Babujanarthanam R, Alarjani KM, Hussein DS, Rasheed RA, et al. Green synthesis of gold nanoparticles using *Jatropha integerrima* Jacq. flower extract and their antibacterial activity. J King Saud Univ Sci. 2022 Apr;34(3):101830.

50. Clarance P, Luvankar B, Sales J, Khusro A, Agastian P, Tack JC, et al. Green synthesis and characterization of gold nanoparticles using endophytic fungi *Fusarium solani* and its in-vitro anticancer and biomedical applications. Saudi J Biol Sci. 2020 Feb;27(2):706–12.

51. Karuppiah C, Palanisamy S, Chen SM, Emmanuel R, Muthupandi K, Prakash P. Green synthesis of gold nanoparticles and its application for the trace level determination of painter's colic. RSC Adv. 2015;5(21):16284–91.

52. Islam NU, Jalil K, Shahid M, Rauf A, Muhammad N, Khan A, et al. Green synthesis and biological activities of gold nanoparticles functionalized with *Salix alba*. Arabian Journal of Chemistry. 2019 Dec;12(8):2914–25.

53. Sadalage PS, Patil R V., Havaldar D V., Gavade SS, Santos AC, Pawar KD. Optimally biosynthesized, PEGylated gold nanoparticles functionalized with quercetin and camptothecin enhance potential anti-inflammatory, anti-cancer and anti-angiogenic activities. J Nanobiotechnology. 2021 Dec 25;19(1):84.

54. El-Azazy M, S. El-Shafie A, Al-Saad K. Introductory Chapter: Infrared Spectroscopy - Principles and Applications. In: Infrared Spectroscopy - Perspectives and Applications. IntechOpen; 2023.

55. Rahman MdM, Hossain MMdK, Rubaya R, Halder J, Karim MdE, Bhuiya AA, et al. Association of Antibiotic Resistance Traits in Uropathogenic *Escherichia coli* (UPEC) Isolates. Canadian Journal of Infectious Diseases and Medical Microbiology. 2022 Mar 16;2022:1–9.

56. Saeloh D, Visutthi M. Efficacy of Thai Plant Extracts for Antibacterial and Anti-Biofilm Activities against Pathogenic Bacteria. Antibiotics. 2021 Nov 30;10(12):1470.

57. Chirumamilla P, Dharavath SB, Taduri S. GC–MS profiling and antibacterial activity of Solanum khasianum leaf and root extracts. Bull Natl Res Cent. 2022 Dec 8;46(1):127.

58. Mbarga Manga Joseph A, Podoprigora Irina V, Anyutoulou Kitio Linda D, Ngah E, Senyagin Alexander N. Urinary tract infections: Virulence factors, resistance to antibiotics, and management of uropathogenic bacteria with medicinal plants—A review. J Appl Pharm Sci. 2020 Jul 5;

59. Baeshen NA, Almulaiky YQ, Afifi M, Al-Farga A, Ali HA, Baeshen NN, et al. GC-MS Analysis of Bioactive Compounds Extracted from Plant *Rhazya stricta* Using Various Solvents. Plants. 2023 Feb 20;12(4):960.

60. Lee JE, Jayakody J, Kim JI, Jeong JW, Choi KM, Kim TS, et al. The Influence of Solvent Choice on the Extraction of Bioactive Compounds from Asteraceae: A Comparative Review. Foods. 2024 Oct 2;13(19):3151.

61. kazemi S, Hosseingholian A, Gohari SD, Feirahi F, Moammeri F, Mesbahian G, et al. Recent advances in green synthesized nanoparticles: from production to application. Materials Today Sustainability. 2023 Dec;24:100500.

62. Şahin A, Altınsoy Ş, Kızılbey K. An approach for cationic dyes removal from wastewater: Green synthesis of iron nanoparticles using *Prunus avium* stems extracts. Kuwait Journal of Science. 2024 Jul;51(3):100226.

63. Sobrado MA, Greaves ED. Leaf secretion composition of the mangrove species *Avicennia germinans* (L.) in relation to salinity: a case study by using total-reflection X-ray fluorescence analysis. Plant Science. 2000 Oct;159(1):1–5.

64. Martínez-Colón M, Alegría H, Huber A, Kubra-Gul H, Kurt-Karakus P. Bioaccumulation and biomagnification of potential toxic elements (PTEs): An *Avicennia germinans*–Uca rapax trophic transfer story from Jobos Bay, Puerto Rico. Ecol Indic. 2021 Feb;121:107038.

65. Muñoz-García A, Arbeli Z, Boyacá-Vásquez V, Vanegas J. Metagenomic and genomic characterization of heavy metal tolerance and resistance genes in the rhizosphere microbiome of *Avicennia germinans* in a semi-arid mangrove forest in the tropics. Mar Pollut Bull. 2022 Nov;184:114204.

66. Zhou Y, Kong Y, Kundu S, Cirillo JD, Liang H. Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and *Bacillus* Calmette-Guérin. J Nanobiotechnology. 2012 Dec 6;10(1):19.

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Integrating the Egyptian Government Excellence Award (EGEA) With The GAHAR Standards for Hospitals for Sustainable Excellence in Egyptian Healthcare Facilities

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ABSTRACT:

The need to implement control standards and quality management in healthcare services has shown significant growth in recent decades to improve the manage-ment of these services and the satisfaction of their users. This will be achieved through creating a framework for implementing the Egyptian government excellence award in hospitals for sustainable services. The study conducted to compare the per-formance of hospitals accredited by GAHAR and non-accredited hospitals on the re-quirements of the Egypt Award for Government Excellence identifies the link and the gap between GAHAR accreditation and the model of the Egypt Government Excel-lence Award.

***Methodology:** A quantitative phase using a questionnaire distributed to 206 healthcare providers from a sample of accredited and non-accredited hospitals to assess the extent to which the Egyptian government excellence award (EGEA) criteria are applied through the implementation of GAHAR (General Authority for Healthcare Accreditation & Regulation) Accreditation standards in Egyptian hospitals. This was followed by a qualitative phase in the form of focus group discussions with experts from EGEA to identify the gap between GAHAR accreditation criteria and the Egyptian Government Excellence Award model.*

***Results:** There was a significant difference between GAHAR-accredited and non-accredited hospitals regarding the Egyptian Government Excellence Award criteria, with a p-value < 0.05. Focus group discussion analysis revealed that the strongest integration opportunity lies in quality and patient safety, as both frameworks prioritize this. Innovation and alignment with Egypt Vision 2030 were also recurring opportunities. Dual implementation increases complexity. Resource limitations and resistance to change are major challenges.*

***Conclusion:** There is convergence between the accreditation model and the Egyptian excellence model, suggesting that accreditation helps the healthcare sector to implement the best management practices that are present in the Egyptian Excellence Model.*

KEYWORDS:

Accreditation, Egyptian Excellence Award; GAHAR Standards for hospitals, Healthcare Quality Management.

1. Introduction

The need to implement control standards and quality management in healthcare services has shown significant growth in recent decades to improve the management of these services and the satisfaction of their users. [1]

There are many quality models possible to be employed in healthcare organizations, such as structured process improvement methods (PDCA, 8D, 5S), accreditation (ONA, JCI, CCHSA, NIAHO), models of organizational excellence (Malcolm Baldrige National Quality Award, European Foundation for Quality Management (EFQM), Healthcare Excellent Quality Management

(HEQMM)); International Standards Organization for Standardization - ISO (ISO 9001:2008; ISO 14000, ISO 26000), among others. [2-5]

In recent years, accreditation has been widely used across Egyptian hospitals to improve quality and safety. [6] Although the Egyptian government excellence award includes several categories, including institutional excellence, it has not specifically discussed hospitals as an independent category. In 2013, the adaptation of Joint Commission International (JCI) accreditation standards for hospitals to the health care excellence model was proposed. Studies in several countries regarding the comparison of the excellence and evaluation models showed the comprehensiveness of the organizational excellence model in comparison with other evaluation and accreditation models. [7]

The Egyptian accreditation system for healthcare organizations will later be a basic requirement for healthcare facilities to obtain in Egypt. Accreditation criteria function as regulations and metrics that establishments are required to fulfill in order to exhibit their dedication to excellence and ongoing enhancement. [8]

Creating a culture of excellence at a practical level, excellence works by identifying problems in the healthcare system and processes so healthcare leaders can build and sustain continuous improvement. [9]

The aim of this study is to compare the performance of GAHAR-accredited and non-accredited hospitals on the requirements of the Egyptian Government Excellence Award, and to analyze the relationship and discrepancies between GAHAR accreditation criteria and the EGEA model.

2. Methodology

Phase 1: Quantitative phase

A cross-sectional survey was conducted, including healthcare providers in GAHAR-accredited and non-accredited hospitals. Two Egyptian governorates were selected (purposive sample), namely Aswan and Luxor, as they were among the first phase governorates implementing the Universal Health Insurance System (UHIS), and they share similar demographic characteristics. A list of hospitals in each governorate was obtained from the health affairs directorate in each governorate. Six hospitals from each governorate were selected randomly (3 accredited hospitals and 3 non-accredited hospitals). In the selected hospitals, the medical, paramedical, and non-medical workers were invited to participate in the study. The questionnaire was distributed to a sample of 300; out of those, 200 submitted a complete response.

Data were collected using a self-administered questionnaire (paper form) developed by the main investigator. The questionnaire was based on best practices for the Egyptian Government Excellence Award for Institutions, approved by the Ministry and published on the official award platform and the Egyptian Government Excellence Award for Institutions Guide. [10,11]

The questionnaire was presented in the form of a number of statements under ten EGEA criteria: Egypt Vision 2030 (three questions, Main Tasks (eight questions), Seven-Star Services (eight questions), Smart Government (two questions), Foreseing the Future (three questions), Innovation Management (five questions), Human Capital (five questions), Assets and Resources (five questions), Governance (four questions), Risk Management and Business Continuity (three questions).

The questionnaire was presented to a committee of three experts working as assessors of the Egyptian Government Excellence Award in coordination with the Productivity and Quality Institute of the Arab Academy for Science &Technology and Maritime Transport to assess its clarity and relevance (content validity). A pilot study was then conducted on a sample of service providers selected randomly from one accredited and one non-accredited hospital. Modifications of a few items to be clearer were made (e.g., adding a category regarding the type of hospital accreditation for registration).

Phase 2: Qualitative study

Based on findings of Phase I, a qualitative approach using focus groups with a sample of assessors in the "Egypt Government Excellence Award" and quality experts (n=13), aiming at identifying the perceived influence of GAHAR accreditation on hospital performance towards the excellence award, and developing a set of recommendations for accredited hospitals to achieve excellence. Two focus group discussions

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were conducted, including a number of participants (n=13). The discussion guide was prepared based on the results of phase 1 and a thorough literature review. It consists of six main points, namely (General understanding, Perceptions of EGEA Supporting Sustainable Hospital Services, Opportunities for Integration between EGEA and GAHAR, Challenges in dual Implementation, Assessment of barriers & enablers of integration between EGEA & GAHAR accreditation, Suggestions for Alignment. The discussion was recorded, and analysis was conducted later.

Informed consent was obtained from each participant before filling the questionnaire, and ethical approval for conducting the research and confidentiality of data were ensured.

Statistical Analysis:

Data was analyzed using Statistical Program for Social Science (SPSS) version 24. Qualitative data were expressed as frequency and percentage. Quantitative data were expressed as mean ±SD, as it was normally distributed.

Scoring of the questionnaire, each statement was answered on a scale of three points, ranging from fully fulfilled, partially fulfilled, or not fulfilled. Each statement was scored as 2 for fully fulfilled,1 for partially fulfilled, and 0 for not fulfilled, and a total score for each domain was calculated.

The following tests were done:

- An independent sample test to compare quantitative data between two groups.
- Chi-square test was used when comparing data between groups regarding qualitative data.
- P-value ≤ 0.05 was considered significant.

Qualitative phase: The collected data were analyzed using thematic content analysis.

3. Results

a. Quantitative phase.

Characteristics of the study participants

Table 1 presents the distribution of the studied participants according to the accreditation status of their hospitals. The sample included participants from both accredited hospitals (n = 111) and non-accredited or registered for accreditation (n = 95),

Participants' current job positions were presented in Table 2.

Table 1: Description of the studied participants according to the hospital's accreditation status.

no		Studied participants (N=206)	
		%	
Accreditation status	Accredited	111	53.9
	Non-accredited	48	23.3
	Registered	47	22.8

Table 2: Description of the studied participants according to their current job

no		Studied participants (N=206)	
		%	
Current job title	Medical staff	75	36.4
	No- medical	46	22,3
	Paramedical	85	41.3

Comparison of Excellence Award criteria by hospital accreditation status

Table 3 demonstrates a clear and consistent pattern of higher mean scores across all Egypt Government Excellence Award (EGEA) criteria among accredited hospitals compared to non-accredited or partially accredited hospitals. The differences between the two groups were statistically significant for all ten main criteria ($p < 0.001$).

Accredited hospitals achieved significantly higher scores in Egypt Vision 2030 alignment, Main Tasks, and Seven-Star Services, indicating stronger strategic orientation, service delivery, and alignment with national development priorities. Similarly, significant differences were observed in Smart Government and Foreseeing the Future, suggesting that accredited hospitals are more advanced in digital transformation, strategic foresight, and preparedness.

Regarding organizational capacity, accredited hospitals scored substantially higher in Innovation Management, Human Capital, and Assets and Resources. Furthermore, governance-related dimensions, including Governance and Risk Management and Business Continuity, also showed significantly higher mean scores among accredited hospitals.

Table (3): Comparison between accredited and non-accredited hospitals regarding Excellence Award criteria

EGEA criteria GAHAR Accredited (N = 111)		Hospital accreditation GAHAR Non-accredited - partially accredited (N = 95)		t-test (P-value)
Main criterion: Egypt Vision 2030	Mean ±SD	7.88 ± 1.34	5.2 ± 1.8	t = 11.7 < 0.001
	Range	3 - 9	3 – 9	
Main criterion 2: Main Tasks	Mean ±SD	22.4 ± 3.3	14.3 ± 4.6	t = 14.4 < 0.001
	Range	8 - 24	8 – 24	
Main criterion 3: Seven-Star Services	Mean ±SD	22.3 ± 3.4	14.9 ± 3.8	t = 14.5 < 0.001
	Range	8 - 24	8 – 24	
Main criterion 4: Smart Government	Mean ±SD	5.57 ± 0.96	4.2 ± 1.08	t = 8.9 < 0.001
	Range	2 - 6	2 – 6	
Main criterion 5: Foreseeing the Future	Mean ±SD	6.8 ± 1.71	5.3 ± 1.8	t = 6.3 < 0.001
	Range	3 - 9	3 – 9	
Main criterion 6: Innovation Management	Mean ±SD	10.6 ± 3.6	8.1 ± 3.1	t = 5.1 < 0.001
	Range	5 - 15	4 – 15	
Main criterion 7: Human Capital	Mean ±SD	13.9 ± 2.2	9.7 ± 2.3	t = 13.1 < 0.001
	Range	5 - 15	5 – 15	
Main criterion 8: Assets and Resources	Mean ±SD	14.06 ± 2.1	9.2 ± 3.06	t = 13.3 < 0.001
	Range	5 - 15	5 – 15	
Main criterion 9: Governance	Mean ±SD	11.2 ± 1.68	8.7 ± 1.9	t = 9.5 < 0.001
	Range	4 - 12	4 – 12	
Main criterion 10: Risk Management and Business Continuity	Mean ±SD	8.4 ± 1.4	5.5 ± 1.9	t = 12.3 < 0.001
	Range	3 - 9	3 – 9	

b. Qualitative findings from focus group discussions

The focus group discussions further contextualized the quantitative results. Most participants perceived EGEA as a supportive framework for achieving sustainable hospital services, particularly when integrated with GAHAR accreditation standards. However, lack of awareness and limited resources were frequently cited as barriers to effective implementation.

Participants identified quality and patient safety as the strongest areas of potential integration between EGEA and GAHAR, followed by innovation and alignment with Egypt Vision 2030. While consultants emphasized strategic alignment, frontline staff highlighted gaps in community engagement. Concerns were also raised regarding the increased complexity associated with dual implementation, compounded by resource constraints and resistance to change.

Despite these challenges, participants acknowledged important enablers, including national policy support and motivated healthcare staff. The most frequently proposed recommendation was the provision of specialized training, alongside the development of a clear implementation roadmap to align both frameworks, supported by incentives and leadership commitment.

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Table 4: Results of focus group discussions (Thematic analysis)

Discussion points	Responses
Perceptions of EGEA Supporting Sustainable Hospital Services	Most participants believed EGEA supports sustainability, especially when combined with GAHAR standards. However, lack of awareness and inadequate resources were identified as barriers.
Opportunities for Integration Between EGEA and GAHAR	The strongest integration opportunity lies in quality and patient safety, as both frameworks prioritize this. Innovation and alignment with Egypt Vision 2030 were also recurring opportunities. Consultants emphasized strategy, while frontline staff highlighted community engagement gaps.
Challenges in Dual Implementation	Participants expressed concern that dual implementation increases complexity. Resource limitations and resistance to change were also strong themes.
Assessment of Barriers and Enablers	Participants emphasized systemic Barriers (strategy gaps, weak participation, lack of funding). Yet, they acknowledged enablers such as national vision support and motivated staff. This indicates potential if resources and training are provided.
Suggestions for Alignment	The most repeated suggestion was specialized training. A roadmap aligning both frameworks was also strongly requested.

3. Discussion

As illustrated in our results, most fields and criteria of the GAHAR accreditation standards are aligned with sub-criteria, guidelines, and supplement points of the EGEA model. These findings are similar to research conducted in Iran, proving that JCI (Joint Commission International) accreditation standards requirements overlap with the healthcare excellence model. (12)

Heaton has compared the Organizational Excellence Model of the European Foundation for Quality Management (EFQM), International Standards of quality management, JCI accreditation standards, and Visitatie Model and stated that JCI standards and the excellence model can be used for self-assessment or external evaluation to investigate and improve healthcare organizations. The results of this research are consistent with our research findings that refer to GAHAR and the Egyptian Excellence Model as independent yet overlapping models and their applicability for continual healthcare improvement. (13)

In partial alignment with our results that the excellence model is comprehensive and suitable for internal evaluation, Shaw et al evaluated organizational excellence and accreditation models. They concluded that standards of evaluation are only valuable as an audit and certification issuance and do not address organizational development. (14)

Patients continue to expect excellence in their healthcare facilities. Although excellence is synonymous with quality, the differences between facilities' approaches to quality and others' approaches to excellence are far-reaching. (12) According to the American Society for Quality, "Excellence is a measure of consistently superior performance that surpasses requirements and expectations without demonstrating significant flaws or waste"[15]. However, organizational excellence is defined as the ongoing effort to establish an internal framework of standards and processes intended to engage and motivate employees to deliver products and services that fulfill customer requirements within business expectations. To ensure excellence, healthcare institutions should continually assess their quality and take necessary actions to enhance quality in their road to excellence [16].

According to Furnival et al, quality improvements can be initiated by collecting data and applying appropriate analytical techniques. Evidence of a performance gap encourages decision makers to improve the current situation. An assessment provides a detailed review of strengths and weaknesses, activates quality initiatives, empowers employees, and compares current performance with that of competitors [17]. Developing a quality assessment tool using the European Foundation for Quality Management (EFQM) model opens the door to indispensable performance discussions. A self-assessment identifies areas for improvement and highlights defective systems and processes. A facility's compliance with accreditation standards, such as United States Joint Commission standards, may also be assessed and measured to determine how well it adheres to each system or chapter, whether organizational or patient-centered. For example, the performance metric is included in the Joint Commission standard as part of the chapter's standards, which includes a section about the Baldrige excellence model. In addition, the EFQM excellence [18]. Both the Egyptian Excellence Award and GAHAR Accreditation are prestigious recognitions in the field of healthcare quality in hospitals. While various models of managerial tools and techniques

are used in organizations, and it seems that some of these models, including JCI standards, are more appropriate than others for health care organizations, the EFQM model provides a comprehensive look at the organization until it is determined how these tools and methods align together and complement each other. Therefore, the organizational excellence model can be used alongside these tools as a comprehensive framework in the development of sustainable excellence [12].

Finally, the results of a questionnaire conducted in Iran of 150 managers, officials, and specialists from different departments of the Ministry of Health and Medical Education and Iran's hospitals. In this questionnaire, a separate section was predicted to assess alignment and integration of the health care excellence model with other hospital models and standards, and one of the questions in this section was related to the JCI accreditation standards. In 95 completed questionnaires, respondents rated 74% in relation to the coverage of the requirements of the JCI accreditation standards via the health care excellence model [12].

I can see that reference 8 is used very frequently in the discussion. Worries of plagiarism may exist.

4. Conclusion

There is convergence between the accreditation model and the Egyptian Excellence Model, suggesting that accreditation can help the healthcare sector to implement the best management practices that are present in the Egyptian Excellence Model. In other words, GAHAR accreditation could be seen as a critical step towards excellence in Egyptian healthcare organizations.

Disclosure:
Nothing to disclose

Conflict of interest:
None

References

1. Busse R, Panteli D, Quentin W. An introduction to healthcare quality: defining and explaining its role in health systems. In: Busse R, Klazinga N, Panteli D, Quentin W, editors. Improving healthcare quality in Europe: Characteristics, effectiveness and implementation of different strategies. Copenhagen, Denmark: WHO Regional Office for Europe & European Observatory on Health Systems and Policies; 2019. p. 3–21.

2. Patel PM, Deshpande VA. Application Of Plan-Do-Check-Act Cycle For Quality And Productivity Improvement-A Review. Int J Res Appl Sci Eng Technol. 2017;5(1).

3. Jafari G, Khalifegari S, Danaie K, Dolatshahi P, Ramazani P, Ramazani M. Accreditation standards for hospitals in Iran. Tehran: Ministry of Health and Medical Education, Deputy of Treatment. 2012;

4. NABITZ U, KLAZINGA N, WALBURG J. The EFQM excellence model: European and Dutch experiences with the EFQM approach in health care. International Journal for Quality in Health Care. 2000 Jun 1;12(3):191–202.

5. Lewis WG, Fai Pun K, Lalla TRM. Empirical investigation of the hard and soft criteria of TQM in ISO 9001 certified small and medium-sized enterprises. International Journal of Quality & Reliability Management. 2006 Oct 1;23(8):964–85.

6. Alraimi AA, Al-Nashmi MM. The interactive effect of the application of accreditation standards (JCIs) and the practice of administrative control in improving the quality of health services: a study on Yemeni hospitals. BMC Health Serv Res. 2024 Nov 14;24(1):1403.

7. Berssaneti FT, Saut AM, Barakat MF, Calarge FA. Is there any link between accreditation programs and the models of organizational excellence? Revista da Escola de Enfermagem da USP. 2016 Aug;50(4):650–7.

8. Alsaleem NAI. The relationship between accreditation standards and organizational excellence and their role in the excellence of administrative leadership. Journal of Economic, Administrative, and Legal Sciences. 2025 Apr 30;9(4):116–26.

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http://dx.doi.org/10.21622/AMPDR.2026.06.1.1894

9. Toussaint John. Creating a culture of excellence How healthcare leaders can build and sustain continuous improvement. KPMG International's Healthcare Practice. 2019;

10. Egyptian Government Excellence Award. Institution award: summary of standards and best practices for each criterion [Internet]. Cairo: EGEA; 2024. Available from: https://egea.gov.eg/

11. Faculty of Medicine, Mansoura University. General organization and administrative regulations (GOAAL) [Internet]. Mansoura: Mansoura University; 2020. Available from: https://medfac.mans.edu.eg/images/files/Files/goaal.pdf

12. Yousefian S, Harat AT, Fathi M, Ravand M. A Proposed adaptation of Joint commission international accreditation standards for hospital- JCI to the health care excellence model. In: Proceedings of the International Conference on Health Care Quality Improvement. 2013.

13. HEATON C. External peer review in Europe: an overview from the ExPeRT Project. International Journal for Quality in Health Care. 2000 Jun 1;12(3):177–82.

14. SHAW CD. External quality mechanisms for health care: summary of the ExPeRT project on visitatie, accreditation, EFQM and ISO assessment in European Union countries. International Journal for Quality in Health Care. 2000 Jun 1;12(3):169–75.

15. Berwick DM. What 'Patient-Centered' Should Mean: Confessions Of An Extremist. Health Aff. 2009 Jan;28(Supplement 1):w555–65.

16. Bassett S, Westmore K. Systems and processes that ensure high quality care. Nurs Manage. 2012;19(6).

17. Spohn R. The Self-Assessment Process and Impacts on the Health Information Management Program Performance: A Case Study. Perspect Health Inf Manag. 2015;12.

18. Marques AI, Santos L, Soares P, Santos R, Oliveira-Tavares A, Mota J, et al. A proposed adaptation of the European Foundation for Quality Management Excellence Model to physical activity programmes for the elderly - development of a quality self-assessment tool using a modified Delphi process. International Journal of Behavioral Nutrition and Physical Activity. 2011;8.

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Hepatoprotective Properties of Psidium Guajava Fruit and Musa Parasidiaca Roots as a Di-Herbal Remedy in Wister Rats

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ABSTRACT:

Oxidative stress is a major factor in the development of chronic liver diseases, prompting interest in plant-based antioxidants. This study investigates the hepatoprotective and antioxidant effects of Psidium guajava fruit and Musa paradisiaca roots, individually and as a di-herbal remedy, against carbon tetrachloride (CCl_4)-induced liver toxicity in Wistar rats. Forty-two rats (n=6 per group) were used. Liver damage was induced with CCl_4 (1 ml/kg) for one month, followed by treatment with methanolic extracts of guava fruit and plantain roots (500 mg/kg individually; 500 mg/kg and 1000 mg/kg combined) for an additional month. Serum levels of ALT, AST, ALP, bilirubin, albumin, and total protein were measured alongside oxidative stress parameters (SOD, CAT, GSH, GPx, NO, MDA). In vitro antioxidant assays (DPPH, ABTS, FRAP, nitric oxide scavenging) were also performed. CCl_4 induction elevated liver enzymes and MDA levels while reducing antioxidant markers. Treatment with the extracts improved protein profiles, restored antioxidant enzyme activity, decreased lipid peroxidation, and demonstrated strong in vitro radical-scavenging activity. Findings indicate that the di-herbal combination offers potential synergistic hepatoprotective and antioxidant effects, supporting further mechanistic and histopathological investigation.

KEYWORDS:

Hepatoprotective, Psidium guajava, Musa parasidiaca, Di-herbal Remedy, Wister Rats.

1. Introduction

Hepatotoxicity refers to impairment or damage to the liver, resulting from an excessive accumulation of xenobiotics or drugs (1). The substances or chemicals responsible for liver damage are referred to as hepatotoxins or hepatoxicants (2). These hepatotoxicants are external compounds with significant clinical importance, encompassing instances such as dietary supplements, drug overdoses, herbal remedies, natural substances, and industrial chemicals (3). Even within recommended therapeutic doses, certain drugs can lead to liver injury. Hepatotoxicity can also arise from immunologically mediated responses that affect epithelial cells, liver vasculature, or reactive metabolites (4).

The liver is an organ susceptible to reactive oxygen species (ROS) attack; ultimately, oxidative stress is a major player in the initiation of liver damage. Furthermore, several investigations (on mice and humans) have shown that oxidative stress reduces the ability of mature hepatocytes to regenerate, leading to cirrhosis (5).

Recent studies (6-8) (Oyelola et al., 2021; Rajesh et al., 2022; Chen et al., 2023) have further emphasized that natural antioxidants from fruits and herbs offer hepatoprotective effects by modulating oxidative pathways and improving biochemical markers of liver function. Plants with antioxidant effects have shown a promising therapeutic effect on liver disorders in animal experiments (9).

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Guava, also known as Psidium guajava, is a popular tropical fruit grown in several different tropical and subtropical climates (10). Although it is a fruit commonly found in Nigeria, it is widely consumed as food and used in traditional medicine in other parts of the globe. Among other health benefits of guava, several studies (10-13) have indicated guava to be rich in antioxidant properties and exceptionally high vitamin C content.

On the other hand, Plantain (*Musa Parasidiaca*) is a perennial herbaceous plant found on every continent, including Africa. In medicine, the roots of plantain are employed as an antibiotic, an antidote, and an antiseptic agent. In addition, the roots are decocted and used to cure various ailments (11). Individually, both plants have been reported to possess hepatoprotective effects. However, despite their popularity in traditional practices, the combined use of P. *guajava* fruit and M. paradisiaca root extracts as a di-herbal therapy lacks scientific evidence. The rationale for combining *Psidium guajava* and *Musa paradisiaca* lies in their complementary phytochemical profiles—guava being rich in vitamin C and flavonoids, while plantain roots contain alkaloids and terpenoids known for anti-inflammatory actions. This di-herbal approach may provide a synergistic mechanism, enhancing both antioxidant and hepatoprotective potential beyond their individual effects.

This study, therefore, investigates the hepatoprotective and antioxidant properties of guava fruit and plantain root extracts, individually and in combination, against CCl_4-induced toxicity in Wistar rats.

2. Methodology

Collection and Extraction of Plant Materials

Guava fruit and Plantain roots were collected from a local farm in Keffi, Nasarawa state, Nigeria. They were both cleaned, dried, and ground into powder individually. To perform methanolic extraction, 400g of guava fruit powder was soaked in 1.5 L of absolute methanol for three days, then filtered. The procedure was iterated with the remaining powder residues. Subsequently, the methanolic extracts underwent concentration through rotary evaporation for a period of 24 hours. 600g of powdered roots from Plantain roots were blended with 2 liters of methanol. The resulting mixture underwent filtration through Whaltman filter paper no. 1. The obtained extract was concentrated through a rotary evaporation over a period of 24 hours. The residue was obtained and refrigerated until required.

Experimental Animals:

Forty-two (42) adult Wistar rats (160–200 g) were kept under standard laboratory conditions (25 ± 2°C; 12-hour light–dark cycle). They were acclimatized for two weeks with free access to food and water. Ethical approval was obtained from NSUK-ACUREC. Animals were randomly assigned to groups using a simple randomization procedure to minimize selection bias.

Experimental Design:

The CCl_4 dose was standardized to 1 ml/kg diluted in olive oil (1:1).
The 42 rats were assigned to seven groups (n=6):
- Group 1: Control (no CCl_4)
- Group 2: Negative control (CCl_4 only)
- Group 3: CCl_4 + 500 mg/kg guava extract
- Group 4: CCl_4 + 500 mg/kg plantain extract
- Group 5: CCl_4 + 250 mg/kg di-herbal combo
- Group 6: CCl_4 + 500 mg/kg di-herbal combo
- Group 7: CCl_4 + 100 mg/kg silymarin.

The silymarin dose of 100 mg/kg was selected based on published studies demonstrating consistent hepatoprotective and antioxidant efficacy at this concentration in carbon tetrachloride–induced liver injury models in rodents (63–65).
Carbon tetrachloride in olive oil was administered intraperitoneally. Meanwhile, all the animals were fed on vital feed and water (H_2O) ad libitum. Each group consisted of six rats; however, biochemical determinations were conducted in five replicates per group due to sample volume constraints and exclusion of technically compromised samples.

Extract Standardization:

The methanolic extracts of guava fruit and plantain roots were standardized based on their total phenolic and flavonoid contents, quantified using gallic acid and quercetin equivalents, respectively. These parameters served as marker indicators of extract potency, ensuring batch-to-batch reproducibility before biological evaluation.

Biochemical Assays:

Liver biomarkers (ALT, AST, ALP, albumin, total protein, and bilirubin) were analyzed using standard methods using standard kits from Randox Laboratory, UK, according to the instructions of the manufacturer. Oxidative stress parameters (SOD, CAT, GSH, GST, MDA, NO) were measured according to validated protocols.

In Vitro Antioxidant Assays:

The DPPH, ABTS, FRAP, and nitric oxide scavenging assays followed established protocols with slight modifications. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging assay was conducted following Brand-Williams *et al.* (1995) (13) with minor adjustments. ABTS antioxidant activity was determined as described by Re et al. (1999)(14). Ferric reducing antioxidant power (FRAP) was estimated spectrophotometrically following the procedure of Benzie and Strain 1996 (15). Nitric oxide generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci *et al.* (1994) (16). Absorbance was read at the required wavelengths after incubation.

Statistical Analysis:

The analysis was performed using SPSS version 16 (Statistical Package for the Social Sciences). The groups' differences were tested for significance by one-way ANOVA followed by the Duncan post hoc test. Data were expressed as the Mean ± SD. P-values <0.05 were considered statistically significant.

3. Results

Total Phenol and Flavonoid present in Plantain roots and Guava fruits methanol extract.

The results of the quantification of Total Phenol and Flavonoid present in the methanol extract of Plantain roots and Guava fruits are presented in Table 1. The total phenol of guava fruits and plantain roots are 288.05 ± 0.01 and 54.1± 0.01, respectively, while their total flavonoid content is 43.30± 0.56 and 31.74± 0.80, respectively.

Table 1: Total Phenol and Total Flavonoid of Plantain roots and Guava fruits methanol extract

Extract	Total Phenol (mg/g GAE)	Total Flavonoids (mg/100g QUE)
Guava fruit	288.05 ± 0.01	43.30 ± 0.56
Plantain roots	54.10 ± 0.01	31.74± 0.80

Values are expressed as Mean ± Standard error of Mean (SEM) for three determinations.

Effect of Guava fruit and Plantain roots on the serum protein concentrations in the experimental groups.

The results of the effects of various concentrations of Guava fruit and Plantain roots individually and as a di-herb combo on the serum protein concentrations in the experimental groups are presented in Table 2. The results showed that CCl_4 induction significantly altered total protein and albumin levels compared to the control group. Treatment with guava fruit and plantain root extracts restored protein values closer to normal.

Table 2: Effect of Guava fruit and Plantain roots on the serum protein concentrations in the experimental groups

Group	Test (Unit)	Total Protein(g/dl)	Albumin (g/dL)	Direct Bilirubin (µmol/L)	Total Bilirubin (µmol/L)
1	Positive control	5.63 ± 0.31^{a}	3.35 ± 0.09^{a}	17.30 ± 0.75^{a}	21.40 ± 1.55^{a}
2	Negative control	7.49 ± 0.30^{b}	2.53 ± 0.16^{b}	19.80 ± 1.03^{b}	25.10 ± 1.61^{b}
3	500mg/kg Guava fruit	5.93 ± 0.19a	2.96 ± 0.09^{c}	20.30 ± 1.76^{c}	21.40 ± 2.04^{a}
4	500mg/kg Plantain roots	5.75 ± 0.23^{a}	2.88 ± 0.08^{c}	16.10 ± 0.77^{d}	18.60 ± 1.02^{c}
5	250mg/kg di-herbal	6.29 ± 0.18^{c}	3.18 ± 0.13^{d}	18.80 ± 1.00^{e}	19.80 ± 1.07^{c}
6	500mg/kg di-herbal	6.15 ± 0.35^{c}	2.89 ± 0.09^{c}	15.80 ± 0.46^{d}	17.90 ± 1.51^{c}
7	100mg/kg silymarin	5.61 ± 0.15^{a}	3.15 ± 0.06^{d}	16.70 ± 1.01^{a}	21.20 ± 1.35^{a}

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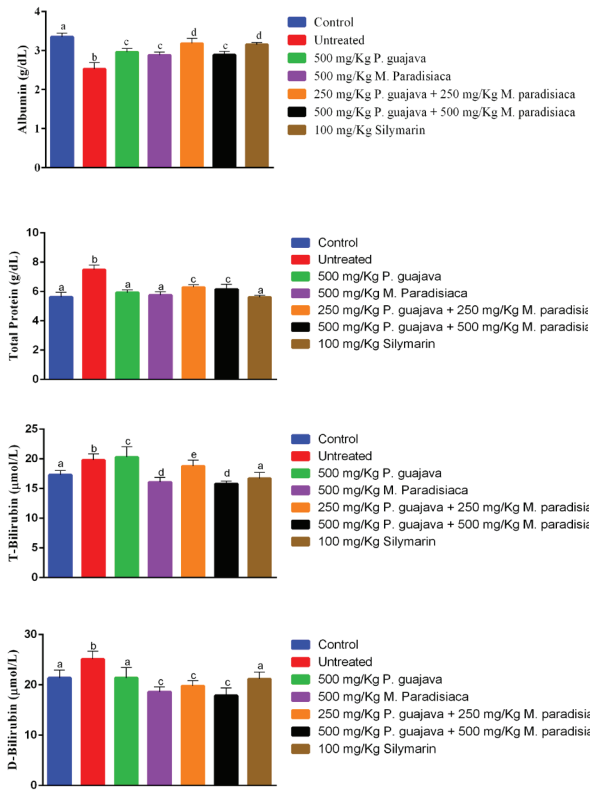
Figure 1: Effect of Guava fruit and Plantain roots on the serum protein concentrations in the experimental groups.



Effect on Liver Enzymes (ALT, AST, ALP):
CCl_4 significantly increased ALT, AST, and ALP levels, indicating severe hepatocellular damage. Treatment groups exhibited reductions in ALT and AST, but ALP levels remained elevated in all extract-treated groups. The results of the effect of Guava fruit and Plantain roots on the serum liver enzymes concentrations in the experimental groups are presented in Table 3.

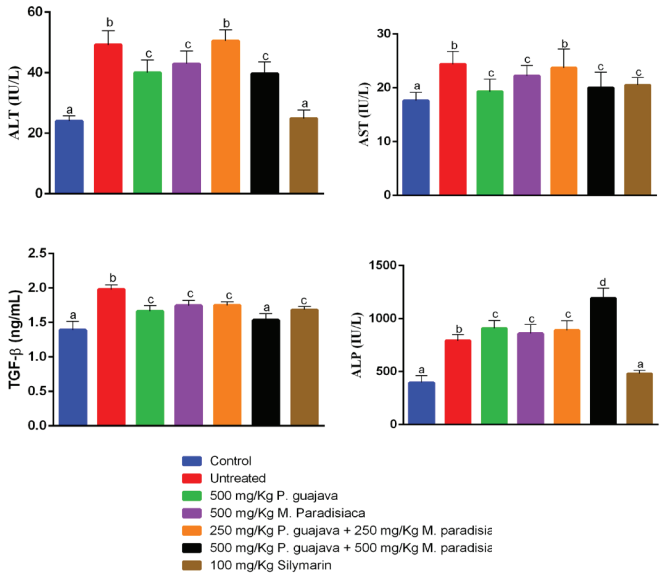
Effect on TGF-β:
TGF-β levels increased following CCl_4 administration, reflecting inflammatory and fibrotic signaling. Treatment with the 500 mg/kg di-herbal combination significantly reduced TGF-β levels. The results of the effect of Guava fruit and Plantain roots on TGF-β concentrations in the experimental groups are presented in Table 3.

Table 3: Effect of Guava fruit and Plantain roots on the serum concentration of liver enzymes and TGF-β in the experimental groups

Group	Test (Unit)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	TGF-β (ng/mL)
1	Positive control	395.6 ± 66.4^a	24.02 ± 1.80^a	17.60 ± 1.56 ^{a}	1.39 ± 0.12^a
2	Negative control	792.40 ± 57.55^b	49.17 ± 4.69^b	24.40 ± 2.33^b	1.98 ± 0.07^b
3	500mg/kg Guava fruit	907.60 ± 75.63^c	40.05 ± 4.19^c	19.30 ± 2.32^c	1.66 ± 0.08^c
4	500mg/kg Plantain roots	859.30 ± 86.80^c	42.90 ± 4.31^c	22.20 ± 1.95^c	1.74 ± 0.07^c
5	250mg/kg di-herbal	889.60 ± 90.48^c	50.50 ± 3.73^b	23.70 ± 3.49^b	1.75 ± 0.05^c
6	500mg/kg di-herbal	1192.80 ± 95.29^d	39.70 ± 3.91^c	20.00 ± 2.89^c	1.54 ± 0.09^a
7	100mg/kg silymarin	480.40 ± 32.58^a	24.86 ± 2.86^a	20.50 ± 1.40^c	1.68 ± 0.05^c

Values that have the same superscript are not significantly different @ p < 0.05 down the table.

Figure 2: Effect of Guava fruit and Plantain roots on the liver enzymes and TGF-β in experimental groups



Effect of Guava fruit and Plantain roots on antioxidant parameters

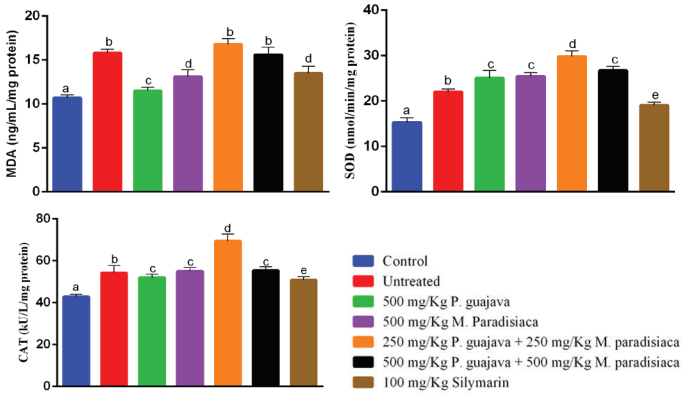
The results of the effect of Guava fruit and Plantain roots on the antioxidant parameters, such as MDA, SOD, and CAT concentrations in the experimental groups, are presented in Table 4. CCl_4 increased MDA levels and reduced SOD and CAT activity. Treatment with guava and plantain extracts significantly reversed these changes, with the di-herbal groups producing the greatest improvement.

Table 4: Concentration of antioxidant parameters

Group	Test (Unit)	MDA (nmol/L)	SOD (nmol/min/mg protein)	CAT (kU/L/mg protein)
1	Positive control	10.70 ± 0.33^{a}	22.00 ± 0.65^{c}	54.33 ± 3.37 ^{c}
2	Negative control	15.80 ± 0.45^{d}	15.27 ± 1.09^{a}	42.89 ± 1.11^{a}
3	500mg/kg Guava	11.50 ± 0.41^{b}	25.10 ± 1.63^{d}	51.97 ± 1.58^{b}
4	500mg/kg Plantain roots	13.10 ± 0.80^{c}	25.41 ± 0.86^{d}	55.01 ± 1.85^{c}
5	250mg/kg di-herb	16.80 ± 0.64^{d}	29.80 ± 1.27^{e}	69.40 ± 3.44^{d}
6	500mg/kg di-herb	15.60 ± 0.86^{d}	26.72 ± 0.98^{c}	55.43 ± 1.78^{c}
7	100mg/kg silymarin	13.50 ± 0.81^{c}	19.09 ± 0.68^{b}	50.79 ± 1.65^{b}

Values with different superscripts down the column are statistically significant at $p < 0.05$

Figure 3: Effect of Guava fruit and Plantain roots on antioxidant parameters



Effect on GST, GSH, and Nitrite:

The results of the effect of Guava fruit and Plantain roots on the antioxidant parameters such as GST, GSH, and Nitrite concentrations in the experimental groups are presented in Table 5. CCl_4 reduced GST and GSH levels while increasing Nitrite, indicating oxidative stress. The di-herbal extract at 250 mg/kg produced the greatest improvement in antioxidant markers.

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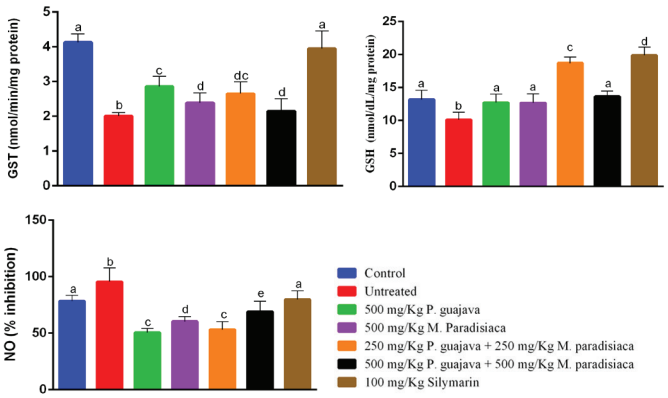
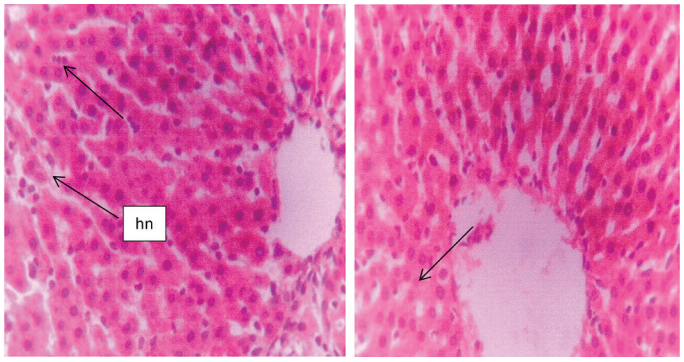
Figure 4: Effect of Guava fruit and Plantain roots methanol extract on GSH, GST, and Nitrite.

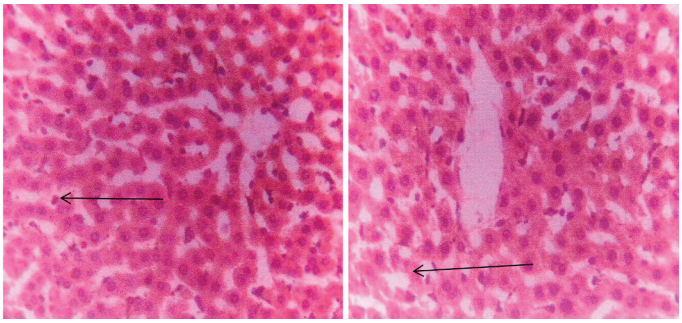


Histopathological Findings

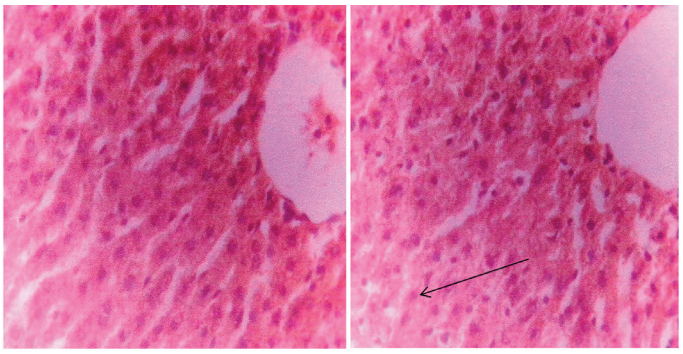
Qualitative histopathological examination revealed normal hepatocytes in control rats. CCl_4-treated rats showed hepatic necrosis (HN), inflammatory cell hyperplasia (LH), and sinusoidal congestion (SC). Treatment groups demonstrated partial restoration of hepatic architecture with mostly normal hepatocytes and only mild residual lesions. These findings support biochemical evidence of hepatocellular recovery and explain persistent ALP elevation due to delayed biliary or vascular normalization.



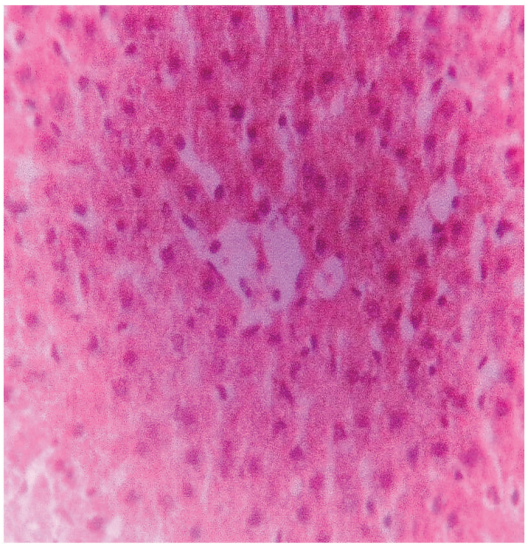
G1 shows sc with slight lh, f2 shows slight hn



D3 shows slight LH, e1 shows slight HN



B3 shows normal hepatocytes, a2 show slight hn



C2 shows normal hepatocytes.
Codes
Hn is hepatic necrosis
LH is a hyperplasia of inflammatory cells
Sc is sinusoidal congestion

4. Discussion

Over the years, phytochemicals have been explored in managing several diseases in folklore medicine (16). The ameliorative properties of several medicinal plants have been related to bioactive principles like phenolic, flavonoids, saponins, and alkaloids (17). In the context of this research, both guava fruit and plantain roots were found to contain flavonoids and phenols. Phenols are known for their noteworthy antioxidant properties, while flavonoids, owing to their specific chemical structure, are identified as the most potent among the phenolic compounds with antioxidant activity (18). The findings of the constituent phytochemicals of plantain roots of this study agree with the studies of (19) and (11). For guava fruit, the findings of this study agree with the study of Atik et al (2019)(20). A correlation between phenolic content and antioxidant activities on fruits and vegetables

has been previously reported (21); (22). Phenolic compounds recovery was largely dependent on the nature and polarity of the solvent. This is because a wide range of phenols are soluble in aqueous methanol mixtures. The TPC and TFC are similar and in line with several studies, such as Thuaytong and Anprung 2011, Lin & Yin (2012), Saeed et al. 2019 and Odubanjo et al. 2022(21,23–25).

The DPPH radical scavenging activity of antioxidants is largely dependent on their hydrogen-donating ability (26). In this study, Guava fruit and Plantain roots exhibited radical scavenging activities against DPPH radical. This was evident in the extracts' proton-donating abilities, making them potential subjects as primary antioxidants against free radicals and reactive oxygen species. Guava fruit showed the most free radical scavenging property in a dose-dependent manner when compared with Ascorbic acid, which is the standard. This agrees with the study of (27) and Lim et al 2006 (28). The results of plantain roots in this study are in line with the work of (Abdel Ghany et al. 2018, 29) for the pseudostem exudate of *M. paradisiaca,* DPPH scavenging % increased with increasing exudate concentrations.

Antioxidant activity has been proposed to be related to reducing power (26). Therefore, Guava fruit and Plantain roots possess antioxidant properties by reducing iron (Fe^{2+}) II to iron III (Fe^{3+}), but not better than the reference compound. This trend was also observed in the Nitric oxide (NO) and ABTS (2,2'-azinobis (3-ethylbenzothiaoline-6-sulfonate) assay and showed free radical scavenging property in a dose-dependent manner, and this is in line with several studies (30–35). The ability of guava fruit and plantain roots to chelate iron and scavenge DPPH, nitric oxide radicals is attributed mainly to the presence of phenolic compounds (35,36). These strong *in vitro* antioxidant properties of the extract of guava fruit and plantain roots may be a result of the functional groups of the compounds present in the extract. Factors modulating these antioxidant activities include the number of hydroxyl groups, methoxy esters, carbohydrate moieties, and phenolic units (37).

After careful evaluation of the individual compounds, the redox property and antioxidant effects of the compounds were found to be related to their functional group characteristics, the amount and positions of hydroxyl groups in their structures (35). Furthermore, the activity of phenolic compounds was suggested to be a synergistic effect on antioxidant capacity when they are together (36,38–40)

The concentration of albumin, bilirubin, and total protein can be used to ascertain different types of liver damage (41). Bilirubin is a product of haemoglobin catabolism with important biological and diagnostic values (42). The significant increase in both the serum total and direct bilirubin in the negative control induced with CCl_4 compared to the positive control suggests that there was a defect in liver function on induction, and this might be a result of haemolysis and impairment of the secretion of conjugated bilirubin into the bile duct in the liver of the rats induced with $CC1_4$. The results also revealed a significant decrease ($p<0.05$) across treatment groups 3, 4, 5, and 6, respectively, compared to the negative control. This suggests that the extracts at different concentrations were able to improve the liver damage, which is in line with the studies of Nirmila *et al*, 2012, and Osman et al., 2011(44,45). For both direct and total bilirubin, Group 6 (500mg/kg guava fruit + 500mg/kg plantain roots) was significantly lower ($p<0.05$) than group 7(100mg/kg silymarin) and positive control, this may suggests that treatment was able to reverse haemolysis and impairment of bilirubin and thus combing the extract might have a positive synergistic effect as evident in the results.

A significant decrease ($p<0.05$) in Albumin concentration in the negative group compared to the positive control suggests that there may be a reduction in the hepatocyte mass due to disease, and this suggests that the functionality of the immune cells has been compromised, and the severity and risk of infections are increased. Therefore, a significant increase ($p<0.05$) in albumin concentration across all the treatment groups suggests that immune function is boosted, hepatocyte volume increased, and the severity of the risk of infection has been mitigated. This agrees with the study by (11), plantain roots increased albumin content of arsenic oxidative damage in rats, and Osman et al. 2011 (45) showed that the ethanolic extract of guava leaf decreased the level of albumin in CCl_4-induced hepatotoxicity.

The assessment of protein concentration in the liver could be used to ascertain the secretory and synthetic functions (46). In this study, total protein (TP) concentrations in the negative control group induced with $CC1_4$ significantly increased

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($p<0.05$) compared to the positive control. This suggests liver damage, thereby making it nearly impossible for the body to process and metabolize protein for utilization. Also, this might be because of increased gamma globulin, indicating the presence of inflammatory diseases and immune disorders, further confirming liver damage. There was a significant decrease across all treatment groups compared to the negative control. It can be deduced that since guava fruit and plantain roots at all treatment concentrations significantly decreased the elevated TP in CCl_4-induced hepatotoxicity, they were able to improve the disease state. This is in line with the study Abbas et al. 2016, and Mohan et al. 2015 (46,47) that revealed P. *guajava* and M.*paradisiaca* extract decreased total protein, respectively. However, the individual groups of the extract, i.e, Group 3 (500mg/kg P. *guajava*) and Group 4 (500mg/kg M.*paradisiaca*), had an optimum effect compared to the di-herbal group (5 and 6).

Alanine aminotransaminases (ALT) and aspartate aminotransferases (AST) are well-known enzymes used as biomarkers to predict possible toxicity to the liver (48). The results from this study showed that on induction with CCl_4 (Negative control), the serum levels of ALT and AST increased significantly ($p<0.05$) when compared with the positive control. For ALT, the most effective treatment was group 6 (500mg/kg guava fruit and 500 mg/kg plantain root di-herb), which significantly ($p<0.05$) decreased ALT compared to the negative control. For AST, the most effective treatment is group 3 (500 mg/kg guava fruit), followed by group 6 (500 mg/kg guava fruit and 500 mg/kg plantain roots di-herb). This suggests that the di-herb combo might have a synergistic effect, as evident in the results. Nirmila et al. 2012 and Oyewole et al. 2015 (11,43)showed that plantain roots reduced ALT and AST levels, whereas Roy and Das (2010 also showed a decrease in AST and ALT levels by guava(49).

The result showed that CCl_4 induction (Negative control) elevated the ALP level significantly ($p<0.05$) in the serum compared to the positive control, while treatment at all concentrations of guava fruit and plantain roots further increased the ALP concentrations. This suggests that guava fruit and plantain roots were not able to protect or restore the integrity of the cell components of the liver. This was also observed in a study by Sambo et al. 2019 where the aqueous extract of guava fruit further increased the level of ALP, suggesting hepatoxicity; however, ALP is not specific to the liver, it is also found in bones, bile ducts, etc(50). Therefore, elevations in total ALP may arise from extra-hepatic tissue activity rather than persistent hepatic injury. Without isoenzyme differentiation, it is not possible to attribute the increased ALP solely to the liver. Histological evidence of mild sinusoidal congestion and inflammatory infiltration supports delayed structural recovery rather than ongoing hepatocellular injury.

Furthermore, the kinetics of ALP clearance differ substantially from those of transaminases. Unlike ALT and AST, which respond rapidly to injury and recovery, ALP has a longer circulating half-life and often normalizes more slowly, especially when the pattern of injury has cholestatic components (51). Thus, the observed persistence of elevated ALP alongside improved ALT/AST likely reflects delayed enzyme resolution rather than ongoing liver damage. Interpretation of liver injury patterns also requires more than ALP alone. Classification frameworks emphasize that cholestatic or mixed patterns typically present with disproportionate ALP elevation, whereas hepatocellular injury is driven primarily by ALT/AST changes (52). In this study, the improvement of ALT/AST and other biochemical parameters suggests hepatocellular recovery despite the isolated ALP elevation.

We acknowledge that, as a limitation, ALP isoenzyme fractionation was not performed. Future studies should include isoenzyme profiling or repeated ALP measurements at later time points to clarify the source of ALP elevation and strengthen mechanistic interpretation.

While having a normal level of human transforming growth factor-beta 1 (TGF-β) indicates a healthy immune system, high levels of this growth factor can indicate several diseases and conditions, such as chronic Liver diseases, chronic inflammatory response syndrome, cancer, tumor cells, fibrosis, and many more. In this study, the induction of CCl_4 significantly increased ($p<0.05$) the levels of TGF-β compared to the positive control; however, it was significantly ($p<0.05$) reduced in group 6 (500 mg/kg di-herbal combo) compared to the negative control group. This suggests that the guava fruit and plantain roots di-herbal combo can boost the immune system, and it could also indicate the amelioration of the disease condition. This is in line with the study by El-Said et al. 2022 (53) on plantain leaves and Shady et al. 2022 (54) on guava seeds, decreasing the level of TGF-β.

Glutathione (GSH), a liver-concentrated tripeptide, serves as a crucial thiol-reducing agent in regulating redox processes. Beyond its role as a free radical scavenger, GSH is implicated in determining outcomes between survival, necrosis, and apoptosis. Moreover, it influences the functionality of signal transduction and transcription factor molecules. In this study, there was a significant ($p<0.05$) decrease in GST and GSH concentration on induction with CCl_4 (Negative control) compared to the positive control; however, guava fruit and plantain roots di-herbal combo or individually significantly increased GST and GSH compared to the negative control. The most effective treatment was Group 5 (250mg/kg di-herb), which significantly increased GST and GSH compared to the negative control. This suggests that the di-herbal combo of guava fruit and plantain roots confers free radical scavenging property. These results agree with Hung et al. 2006 (55) and Suneetha et al.2010 (56), who showed that CCl_4-treated rats significantly decreased the GSH content in the liver. Nitrite levels were elevated in the negative control, indicating increased oxidative stress and nitric oxide production. Treatments with guava fruit and plantain roots, either alone or in combination, reduced nitrite levels, suggesting a potential ability to attenuate nitric oxide production and oxidative stress. This agrees with the studies by Cavichioli et al. 2022 and Rao et al. 2016 (57).

CCl_4 multiorgan toxicity is linked to a depleted antioxidant defense because of excessive O_2 and H_2O_2 generation (58). It is interesting to note that research has linked liver damage to oxidative stress (59). The increase in SOD, CAT, and MDA activity seen in this study on induction with CCl_4 is consistent with earlier research (49,60) and consequent to its increase in liver tissue in response to the liberation of reactive oxygen species. This suggests that products of free radical reactions might be involved in the pathogenesis and/or progression of medical cholestasis, and that these molecules might attempt to minimise the liver injury. Both SOD and Catalase are antioxidant enzymes that help neutralize reactive oxygen species (ROS). Treatments with guava fruit and plantain roots increased the levels of SOD and Catalase significantly ($p<0.05$) compared to the negative control. This suggests treatments might enhance antioxidant enzyme activity and protect against oxidative stress. Suneetha et al. 2010 (56) and Osman et al. 2011 (45) showed a decrease after induction with CCl_4 in SOD and CAT, and an increase after treating with guava leaves. A study by (Vijayakumar et al. 2008, 61) showed that plantain significantly increased CAT and SOD, respectively. The most effective treatment that significantly decreased SOD and CAT is the di-herbal groups (5 and 6), suggesting the di-herbal combo has a positive synergistic effect, as seen in the result. However, there was a significant decrease in MDA following treatment with group 3 (500mg/kg *P. guajava*) compared with the negative control and even better than silymarin 100mg. This shows it is effective against lipid peroxidation in the hepatocyte membrane. This result agrees with data from Osman et al. 2011, where guava significantly reduces MDA, and there is no significant difference between treatment and positive control (62).

5. Conclusion

The present study demonstrates that *Psidium guajava* fruit extract and *Musa paradisiaca* root extract possess significant hepatoprotective and antioxidant activities against CCl_4-induced liver injury in Wistar rats. Both extracts improved liver function biomarkers, reduced oxidative stress, and enhanced endogenous antioxidant defenses. Notably, the combined di-herbal extract at 500 mg/kg produced the most pronounced protective effects, suggesting a synergistic interaction between the phytochemicals of both plants.

These findings support the traditional use of *P. guajava* and *M. paradisiaca* in managing liver disorders and highlight their potential for development as complementary therapeutic agents. However, further studies incorporating expanded dose–response assessments and molecular investigations are recommended to validate these results and better understand the mechanisms involved.

6. Limitations and Recommendations

This study has several limitations that should be acknowledged. First, the relatively short study duration and modest sample size may have restricted the full expression of biochemical recovery or injury patterns, particularly for biomarkers such as ALP, which have slower clearance kinetics. Longer observation periods with larger cohorts will be essential to determine whether the biochemical alterations observed here persist or resolve with extended treatment.

Second, the study did not investigate the

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molecular mechanisms underlying the observed effects. Although the findings suggest potential hepatoprotective and antioxidant activity, the precise pathways involved remain unclear. Future work should therefore explore oxidative stress signaling, inflammatory mediators, and apoptotic pathways, and evaluate mitochondrial and enzymatic responses using both in vitro and in vivo models.

Third, although the extract was confirmed to contain broad classes of phytochemicals, the specific bioactive constituents responsible for the biological effects were not isolated or quantified. Isolation, purification, and characterization of these active compounds will be crucial for identifying the molecules responsible for the therapeutic effects and for optimizing dosage and treatment regimens.

Additionally, Histopathological evaluation was qualitative; quantitative scoring and photomicrographs were not included. Future studies should incorporate standardized histological grading and molecular pathway investigations.

Finally, total ALP activity was measured without isoenzyme fractionation, making it difficult to distinguish hepatic from extra-hepatic sources of elevation. Isoenzyme profiling should therefore be included in subsequent research to clarify the origin of ALP changes.

Furthermore, comprehensive toxicity studies—both in vitro and in vivo—are recommended to establish the safety profile of *Psidium guajava* and *Musa paradisiaca* extracts at varying concentrations, especially if they are to be considered for therapeutic development.

References

1. Navarro VJ, Senior JR. Drug-Related Hepatotoxicity. New England Journal of Medicine. 2006 Feb 16;354(7):731–9.

2. Willett KL, Roth RA, Walker L. Workshop Overview: Hepatotoxicity Assessment for Botanical Dietary Supplements. Toxicological Sciences. 2004 May;79(1):4–9.

3. Papay JI, Clines D, Rafi R, Yuen N, Britt SD, Walsh JS, et al. Drug-induced liver injury following positive drug rechallenge. Regulatory Toxicology and Pharmacology. 2009 Jun;54(1):84–90.

4. Singh SK, Kesari AN, Rai PK, Watal G. Assessment of glycemic potential ofMusa paradisiaca stem juice. Indian Journal of Clinical Biochemistry. 2007 Sep;22(2):48–52.

5. Cichoż-Lach H. Oxidative stress as a crucial factor in liver diseases. World Journal of Gastroenterology. 2014;20(25):8082.

6. Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, et al. Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. Frontiers in Pharmacology. 2022 Feb 14;13(1).

7. Caldas APS, Coelho OGL, Bressan J. Cranberry antioxidant power on oxidative stress, inflammation and mitochondrial damage. International Journal of Food Properties. 2018 Jan 1;21(1):582–92.

8. Yau Yan L. ANTIOXIDANT PROPERTIES OF GUAVA FRUIT: COMPARISON WITH SOME LOCAL FRUITS. Sunway Academic Journal [Internet]. 2006 [cited 2025 Sep 22];3:9–20. Available from: http://eprints.sunway.edu.my/28/1/lim3.pdf

9. Hartati R, Nadifan HI, Fidrianny I. Crystal Guava (*Psidium guajava* L. "Crystal"): Evaluation of *In Vitro* Antioxidant Capacities and Phytochemical Content. The Scientific World Journal. 2020 Sep 1;2020:1–7.

10. Bouchoukh, Imane , Hazmoune, Tahar , Boudelaa, Mokhtar, Bensouici, Chawki , Zellagui, Amar . Anticholinesterase and antioxidant activities of foliar extract from a tropical species: *Psidium guajava* L. (Myrtaceae) grown in Algeria. Current Issues in Pharmacy and Medical Sciences. 2019 Sep 1;32(3):160–7.

11. Israel Oyewole O. Hepatorenal Protective Activities of Plantain Root (Musa paradisiaca) on Arsenic-induced Oxidative Damage in Rat. Advances in Biochemistry. 2015;3(2):30.

12. Yadav N, Chattopadhyay S, Pal A, Chanda D, Gupta A. Hepatoprotective effects and safety evaluation of coumarinolignoids isolated from Cleome viscosa seeds. Indian Journal of Pharmaceutical Sciences. 2010;72(6):759.

13. Brand-Williams W, Cuvelier ME, Berset C. Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT - Food Science and Technology [Internet]. 1995 Jan [cited 2025 Sep 22];28(1):25–30. Available from: https://www.sciencedirect.com/science/article/pii/S0023643895800085

14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999 May;26(9-10):1231–7.

15. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochemical and Biophysical Research Communications [Internet]. 1994 Jun 15 [cited 2025 Sep 22];201(2):748–55. Available from: https://pubmed.ncbi.nlm.nih.gov/8003011/

16. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances. 2015 Dec;33(8):1582–614.

17. Yakubu MT, Sunmonu TO, Lewu FB, Ashafa AOT, Olorunniji FJ, Eddouks M. Medicinal Plants Used in the Management of Diabetes Mellitus 2015. Evidence-Based Complementary and Alternative Medicine. 2015;1–2.

18. Bamidele TO, Enemali MO, Ijeomah AU. HEPATOPROTECTIVE ACTIVITY OF ETHANOL EXTRACT OF PURPLE ONION BULB (Allium cepa Linn) ON CCl4 INDUCED HEPATOTOXICITY IN WISTAR RATS [Internet]. Ftstjournal.com. FUW Trends in Science & Technology Journal; 2018 [cited 2025 Sep 22]. Available from: https://www.ftstjournal.com/

19. Amutha K, Selvakumari U. Wound healing activity of methanolic stem extract ofMusa paradisiaca Linn.(Banana) in Wistar albino rats. International Wound Journal. 2014 Sep 16;13(5):763–7.

20. Atik N, Muda I, Rahmadi AR, Achadiyani A, Djunaedi DD. PHYTOCHEMICAL SCREENING AND HISTOLOGY APPEARANCE OF ACUTE ORAL TOXICITY STUDY ON ETHANOL EXTRACT OF PSIDIUM GUAJAVA LINN. FRUIT IN MICE. Asian Journal of Pharmaceutical and Clinical Research [Internet]. 2019 Jan 7 [cited 2025 Sep 22];12(1):351. Available from: https://repository.unmul.ac.id/bitstream/handle/123456789/8407/document(2).pdf?sequence=1

21. Saeed A, Marwat MS, Shah AH, Na R, Zain-Ul-Abidi S, Akbar S, et al. Assessment of total phenolic and flavonoid contents of selected fruits and vegetables. Indian Journal of Traditional Knowledge (IJTK), [Internet]. 2019;18(4):686–93. Available from: http://op.niscpr.res.in/index.php/IJTK/article/view/28999

22. Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, et al. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. The Journal of Nutrition [Internet]. 2003 Sep 1;133(9):2812–9. Available from: https://pubmed.ncbi.nlm.nih.gov/12949370/

23. Thuaytong W, Anprung P. Bioactive Compounds and Prebiotic Activity in Thailand-Grown Red and White Guava Fruit (Psidium guajava L.). Food Science and Technology International. 2011 Jun;17(3):205–12.

24. Lin CY, Yin MC. Renal Protective Effects of Extracts from Guava Fruit (Psidium guajava L.) in Diabetic Mice. Plant Foods for Human Nutrition. 2012 May 12;67(3):303–8.

25. Odubanjo VO, Shodehinde SA, Adedeji FA, Daniel JA, Olayinka AA, Akinnusi PA, et al. Starch Content, Antioxidant Activity and Inhibition of Starch Hydrolyzing Enzymes by Unripe Musa paradisiaca and Musa acuminata. Science Letters. 2022 Jun 14;10(2):67–75.

26. Oboh, G. and Rocha, J. (2007) Antioxidant in Foods A New Challenge for Food Processors. In Leading Edge Antioxidants Research, Nova Science Publishers Inc., New York, 35-64. - References - Scientific Research Publishing [Internet]. Scirp.org. 2020 [cited 2025 Sep 22]. Available from: https://www.scirp.org/reference/referencespapers?referenceid=2747009

27. Gull J, Sultana B, Anwar F, Naseer R, Ashraf M, Ashrafuzzaman M. Variation in Antioxidant Attributes at Three Ripening Stages of Guava (Psidium guajava L.) Fruit from Different Geographical Regions of Pakistan. Molecules. 2012 Mar 14;17(3):3165–80.

28. Lim, Y. Y., Lim, T. T., & Tee, J. J. Antioxidant properties of guava fruit: comparison with some local fruits. Sunway Academic Journal ; 2006.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1693

29. Abdel Ghany TM, Ganash M, Alawlaqi MM, Al-Rajhi AMH. Antioxidant, Antitumor, Antimicrobial Activities Evaluation of Musa paradisiaca L. Pseudostem Exudate Cultivated in Saudi Arabia. BioNanoScience [Internet]. 2018 Dec 6;9(1):172–8. Available from: https://link.springer.com/article/10.1007/s12668-018-0580-x

30. SOWMYA BH, ANANDHI DU. Quantification of Total Phenolics, Flavonoids and Evaluation of in vitro Free Radical Scavenging Activities in Psidium guajava L. Indian Journal of Pharmaceutical Sciences. 2020;82(4).

31. Ademiluyi AO, Oboh G, Ogunsuyi OB, Oloruntoba FM. A comparative study on antihypertensive and antioxidant properties of phenolic extracts from fruit and leaf of some guava (Psidium guajava L.) varieties. Comparative Clinical Pathology [Internet]. 2016 Mar 1;25(2):363–74. Available from: https://link.springer.com/article/10.1007%2Fs00580-015-2192-y

32. Ashok Kumar BS, Lakshman K, Jayaveea KN, Sheshadri Shekar D, Saleemulla Khan, Thippeswamy BS, et al. Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of Amaranthus viridis Linn in alloxan induced diabetic rats. Experimental and Toxicologic Pathology. 2012 Jan;64(1-2):75–9.

33. Loganayaki N, Rajendrakumaran D, Manian S. Antioxidant capacity and phenolic content of different solvent extracts from banana (Musa paradisiaca) and mustai (Rivea hypocrateriformis). Food Science and Biotechnology. 2010 Oct;19(5):1251–8.

34. Thaipong K;Boonprakob U;Cisneros-Zevallos L;Byrne DH. Hydrophilic and lipophilic antioxidant activities of guava fruits. The Southeast Asian journal of tropical medicine and public health [Internet]. 2018;36 Suppl 4. Available from: https://pubmed.ncbi.nlm.nih.gov/16438219/

35. Chen HY, Yen GC. Antioxidant activity and free radical-scavenging capacity of extracts from guava (Psidium guajava L.) leaves. Food Chemistry. 2007 Jan;101(2):686–94.

36. Azofeifa G, Quesada S, Boudard F, Morena M, Cristol JP, Pérez AM, et al. Antioxidant and Anti-inflammatory in Vitro Activities of Phenolic Compounds from Tropical Highland Blackberry (Rubus adenotrichos). Journal of Agricultural and Food Chemistry. 2013 Jun 10;61(24):5798–804.

37. Medina, M., et al. (2014) Health Promotion and Chronic Disease Prevention What Are Family Health Teams Doing Saúde Debate, 38, 69-82. - References - Scientific Research Publishing [Internet]. Scirp.org. 2016. Available from: https://www.scirp.org/reference/referencespapers?referenceid=1948011

38. Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal. 2013;2013(162750):1–16.

39. Aparadh VT, Naik VV, Karadge BA. ANTIOXIDATIVE PROPERTIES (TPC, DPPH, FRAP, METAL CHELATING ABILITY, REDUCING POWER AND TAC) WITHIN SOME CLEOME SPECIES [Internet]. 2012 [cited 2025 Sep 22]. Available from: https://rosa.uniroma1.it/rosa04/annali_di_botanica/article/view/9958

40. Ibrahim M, Muhammad N, Naeem M, Deobald AM, Kamdem JP, Rocha JBT. In vitro evaluation of glutathione peroxidase (GPx)-like activity and antioxidant properties of an organoselenium compound. Toxicology in Vitro. 2015 Aug;29(5):947–52.

41. Liang N, Kitts D. Antioxidant Property of Coffee Components: Assessment of Methods that Define Mechanisms of Action. Molecules. 2014 Nov 19;19(11):19180–208.

42. Adebayo A. Protective Properties of Citrullus lanatus on Carbon Tetrachloride Induced Liver Damage in Rats. European Journal of Medicinal Plants. 2014;4(8):979–89.

43. Hopkins PN, Wu L, Hunt SC, James BC, Vincent G, Williams RR. Higher Serum Bilirubin Is Associated With Decreased Risk for Early Familial Coronary Artery Disease. 1996 Feb 1;16(2):250–5.

44. Nirmala M, Girija K, Lakshman K, Divya T. Hepatoprotective activity of Musa paradisiaca on experimental animal models. Asian Pacific Journal of Tropical Biomedicine. 2012 Jan;2(1):11–5.

45. Osman M, Ahmed M, Mahfouz S, Elaby S. Biochemical Studies on the Hepatoprotective Effects of Pomegranate and Guava Ethanol Extracts. New York Science Journal. 2011;4(3):27–41.

46. Khizar A, Ghazala HR, Hina Z, Tariq MJ. Evaluation of Hepatic activity of various morphological

parts of Musa paradisiaca L. African Journal of Pharmacy and Pharmacology. 2016 May 22;10(19):419–29.

47. Mohan M, Shashank B, Priya A. Protective effect of Psidium guajava L. leaves ethanolic extract on doxorubicin-induced nephrotoxicity in rats. Indian Journal of Natural Products and Resources [Internet]. 2014;5(2):129–33. Available from: https://nopr.niscpr.res.in/bitstream/123456789/29070/1/IJNPR%205%282%29%20129-133.pdf

48. Rahmat A, Bakar M, Hambali Z. The effects of guava (*Psidium guajava) consumption on total antioxidant and lipid profile in normal male youth*. African Journal of Food, Agriculture, Nutrition and Development. 2011 Nov 9;6(2).

49. Roy CK, Das AK. Comparative evaluation of different extracts of leaves of Psidium guajava Linn. for hepatoprotective activity. Pakistan journal of pharmaceutical sciences [Internet]. 2010 Jan;23(1):15–20. Available from: https://pubmed.ncbi.nlm.nih.gov/20067861/

50. Sambo N, Garba SH, Timothy H. Effect of the aqueous extract of *Psidium guajava* on erythromycin-induced liver damage in rats. Nigerian Journal of Physiological Sciences [Internet]. 2010 Mar 16;24(2):171–6. Available from: https://www.ajol.info/index.php/njps/article/view/52928

51. Iluz-Freundlich D, Zhang M, Uhanova J, Minuk GY. The relative expression of hepatocellular and cholestatic liver enzymes in adult patients with liver disease. Annals of Hepatology. 2020 Mar;19(2):204–8.

52. Lala V, Minter DA, Zubair M. Liver Function Tests [Internet]. Nih.gov. StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482489/

53. Karim Samy El-Said, Hussein S, Alrashdi BM, Mahmoud HA, Ibrahim MA, Elbakry M, et al. Musa sp. Leaves Extract Ameliorates the Hepato-Renal Toxicities Induced by Cadmium in Mice. Molecules. 2022 Jan 16;27(2):559–9.

54. Shady NH, Abdullah HS, Maher SA, Albohy A, Elrehany MA, Mokhtar FA, et al. Antiulcer Potential of Psidium guajava Seed Extract Supported by Metabolic Profiling and Molecular Docking. Antioxidants. 2022 Jun 23;11(7):1230.

55. Hung MY, Timothy Yu-Chi Fu, Ping Hsiao Shih, Lee CP, Yen GC. Du-Zhong (Eucommia ulmoides Oliv.) leaves inhibits CCl4-induced hepatic damage in rats. 2006 Aug 1;44(8):1424–31.

56. Suneetha B, Prasad K, Sujatha D.

57. Rao M, Ahmad BA, Mohd KS. IN VITRO NITRIC OXIDE SCAVENGING AND ANTI INFLAMMATORY ACTIVITIES OF DIFFERENT SOLVENT EXTRACTS OF VARIOUS PARTS OF Musa paradisiaca. Malaysian Journal of Analytical Science [Internet]. 2016 Jan 1;20(5):1191–202. Available from: https://www.researchgate.net/publication/327344928_IN_VITRO_NITRIC_OXIDE_SCAVENGING_AND_ANTI_INFLAMMATORY_ACTIVITIES_OF_DIFFERENT_SOLVENT_EXTRACTS_OF_VARIOUS_PARTS_OF_Musa_paradisiaca

58. Marchionatti AM, Perez AV, Diaz de Barboza GE, Pereira BM, Tolosa de Talamoni NG. Mitochondrial dysfunction is responsible for the intestinal calcium absorption inhibition induced by menadione. Biochimica et biophysica acta [Internet]. 2008 Feb;1780(2):101–7. Available from: https://pubmed.ncbi.nlm.nih.gov/18053815/

59. Farombi E, Akintunde J, Nzute N, Adedara I, Arojojoye O. Municipal landfill leachate induces hepatotoxicity and oxidative stress in rats. Toxicology and Industrial Health. 2011 Sep 21;28(6):532–41.

60. Sirisha K, Goverdhan P, Hima G, Madhavi M. Kalam Sirisha et al / Int. J Pharm Phytopharmacol Res. 2013;3(3):190–5.

61. Vijayakumar S, Presannakumar G, Vijayalakshmi NR. Antioxidant activity of banana flavonoids. Fitoterapia [Internet]. 2008 Jun;79(4):279–82. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0367326X08000464

62. Osman M, Ahmed M, Mahfouz S, Elaby S. Biochemical Studies On The Hepatoprotective Effects Of Pomegranate And Guava Ethanol Extracts. New York Science Journal [Internet]. 2011;4(3):27–39. Available from: https://www.sciencepub.net/newyork/ny0403/04_4647ny0403_27_39.pdf

63. Mourelle M, Muriel P, Favari L, Franco TT. PREVENTION OF CCL_4-INDUCED LIVER CIRRHOSIS BY SILYMARIN. 1989 May 6;3(3):183–91.

64. Ramachandra R, Subramanian S, Govindasamy S. Protective role of silymarin on

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http://dx.doi.org/10.21622/AMPDR.2026.06.1.1693

liver damage induced by carbon tetrachloride in rats. Indian J Exp Biol. 2007;45:1053–7.

65. Abenavoli L, Izzo AA, Milić N, Cicala C, Santini A, Capasso R. Milk thistle (Silybum marianum): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. Phytotherapy Research. 2018 Aug 6;32(11):2202–13.

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Fungal Antimicrobial Activity: Potential Use and Applications in the Food Industry

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ABSTRACT:

Food spoilage caused by microorganisms remains a major challenge for global food systems, leading to economic losses and increasing the risk of foodborne illnesses. Although synthetic preservatives and antibacterial agents are widely used, their role in promoting antimicrobial resistance is limited and context-dependent; however, concerns persist regarding consumer acceptance, regulatory restrictions, and potential stress responses they induce in microorganisms. These limitations have intensified interest in natural alternatives that align with clean-label and sustainable food production trends. Fungal species produce a broad range of secondary metabolites—including organic acids, peptides, enzymes, volatile compounds, and pigments—with documented antimicrobial activity against food-spoiling bacteria and fungi. These metabolites are synthesized during fungal growth or in response to environmental stress, and several have been evaluated for their ability to inhibit microbial contamination or extend product shelf life. Despite this promise, only a limited number of fungal-derived compounds have received regulatory authorization for food use, due to concerns related to toxicity, co-production of mycotoxins, sensory impacts, and regulatory constraints. This review critically examines the antimicrobial potential of fungal metabolites, outlining their chemical diversity, mechanisms of action, and demonstrated applications in food systems. It also discusses essential safety, toxicological, and regulatory authorization requirements necessary for their adoption as bio-preservatives in the food industry. Through this comprehensive evaluation, the review highlights both the opportunities and the challenges associated with integrating fungal-derived antimicrobials into modern food preservation strategies.

KEYWORDS:
Food security; bio-preservatives; biocontrol; Sustainable development; Industrial applications.

1. Introduction

Microbial contamination and spoilage remain major challenges for the global food industry, causing substantial economic losses and posing significant public health risks through foodborne illnesses. To control these hazards, the food sector relies on a combination of good manufacturing practices (GMP), good hygienic practices (GHP), and effective preservation technologies. Traditional preservation methods—including refrigeration, freezing, thermal processing, pH reduction, and water activity control—form the backbone of food safety systems. Chemical preservatives such as sorbates, benzoates, and propionates also play an important but complementary role in inhibiting microbial growth in specific food matrices [1].

Growing consumer demand for minimally processed, "clean-label" foods, coupled with the expansion of ready-to-eat and fresh products with inherently short shelf lives, has increased interest in natural alternatives to synthetic additives [2]. In response, modern preservation

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approaches—such as bio-preservation, irradiation, and multi-hurdle technologies—have gained prominence. Bio-preservation, in particular, employs beneficial microbes or their metabolites to suppress spoilage and pathogenic microorganisms, offering potential advantages in maintaining product quality and meeting consumer expectations for natural solutions [3,4]. However, bio-preservation strategies must be evaluated carefully to ensure they do not negatively affect sensory properties or stability in complex food systems.

Although synthetic preservatives are generally recognized as safe at regulated levels, consumer preference for natural alternatives has driven exploration of antimicrobial compounds derived from plants, bacteria, and fungi. Food preservatives contribute minimally to global antimicrobial resistance (AMR), yet certain antimicrobials and processing stresses may trigger adaptive responses in microorganisms. More critically, AMR concerns along the food chain arise from contamination with antibiotic-resistant bacteria originating from agricultural or environmental sources. This challenge underscores the importance of integrated "One Health" strategies that link food safety, environmental stewardship, and human health (**Fig. 1**) [5,6].

Fungal-derived metabolites have emerged as promising candidates for natural bio-preservation. Filamentous fungi and macrofungi synthesize a diverse array of secondary metabolites—including organic acids, enzymes, peptides, volatile compounds, and pigments—that exhibit antimicrobial activity against major foodborne pathogens and spoilage organisms such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Bacillus cereus*, and *Clostridium botulinum* [7,8]. These compounds are produced during normal growth or under environmental stress, providing ecological advantages by suppressing competing microorganisms.

Fungi are already well established in industrial biotechnology, particularly in enzyme production, fermentation processes, and microbial biomass generation [3]. Interest in fungal pigments and bioactive colorants has also expanded due to their natural origin and multifunctional properties.

However, the practical use of fungal metabolites in foods requires rigorous assessment of safety, toxicity, potential co-production of mycotoxins, and compliance with regulatory standards. Notably, natamycin—a widely used antifungal preservative—is produced by *Streptomyces natalensis*, not by fungi, and is included here only as a benchmark for comparison [4].

This review critically evaluates fungal-derived antimicrobial metabolites as natural alternatives to synthetic preservatives. It examines their chemical diversity, mechanisms of action, demonstrated applications in food systems, and the safety and regulatory considerations necessary for their successful adoption. Through this analysis, we aim to clarify both the opportunities and the limitations associated with integrating fungal antimicrobials into modern food preservation strategies.

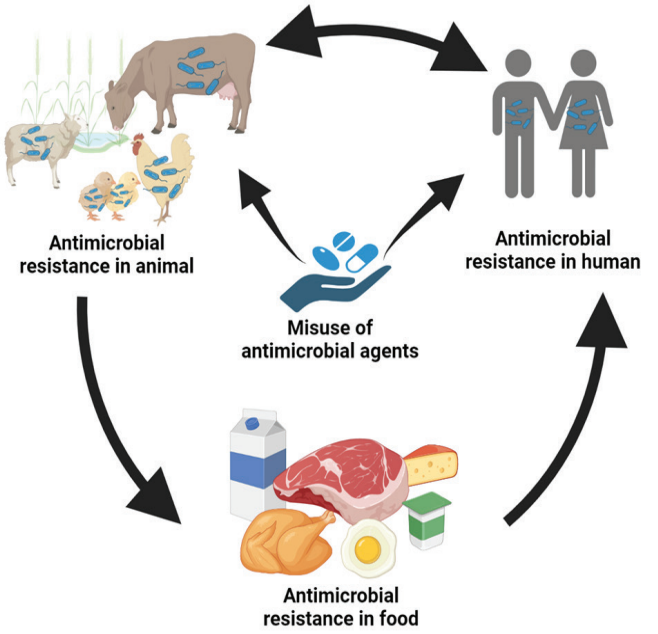

Figure 1: Conceptual illustration of antimicrobial resistance (AMR) transmission within the One Health framework, highlighting interconnected pathways between humans, animals, and the food system, and the selective pressure created by the use and misuse of antimicrobial agents.

2. Traditional Preservation Methods Used in the Food Industry

Traditional preservation methods remain fundamental in sustaining food safety and extending shelf life across the global food industry. These approaches generally fall into three main categories—chemical, biological, and physical—each contributing in different ways to the control of microbial growth and product stability (**Fig. 2**).

2.1: Chemical Methods

Chemical preservatives are widely employed to inhibit microbial proliferation, delay enzymatic spoilage, and prevent oxidative deterioration. Commonly used compounds include sorbic acid and its salts, benzoic acid and benzoates, propionic acid and propionates, and parabens (methyl, ethyl-, and propyl-p-hydroxybenzoates). Their effectiveness depends on food composition and pH, and they are often incorporated into bakery goods, beverages, dairy products, and acidic foods.

Other preservation-related treatments rely on modifying water activity or acidity. Salt curing, for example, reduces water availability and restricts microbial growth; however, salt alone is insufficient to prevent *Clostridium botulinum* in cured meats, making nitrites essential in such products. Sugar creates similar preservative conditions in jams and syrups by lowering water activity. Acidic solutions such as vinegar also contribute to microbial inhibition in pickled foods. Lye (sodium hydroxide) is used in specific traditional products—such as olives and pretzels—to induce characteristic textural or sensory properties, though it is not considered a primary antimicrobial preservation strategy [9,10].

2.2: Biological Methods

Biological preservation relies on microorganisms or their natural metabolites to control spoilage and pathogenic organisms. Lactic acid bacteria (LAB) remain the most widely applied biological preservative agents due to their ability to produce organic acids, antimicrobial peptides such as bacteriocins, hydrogen peroxide, and other inhibitory compounds. LAB fermentation has been used for centuries to preserve dairy products, vegetables, and meats, offering natural inhibition of a wide range of microorganisms. Despite these benefits, fermentation can alter sensory characteristics, and its effectiveness depends heavily on the food matrix and the strains employed [9,10].

2.3: Physical Methods

Physical preservation encompasses both thermal and non-thermal technologies. Thermal treatments such as pasteurization, sterilization, blanching, and boiling inactivate spoilage organisms and pathogens by heat, whereas dehydration removes moisture to reduce water activity and limit microbial survival while also decreasing product weight.

Low-temperature storage methods also play an important role. Refrigeration slows microbial growth, while freezing halts microbial proliferation entirely. However, freezing does not eliminate most microorganisms; they may recover once the product is thawed, necessitating careful handling during storage and distribution.

A range of non-thermal physical methods has been developed to enhance food preservation without significant heat exposure. Irradiation using gamma rays, electron beams, or X-rays effectively reduces microbial load while maintaining product quality. High-pressure processing (HPP) achieves microbial inactivation by exposing foods to very high hydrostatic pressures, making it suitable for heat-sensitive products. Modified-atmosphere and vacuum packaging reduce oxygen availability, although these approaches require proper controls to prevent risks associated with anaerobic pathogens such as *Clostridium botulinum*. Smoking imparts antimicrobial and antioxidant compounds to food surfaces, whereas pickling combines acidic or salty conditions to inhibit microbial growth. These traditional preservation methods continue to form the foundation of food safety and shelf-life extension, supporting modern food systems and complementing emerging natural preservation strategies [9,10].

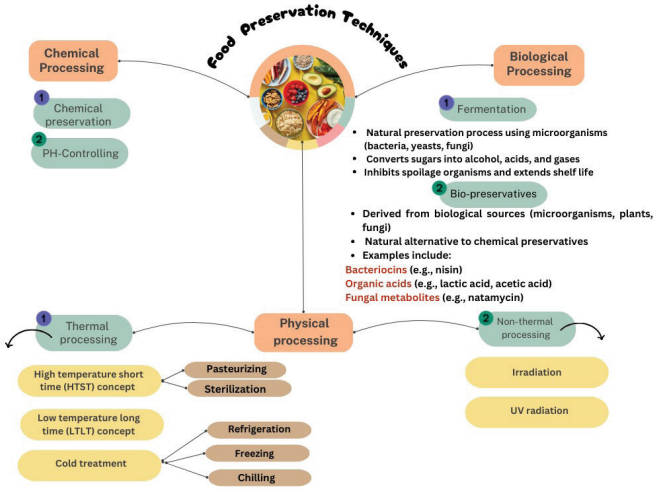

Figure 2: Flow chart presenting the traditional preservatives used in food industries, including chemical, biological, and physical.

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3. Fungal Metabolites and Their Antimicrobial Properties

Fungal metabolites have gained increasing attention as potential natural preservatives due to their chemical diversity and their ability to inhibit a broad range of microorganisms. Many filamentous fungi—particularly those belonging to the *Ascomycota*—produce secondary metabolites with antibacterial and antifungal activity, reflecting their ecological role in competing within complex microbial environments [11,12]. Figure 3 summarizes major classes of fungal metabolites with representative examples relevant to food preservation.

Among these bioactive compounds, fungal pigments have been of particular interest. Although traditionally valued as natural colorants, several fungal pigments also possess noteworthy antimicrobial properties. Species such as *Monascus purpureus*, long used in Asian fermented foods, produce yellow, orange, and red pigments that exhibit inhibitory effects against a variety of fungi, including Aspergillus, Trichoderma, Mucor, Penicillium, and Fusarium, as well as against bacterial genera such as Bacillus, Pseudomonas, Escherichia, and Streptomyces [13]. Pigments from other fungi—such as *Monodictys castaneae*, *Sporobolomyces* spp., *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp.—have also demonstrated broad-spectrum activity [14], underscoring the potential of fungal colorants as multifunctional bio-preservatives.

The antimicrobial efficacy of fungal pigments can vary depending on their chemical composition and mode of extraction. Studies have shown that modifying culture conditions, such as supplementing growth media with specific amino acids, can enhance pigment bioactivity, particularly against Gram-positive bacteria. Minimum inhibitory concentration (MIC) values have been reported for several pigment derivatives, demonstrating their capacity to suppress microbial growth in food contexts [13]. Applications have been explored in meat products—including sausages and beef burgers—where *Monascus* pigments have contributed both color and antimicrobial effects [15]. Nevertheless, careful control of pigment concentration and purity is necessary to ensure both safety and effectiveness.

Despite their promise, fungal pigments face several obstacles on the path to commercial adoption. Safety concerns—most notably the risk of co-producing the mycotoxin citrinin—have restricted the use of *Monascus* pigments in Europe and the United States. As a result, research has focused on developing production strategies that suppress mycotoxin biosynthesis while maintaining pigment yield and bioactivity [16]. These efforts remain essential for ensuring regulatory compliance and consumer safety.

Beyond pigment-producing fungi, species inhabiting environmentally stressed or polluted habitats have also emerged as valuable sources of bioactive metabolites. Such environments exert selective pressure that may promote the production of potent antimicrobial compounds. Recent studies have isolated filamentous fungi from heavily polluted streams in the Amazon region and demonstrated that their metabolites can inhibit extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, highlighting their potential as sources of novel antimicrobial agents [11,17]. Although these findings expand the range of fungi with promising antimicrobial properties, isolates from polluted environments require rigorous toxicological evaluation before any consideration for food-related applications.

Overall, fungal metabolites represent a rich reservoir of compounds with antimicrobial activity relevant to food preservation. However, their practical deployment requires careful attention to safety, purity, regulatory approval, and reliable production methods.

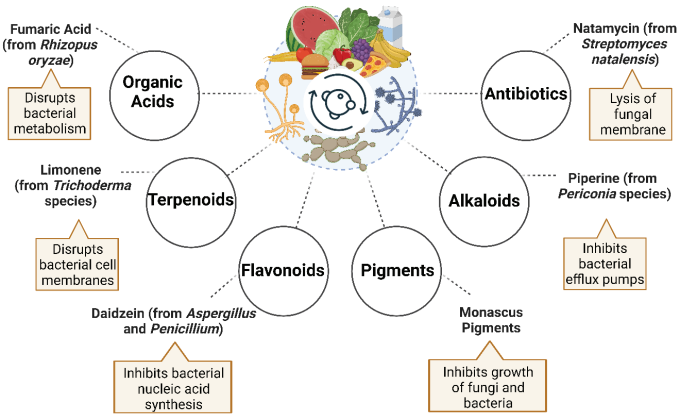

Figure 3: Types of fungal metabolites used in food industries with their mechanism of action as antimicrobials.

3.1: Antimicrobial Activity of Fungal Isolates

Segundo et. al. (2023) studied the antimicrobial activity of 67 fungal isolates that were subjected to a 14-day submerged bioprocessing. Hexane- and ethyl acetate (EtOAc)-based extraction methods were employed to isolate

the metabolites, and their antibacterial effect was examined by agar diffusion against *E. coli* (ESBL-positive) and *E. coli* [17]. These findings demonstrated that both hexane- and EtOAc-based extracts exhibited antibacterial activity, with inhibition zones ranging from 1 to 35.9 mm. Notably, *A. stygium* and *C. rosea* exhibited the largest inhibition zones against *E. coli* and ESBL-positive *E. coli* [17]. The MICs of the EtOAc extracts from *A. stygium*, *C. rosea*, and *C. rosea* were determined using a microdilution assay. The MIC for both *E. coli* and the ESBL-positive *E. coli* strain was found to be 400 µg/mL [17]. Natamycin is a prominent example of a microbial-derived antifungal preservative, which, although not of fungal origin, is included here as a reference compound due to its extensive application in food preservation.

Natamycin (E235) is a natural polyene macrolide antifungal produced by the bacterium *Streptomyces natalensis* (actinobacteria). It is currently the only antimicrobial metabolite derived from microorganisms that is widely and commercially used as a food preservative. Natamycin is approved in many countries for surface treatment of dairy products, particularly cheese [16], where it effectively inhibits the growth of molds and yeasts without adversely affecting the sensory properties of food, making it a benchmark antifungal preservative in the dairy industry [18].

Further analysis identified two active metabolites: daidzein and nodulisporone. Nodulisporone, a tetralone derived from the *Nodulisporium* genus (Ascomycota), displayed antimicrobial activity, with inhibition zones of 13 mm against *Trichoderma harzianum* and 10 mm against *Candida albicans* [17]. Tetralones are known to inhibit DNA replication by blocking cellular DNA topoisomerase, thereby affecting gene expression and recombination in microorganisms [17]. Daidzein, a well-known flavonoid commonly found in plants, was also detected in the genera *Aspergillus*, *Penicillium*, and *Trichoderma*. Flavonoids are recognized for their ability to inhibit the formation of bacterial nucleic acids, metabolism, and interfere with cell membrane permeability [19].

Various classes of antimicrobial metabolites, including alkaloids, terpenoids, flavonoids, phenols, steroids, and volatile oils, have been extracted from endophytic fungi [20,21]. One noteworthy bioactive substance, 7-amino-4-methylcoumarin, produced by the endophytic fungus *Xylaria* sp., found in association with *Ginkgo biloba* L., has demonstrated wide inhibitory effects against a variety of bacterial and fungal infections responsible for foodborne illnesses and spoilage. As a result, it has been suggested as a potential natural food preservative [22]. Additionally, *Fusarium* sp. displayed antagonistic properties against infection caused by bacteria and fungi. Similarly, *Phoma* sp., derived from *Dendrobium devonianum* and *D. thyrsiflorum*, showed a potent inhibitory effect against multiple human-infecting agents, including *E. coli*, *Bacillus subtilis*, *S. aureus*, *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* [23].

3.2: Application of Filamentous Fungi in the Meat Industry

Non-toxigenic species like *P. chrysogenum* and *P. nalgiovense*, which are frequently utilized in the preparation of meat, have the potential to be exploited as biocontrol agents, according to additional research on filamentous fungi. Strains of *P. chrysogenum* purified from dry-cured ham have been shown to inhibit the growth and formation of ochratoxin A (OTA) by *P. verrucosum* in vitro. Similarly, *P. nalgiovense* strains from the commercial culture TEXEL PN1 have been found to limit the growth of common spoilage fungi, such as *A. flavus* and *P. restrictum* [17].

3.3: Marine-Derived Fungi and Their Antimicrobial Compounds

Fungi derived from marine sources serve as valuable sources of bioactive compounds with notable antimicrobial activities, especially from the genera *Neosartorya* and *Aspergillus*. Different compounds were isolated from the soil fungus *Neosartorya siamensis*, marine *N. takakii*, coral-associated *N. laciniosa*, and marine-derived *Aspergillus elegans*, which was obtained from the marine sponge *Monanchora unguiculata*. These compounds were assessed for their antimicrobial activity, particularly focusing on their MICs to evaluate their role as bacterial efflux pump inhibitors. Several of the compounds demonstrated inhibitory effects on the growth of *S. aureus*, although none were effective against *Salmonella enterica* serovar Typhimurium [24]. Yeasts exhibiting antifungal characteristics were sourced from marine ecosystems, indicating significant potential as bioprotective materials for postharvest treatment of fruits and vegetables. According to a study conducted in the South China Sea, 56% of the isolates from deep-sea

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fungi had antibiotic activity against at least one pathogenic bacterium. Such as *Arthrinium*, *Aspergillus*, and *Penicillium* demonstrated both antibacterial and antifungal properties, whereas *Acremonium*, *Cladosporium*, *Geomyces*, and *Phaeosphaeriopsis* exhibited only antifungal effect [25]. Additionally, the marine yeast *Rhodosporidium paludigenum*, sourced from the East China Sea, demonstrated effective inhibition of *Penicillium expansum* on pears and *Alternaria alternata* on jujube fruits [26].

4. Applications in the Food Industry

In the realm of food preservation, fungal metabolites have demonstrated considerable potential due to their capacity to inhibit spoilage organisms and pathogenic microbes, thereby extending shelf life and enhancing food safety. While bacteria are traditionally recognized in the synthesis of various antimicrobial agents, there has been increasing interest in the antifungal properties of yeasts. Yeasts, present in various environments including cereals, vegetables, fruits, meat, dairy, and processed foods, have long played a role in preserving food by the fermentation of products such as wine, beer, dough, and certain cheeses, contributing to both preservation and flavor enhancement [27]. Yeasts exert antimicrobial activity by competing for nutrients, acidifying their environment, resisting stressors like ethanol, and producing antimicrobial molecules, known as mycocins or killer proteins, that inhibit fungal growth. Furthermore, yeasts can colonize fruits, seeds, berries, and leaves, competing effectively with other microorganisms for space and nutrients, rendering them useful biocontrol agents in reducing postharvest decay [28,29].

Extensive research has explored the use of yeasts that produce mycocin to prevent fungal spoilage, with applications in foods and beverages, including wine, olives, soy sauce, and salted vegetables [30]. However, careful consideration must be given to the selection of yeast strains, as they may negatively impact product quality. Yeasts such as *Meyerozyma guilliermondii*, *Candida fructus*, *Issatchenkia orientalis*, and *C. quercitrusa* have been identified on fruits and plant surfaces, displaying antagonistic effects against fungal pathogens [31]. *Wickerhamomyces anomalus*, isolated from avocados, has been shown to inhibit *Colletotrichum gloeosporioides* and *C. acutatum*, the causative agents of avocado anthracnose. Similarly, *Pichia membranifaciens*, either alone or combined with chitosan, has been demonstrated to suppress *C. gloeosporioides* in citrus anthracnose by reducing mycelial growth and spore germination [32]. Additionally, *Hanseniaspora uvarum*, extracted from grapes, has been documented as an effective bio-preservative, controlling gray mold (*Botrytis cinerea*) in postharvest grapes [33] and inhibiting green mold (*Penicillium digitatum*) that affects citrus fruits [34]. Recently, yeast antifungal applications expanded into dry-cured ham [35] and sausages [36], where they are used to control toxigenic *Penicillium* species responsible for spoilage and OTA production. Strains of *Debaryomyces hansenii* have demonstrated antifungal effect against *Penicillium verrucosum* and *P. nordicum*, reducing OTA levels. Although *D. hansenii* can cause spoilage of fresh cheese and cream, it is also widely used as a starter culture to produce surface-ripened cheese and naturally occurs in meat and fruit products [16].

Certain filamentous fungi, like *Penicillium nalgiovense* and *P. chrysogenum*, play a positive role in enhancing the taste and visual quality of fermented foods, such as surface-ripened cheeses [37] and dry-cured ham [38]. These fungi are also employed as antifungal agents, particularly in low-water-activity environments where LAB is unable to thrive. Commercial strains of *P. nalgiovense* and *P. chrysogenum* are widely used in the meat industry [39]. Recent research has demonstrated that *P. chrysogenum*, sourced from dry-cured ham, inhibits spoilage fungi such as *Aspergillus flavus* and *P. restrictum* through the production of antifungal proteins [40]. Similarly, *P. nalgiovense* from the TEXEL PN1 culture has been shown to limit the multiplication and OTA secretion of *P. verrucosum* [41]. Furthermore, combining *P. chrysogenum* with reduced water activity (aw) during the ripening process has been proposed as a strategy to prevent black spot formation in dry-cured ham [38]. Overall, both yeasts and filamentous fungi produce antimicrobial peptides (AMPs), which offer a valuable approach for limiting fungal spoilage and mycotoxin production.

The use of fungal metabolites in food production is critical to reducing food contamination during the agricultural process and in industry. These biocontrol agents diminish the reliance on chemical preservatives, thereby supporting more sustainable agricultural practices and food processing. Furthermore, their use aligns with the United Nations' Sustainable Development

Goals (SDGs), which target food security and agricultural sustainability by 2030. Organic acids like fumaric acid, produced by fungi such as *Rhizopus oryzae*, are also employed in food preservation. Fumaric acid disrupts bacterial metabolism and inhibits the growth of foodborne pathogens like *Salmonella* and *E. coli*. This organic acid is commonly applied in products such as baked goods, beverages, and processed meats, contributing to extended shelf life and improved food safety [42].

Furthermore, endophytes serve as highly effective biocontrol agents by protecting host plants from phytopathogens through mechanisms such as antibiosis, parasitism, and competition. These organisms offer a sustainable substitute to chemical fungicides, which contribute to the emergence of resistant strains and environmental degradation. In addition to disease control, endophytes produce a wide array of bioactive compounds with antimicrobial, insecticidal, and other pharmacological properties, underscoring their importance in promoting sustainable agriculture and plant health management [43]. Among the most studied endophytic fungi are those from the *Trichoderma* genus, widely recognized for their diverse biocontrol traits. These include parasitism, antibiosis, secondary metabolite production, and the induction of plant defense systems. *Trichoderma* species, in particular, produce compounds such as terpenoids that demonstrate potent antifungal activity against plant pathogens, making them valuable tools for disease control in agricultural systems [44].

5. Mechanisms of Fungal Metabolites Antimicrobial Action

In dry-fermented foods, specific yeasts and molds act as effective antifungal agents by inhibiting the growth of spoilage fungi. Their efficacy stems from their ability to compete for space and nutrients and the production of antifungal agents, including antifungal proteins and volatile organic compounds [45]. *Debaryomyces hansenii* isolates produce 2-methyl-1-butanol and other volatile compounds, including unidentified diffusible molecules, that inhibit *Penicillium verrucosum* in dry-cured ham [35]. Similarly, *Meyerozyma guilliermondii*, when combined with *Lactobacillus plantarum* and *Wickerhamomyces anomalus*, generates ethyl acetate and β-1,3-glucanase during dough fermentation, successfully protecting bread from fungal contamination while preserving its taste and texture [16].

As reviewed by Delgado et al, the antifungal peptides produced from molds, such as cysteine-rich antifungal proteins (AFPs), have molecular weights between 5.5 and 10 kDa. These AFPs are highly stable under acidic conditions, heat, and proteolysis, making them suitable for use in fermented foods like cheese and meat [45,46]. Once bound to fungal cell walls or internalized, AFPs disrupt chitin synthesis or increase intracellular reactive oxygen species (ROS) levels, resulting in membrane permeabilization and cell death [45]. The mechanisms of action of antifungal yeasts and *Bacillus* species used to control postharvest diseases are multifaceted. They involve competition for essential nutrients like carbon and iron [47,48], the release of antifungal peptides and lipopeptides to inhibit pathogens [49], and mycoparasitism, where lytic enzymes such as glucanases, chitinases, and proteases break down other fungi, and induction of host resistance [27]. Cyclic lipopeptides like surfactins, fengycins, and iturins, produced by *Bacillus subtilis* and related species, aid in surface colonization and have strong antifungal effects, with fengycins and iturins being particularly harmful to fungi [50].

Antifungal volatile compounds produced by these microorganisms offer another promising mode of action, with potential applications in bio-fumigation for food spoilage control, provided safety can be ensured [51]. Compounds such as 2-phenylethanol, 2-methyl-1-butanol, and 3-methyl-1-butanol were identified for their antifungal properties. Notably, 2-phenylethanol produced by *Penicillium expansum* R82 inhibits various postharvest fungal pathogens, and its mechanism against *Penicillium digitatum* and *Penicillium italicum* has been well-documented [52].

Yeasts used to control grape pathogens employ various strategies, such as producing laminarians, antifungal volatiles, growth-inhibiting compounds, and competing for carbon and iron [53]. Many yeast strains show multiple antifungal actions. For example, *Pichia anomala* strain WRL-076, which is used to reduce aflatoxin in tree nuts, produces 2-phenylethanol, a compound that prevents spore germination, fungal growth, and aflatoxin production in *Aspergillus flavus* [54]. Fungal secondary metabolites, naturally occurring compounds not essential for fungal growth or reproduction,

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demonstrate significant antimicrobial activity due to their structural diversity [55]. These metabolites can be categorized by their biosynthetic pathways, including terpenoids, alkaloids, and flavonoids. Investigating these compounds can help discover new mechanisms to combat AMR, improve healthcare, and control foodborne and waterborne pathogens [56].

The increasing threat of foodborne and waterborne diseases, exacerbated by rising antimicrobial resistance, poses a considerable challenge. However, fungal secondary metabolites offer effective solutions through their diverse mechanisms of action. For example, monoterpenes such as limonene ($C_{10}H_{16}$), due to their small molecular size, exhibit enhanced membrane penetration. The hydrophobic nature of their methyl groups allows them to interact with the phospholipid bilayer, disrupting membrane fluidity, leading to the loss of intracellular contents, and causing a breakdown of membrane potential. This results in potent bactericidal activity [57].

Limonene, derived from *Trichoderma* species, shows strong inhibitory effects against gastroenteritis-causing bacteria, such as *E. coli*, rendering it a promising candidate for food preservation. Fumaric acid is also a fungal secondary metabolite used as a preservative that exhibits activity against various bacterial species, including *E. coli* and *Salmonella* spp., through multiple mechanisms. One such mechanism involves the disruption of bacterial energy metabolism. Fumaric acid acts as an intermediate in the Krebs cycle, positioned between succinate and malate. Its molecular structure, containing two carboxyl groups, enables it to serve as an electron acceptor during anaerobic respiration, allowing its reduction to succinate. Additionally, the trans double bond in fumaric acid enables effective binding to enzyme active sites [58]. When present in excess, fumaric acid overwhelms the fumarate reductase enzyme, inhibiting anaerobic respiration, reducing ATP production, and ultimately suppressing bacterial growth [28].

The above study concludes the critical role of fungal secondary metabolites in healthcare due to their natural origin and lower toxicity compared to conventional antimicrobial agents. These compounds inhibit bacterial mycotoxins and contribute to the global effort to combat AMR, a major public health concern today, as presented in Table 1.

Table 1: Major classes of fungal secondary metabolites with antimicrobial activity and their potential applications in food systems

Category	Metabolite	Producing fungus	Target micro-organisms	Mechanism of action	Potential application /limitations	Referenc-es
Terpenoids (VOCs)	Monoter-penes (e.g., α-pinene, limonene)	*Trichoderma* spp.	*Escherichia coli*, spoilage fungi	Disruption of cell membrane integrity	Indirect application via active or modified-atmo-sphere packaging; limited direct use due to volatility and rapid dissipation	[59,60]
Alkaloids	Peri-conicin-type alkaloids	*Periconia* sp.	*Staphylococ-cus aureus*	Inhibition of efflux pumps and cellular stress pathways	Experimental antimicro-bial agents; safety and regulatory approval not established	[61,62]
Polyketides (phenolic compounds)	Flavonoid-like polyketides	*Colletotrichum gloeosporioi-des*	*E. coli*	Interference with ATP synthesis and metabolic enzymes	Laboratory-scale studies only; sensory and toxico-logical evaluation required	[63]
Organic acids	Fumaric acid	*Rhizopus oryzae*	*Salmonella* spp.	Acidification and disruption of bacte-rial metabolism	Approved acidulant; already used in food systems within regulated limits	[28,58]
Quinones (polyketides)	Ustic acid	*Aspergillus ustus*	*E. coli*	Redox cycling and disruption of cell wall integrity	Experimental antimicrobial compound; co-production of toxic metabolites limits food application	[61,64]

6. Global food preservatives market

Rapid technological advancement and globalization are increasing consumer demand for safe, healthy food, as the presence of harmful chemicals, such as artificial additives, continues to rise. The key drivers of the market include the growing global population, changing consumer lifestyles, and heightened awareness of food safety. Consumers' demands drive companies to start reducing synthetic preservatives and using natural and organic food preservatives like essential oils, plants extract, and organic acids. The global food preservatives market is projected to experience a significant increase from 2018 to 2030, with both natural and synthetic preservatives playing key roles. Natural preservatives, including edible oils, rosemary extracts, natamycin, vinegar, and chitosan, are gaining popularity due to their perceived health benefits and the rising demand for clean-label products. Synthetic preservatives such as propionates, sorbates, and benzoates remain widely used in food production because of their cost-effectiveness and strong antimicrobial effect. The market outlook for food preservatives is also categorized by function, with antimicrobial and antioxidant properties being the primary drivers of their use. Antimicrobial preservatives are essential for preventing microbial spoilage, while antioxidants protect food products from oxidative damage, thereby extending shelf life. In terms of application, preservatives are extensively used across various sectors, including meat and poultry, bakery products, dairy, beverages, and snacks. These sectors rely on preservatives to ensure product safety, maintain quality, and extend shelf life, with the meat and poultry industry representing a significant share of the market. The market is expected to continue expanding across these segments due to growing global demand for processed and convenient foods [65,66].

The demand for extended shelf-life food products is driven by increasing urbanization and a growing working population, particularly in cities. By 2050, the global population is expected to reach 9.7 billion, with 70% residing in urban areas, prompting a rise in the consumption of convenience foods. To meet this demand, food manufacturers are incorporating natural preservatives, such as vinegar and rosemary extract, to extend product shelf life while maintaining quality, especially with the increasing preference for clean-label products. In the Asia Pacific, the food processing industry is expanding rapidly, particularly in India and China, due to rising populations and evolving consumption trends. Government support has fueled the sector's growth, with India's food processing industry contributing significantly to both domestic and export markets. In 2020, it accounted for 32% of the country's total food market. However, the demand for preservative-free foods is also increasing as consumers become more health-conscious, particularly in the U.S., Europe, and China. While synthetic preservatives are considered generally safe, some consumers express concerns about their potential health risks, including links to attention deficit hyperactivity disorder (ADHD), cardiovascular diseases, and obesity, especially in children. Despite these concerns, there remains a strong preference for high-quality, flavorful food products [66].

7. Safety, Sensory Impact, Toxicology, and Dose–Response Validation of Antifungal Metabolites

Despite the growing interest in fungal- and microbial-derived antifungal metabolites as natural alternatives to synthetic food preservatives, comprehensive safety evaluations remain limited for many candidate compounds. While numerous studies report strong antifungal efficacy *in vitro*, systematic assessments of toxicological safety, sensory impact, and dose–response relationships under realistic food conditions are largely absent, representing a major barrier to regulatory approval and industrial adoption.

7.1: Safety and Toxicological Considerations

Many antifungal metabolites produced by filamentous fungi belong to chemically diverse classes such as polyketides, non-ribosomal peptides, terpenoids, and alkaloids, some of which are structurally related to known mycotoxins. This structural similarity raises justified safety concerns, as several fungal secondary metabolites exhibit cytotoxic, genotoxic, or immunosuppressive effects at higher concentrations [64,67]. For example, compounds such as chaetoglobosins and epipolythiodioxopiperazines (ETPs), while demonstrating antifungal or antibacterial activity, have been reported to interfere with mammalian cellular redox balance and immune responses, limiting their direct application in foods [68–70].

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Toxicological evaluations, including acute and chronic toxicity, mutagenicity, teratogenicity, and carcinogenicity studies, are rarely conducted for emerging antifungal metabolites. Regulatory agencies such as the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration (FDA) require such data before approval; however, for most fungal-derived antifungal compounds, no acceptable daily intake (ADI) values or no-observed-adverse-effect levels (NOAELs) have been established [71–73].

7.2: Dose–Response Validation and Sensory Impact

A critical limitation in the development of antifungal metabolites as food preservatives is the lack of robust dose–response validation in real food matrices. Antifungal activity is frequently reported at concentrations determined under in vitro conditions, while corresponding safety margins and sensory thresholds are rarely assessed in parallel. Moreover, MICs obtained in laboratory media often fail to translate directly to complex food systems, where interactions with lipids, proteins, and carbohydrates can significantly reduce antimicrobial efficacy or alter compound bioavailability [74]. In the absence of validated dose–response relationships, it remains unclear whether effective antifungal concentrations can be achieved within acceptable toxicological and sensory limits.

Sensory impact represents an additional, yet underexplored, constraint on the practical application of antifungal metabolites. Many fungal-derived compounds possess intense pigmentation, bitterness, or characteristic odors, which may adversely affect food appearance, flavor, and overall consumer acceptance. For example, pigmented antifungal metabolites such as pulcherrimin and azaphilone derivatives can induce visible color changes in food products, limiting their suitability for applications where visual quality is critical [16,75].

Bitterness is particularly problematic for several classes of antifungal peptides and alkaloids. Defensin-like peptides and non-ribosomal lipopeptides, produced by fungi and bacteria, have been reported to impart bitter or metallic off-flavors. In particular, iturin- and fengycin-type lipopeptides, despite their strong antifungal efficacy, have been associated with perceptible bitterness at concentrations in the low mg/L range in food model systems [76,77]. Similarly, peptaibols, such as alamethicin produced by *Trichoderma* species, are short, highly hydrophobic antifungal peptides rich in α-aminoisobutyric acid; their rigid helical structures and hydrophobic side chains are strongly correlated with bitter taste perception [78,79]. Alkaloids represent another class of antifungal metabolites with pronounced sensory effects. Ergot alkaloids (e.g., ergometrine and ergine), produced by *Claviceps* species, are intensely bitter and have historically been associated with strong aversive sensory properties even at trace concentrations. Although their toxicity precludes their use as food preservatives, they illustrate the potent sensory impact that antifungal alkaloids can exert [80].

Despite these well-documented sensory challenges, formal sensory evaluation studies, including trained panel assessments and consumer acceptability testing, are seldom incorporated into antifungal metabolite research. Most studies continue to focus predominantly on antimicrobial efficacy, highlighting the need for integrated experimental approaches that simultaneously address dose–response behavior, toxicological safety, and sensory impact to support the realistic application of antifungal metabolites in food preservation.

8. Conclusion, challenges, and future perspectives

There has been significant scientific interest in exploring fungal metabolites as potential antimicrobial agents for food preservation, primarily driven by the need to control spoilage microorganisms and extend shelf life under increasingly restrictive regulatory frameworks for synthetic preservatives. Laboratory-scale studies have demonstrated that fungi are capable of producing a diverse array of secondary metabolites—including organic acids, peptides, enzymes, volatile compounds, and pigments—with measurable antimicrobial activity against foodborne bacteria and spoilage fungi. However, the translation of these findings into practical food applications remains limited, and the current contribution of fungal-derived metabolites to commercial food preservation is modest.

A major constraint to the adoption of fungal metabolites as food preservatives is their safety and toxicological profile. Many fungal secondary metabolites are structurally related to, or co-produced with, known mycotoxins, raising

significant concerns regarding cytotoxicity, genotoxicity, and chronic exposure risks. Consequently, only a very limited number of microbial-derived antifungal compounds have received regulatory authorization for food use, and the majority of fungal metabolites discussed in the literature lack Generally Recognized as Safe (GRAS) status or approval by regulatory authorities such as the EFSA or the FDA. These regulatory limitations underscore the importance of distinguishing between experimental antimicrobial activity observed in vitro and compounds that are legally and toxicologically acceptable for use in real food systems.

In addition to safety concerns, technological and sensory challenges further restrict industrial feasibility. The stability of fungal secondary metabolites within complex food matrices is often uncertain, as interactions with food components, processing conditions, and storage environments can reduce antimicrobial efficacy or lead to degradation products with unknown safety profiles. Moreover, several fungal metabolites, particularly pigmented compounds and hydrophobic peptides, may negatively affect food color, flavor, or aroma, thereby limiting consumer acceptance. These factors highlight the need for cautious evaluation of fungal metabolites beyond antimicrobial potency alone.

At present, compounds with realistic pathways toward application are largely restricted to well-characterized and tightly regulated examples, such as natamycin—which, although of bacterial origin, serves as an important benchmark for antifungal biopreservation—or carefully purified fungal-derived proteins and enzymes that can be produced under controlled conditions with minimal risk of toxic co-metabolite formation. Broad generalizations regarding the commercial readiness of fungal metabolites as bio-preservatives are therefore not supported by the current regulatory and toxicological landscape.

Future research should focus on addressing these fundamental limitations through integrated, multidisciplinary approaches. Priority areas include comprehensive toxicological evaluation, assessment of mycotoxin co-production, establishment of dose–response relationships in relevant food matrices, and formal sensory analysis. In parallel, standardized extraction and purification protocols, alongside rigorous strain selection and characterization, are essential to ensure reproducibility and safety. Advances in genomics, transcriptomics, and metabolomics offer valuable tools for identifying fungal strains and metabolic pathways capable of producing antimicrobial compounds while minimizing safety risks. Ultimately, aligning antifungal efficacy with regulatory authorization, toxicological safety, and sensory acceptability will be critical to determining whether fungal metabolites can transition from promising laboratory findings to credible and limited roles in modern food preservation systems.

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References

1. Disanto C, Celano G, Dambrosio A, Quaglia NC, Bozzo G, Tritto A, et al. Food safety in collective catering: knowledge, attitudes and correct application of GHP/GMP knowledge among foodservice workers. Ital J Food Saf. 2021 Jan 19;9(4).

2. Bouarab Chibane L, Degraeve P, Ferhout H, Bouajila J, Oulahal N. Plant antimicrobial polyphenols as potential natural food preservatives. J Sci Food Agric. 2019 Mar 15;99(4):1457–74.

3. Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control. 2014 Dec;46:412–29.

4. Singh VP. Recent approaches in food bio-preservation - a review. Open Vet J. 2018 Mar 29;8(1):104.

5. Giacometti F, Shirzad-Aski H, Ferreira S. Antimicrobials and Food-Related Stresses as Selective Factors for Antibiotic Resistance along the Farm to Fork Continuum. Antibiotics. 2021 Jun 4;10(6):671.

6. Onyeaka H, Ghosh S, Obileke K, Miri T, Odeyemi OA, Nwaiwu O, et al. Preventing chemical contaminants in food: Challenges and prospects for safe and sustainable food production. Food Control. 2024 Jan;155:110040.

7. Sun Y, Zhao X, Ma Y, Guan H, Liang H, Wang D. Inhibitory effect of modified atmosphere packaging on Escherichia coli O157:H7 in fresh-cut cucumbers (Cucumis sativus L.) and effectively maintain quality during storage. Food Chem. 2022 Feb;369:130969.

8. Bergwerff AA, Debast SB. Modernization of Control of Pathogenic Micro-Organisms in the Food-Chain Requires a Durable Role for Immunoaffinity-Based Detection Methodology—A Review. Foods. 2021 Apr 11;10(4):832.

9. Obahiagbon EG, Ogwu MC. Organic Food Preservatives: The Shift Towards Natural Alternatives and Sustainability in the Global South's Markets. In: Food Safety and Quality in the Global South. Singapore: Springer Nature Singapore; 2024. p. 299–329.

10. Abdulmumeen HA, Risikat AN, Sururah AR. Food: Its preservatives, additives and applications. IJCBS. 2012;1.

11. Svahn KS, Göransson U, El-Seedi H, Bohlin L, Larsson DGJ, Olsen B, et al. Antimicrobial activity of filamentous fungi isolated from highly antibiotic-contaminated river sediment. Infect Ecol Epidemiol. 2012 Jan 24;2(1):11591.

12. Conrado R, Gomes TC, Roque GSC, De Souza AO. Overview of Bioactive Fungal Secondary Metabolites: Cytotoxic and Antimicrobial Compounds. Antibiotics. 2022 Nov 11;11(11):1604.

13. Ferdes M, Ungureanu C, Radu N, Chirvase AA. Antimicrobial effect of Monascus purpureus red rice against some bacterial and fungal strains. N Biotechnol. 2009 Sep;25:S194.

14. Manimala MRA, Murugesan R. In vitro antioxidant and antimicrobial activity of carotenoid pigment extracted from Sporobolomyces sp. isolated from natural source. Journal of Applied and Natural Science. 2014 Dec 1;6(2):649–53.

15. El-Kholie EM, El Shaer MK, Abdelreheem MAT, Gharib MA. Detailed evaluation of a newly attained fungal pigment from *Monascus purpureus* in meat burgers. Int J Food Sci Nutr. 2012 Nov 7;63(7):860–5.

16. Leyva Salas M, Mounier J, Valence F, Coton M, Thierry A, Coton E. Antifungal Microbial Agents for Food Biopreservation—A Review. Microorganisms. 2017 Jul 8;5(3):37.

17. Segundo WOPF, de Oliveira RS, Lima RM, Santiago PAL, de Oliveira LA, Cortez ACA, et al. Antimicrobial Potential of Metabolites in Fungal Strains Isolated from a Polluted Stream: Annulohypoxylon stygium WL1B5 Produces Metabolites against Extended-Spectrum Beta-Lactamase-Positive Escherichia coli. Antibiotics. 2022 Dec 24;12(1):27.

18. Azhdari S, Moradi M. Application of antimicrobial coating based on carboxymethyl cellulose and natamycin in active packaging of cheese. Int J Biol Macromol. 2022 Jun;209:2042–9.

19. Singhvi MS, Zinjarde SS. Production of pharmaceutically important genistein and daidzein from soybean flour extract by using β-glucosidase derived from Penicillium janthinellum NCIM 1171. Process Biochemistry. 2020 Oct;97:183–90.

20. Abo Nouh FA, Gezaf SA, Abdel-Azeem AM. Recent Advances in Fungal Antimicrobial Molecules. In 2021. p. 177–203.

21. Abdel-Azeem AM, Yadav AN, Yadav N, Sharma M. Industrially Important Fungi for Sustainable Development. Abdel-Azeem AM, Yadav AN, Yadav N, Sharma M, editors. Vol. 2. Cham: Springer International Publishing; 2021.

22. Pimentel MR, Molina G, Dionísio AP, Maróstica Junior MR, Pastore GM. The Use of Endophytes to Obtain Bioactive Compounds and Their Application in Biotransformation Process. Biotechnol Res Int. 2011 Sep 26;2011:1–11.

23. Xing YM, Chen J, Cui JL, Chen XM, Guo SX. Antimicrobial Activity and Biodiversity of Endophytic Fungi in Dendrobium devonianum and Dendrobium thyrsiflorum from Vietman. Curr Microbiol. 2011 Apr 22;62(4):1218–24.

24. Durães F, Szemerédi N, Kumla D, Pinto M, Kijjoa A, Spengler G, et al. Metabolites from Marine-Derived Fungi as Potential Antimicrobial Adjuvants. Mar Drugs. 2021 Aug 25;19(9):475.

25. Zhang XY, Zhang Y, Xu XY, Qi SH. Diverse Deep-Sea Fungi from the South China Sea and Their Antimicrobial Activity. Curr Microbiol. 2013 Nov 5;67(5):525–30.

26. Wang YT, Xue YR, Liu CH. A Brief Review of Bioactive Metabolites Derived from Deep-Sea Fungi. Mar Drugs. 2015 Jul 23;13(8):4594–616.

27. Muccilli S, Restuccia C. Bioprotective Role of Yeasts. Microorganisms. 2015 Oct 10;3(4):588–611.

28. Gu S, Li J, Chen B, Sun T, Liu Q, Xiao D, et al. Metabolic engineering of the thermophilic filamentous fungus Myceliophthora thermophila to produce fumaric acid. Biotechnol Biofuels. 2018 Dec 3;11(1):323.

29. Hajji-Hedfi L, Wannassi T, El-Maradny YA. Fungal endophytes: Insight into evolution, classification, and ecological functions in plants. Microbial Biosystems. 2025 Mar 1;10(1):51–67.

30. Janisiewicz WJ, Korsten L. BIOLOGICAL CONTROL OF POSTHARVEST DISEASES OF FRUITS. Annu Rev Phytopathol. 2002 Sep;40(1):411–41.

31. Chanchaichaovivat A, Panijpan B, Ruenwongsa P. Putative modes of action of Pichia guilliermondii strain R13 in controlling chilli anthracnose after harvest. Biological Control. 2008 Nov;47(2):207–15.

32. Campos-Martínez A, Velázquez-del Valle MG, Flores-Moctezuma HE, Suárez-Rodríguez R, Ramírez-Trujillo JA, Hernández-Lauzardo AN. Antagonistic yeasts with potential to control Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. and Colletotrichum acutatum J.H. Simmonds on avocado fruits. Crop Protection. 2016 Nov;89:101–4.

33. Qin X, Xiao H, Xue C, Yu Z, Yang R, Cai Z, et al. Biocontrol of gray mold in grapes with the yeast Hanseniaspora uvarum alone and in combination with salicylic acid or sodium bicarbonate. Postharvest Biol Technol. 2015 Feb;100:160–7.

34. Li W, Zhang H, Li P, Apaliya MT, Yang Q, Peng Y, et al. Biocontrol of postharvest green mold of oranges by Hanseniaspora uvarum Y3 in combination with phosphatidylcholine. Biological Control. 2016 Dec;103:30–8.

35. Núñez F, Lara MS, Peromingo B, Delgado J, Sánchez-Montero L, Andrade MJ. Selection and evaluation of Debaryomyces hansenii isolates as potential bioprotective agents against toxigenic penicillia in dry-fermented sausages. Food Microbiol. 2015 Apr;46:114–20.

36. Andrade MJ, Thorsen L, Rodríguez A, Córdoba JJ, Jespersen L. Inhibition of ochratoxigenic moulds by Debaryomyces hansenii strains for biopreservation of dry-cured meat products. Int J Food Microbiol. 2014 Jan;170:70–7.

37. Banjara N, Suhr MJ, Hallen-Adams HE. Diversity of Yeast and Mold Species from a Variety of Cheese Types. Curr Microbiol. 2015 Jun 19;70(6):792–800.

38. Alía A, Andrade MJ, Rodríguez A, Reyes-Prieto M, Bernáldez V, Córdoba JJ. Identification and control of moulds responsible for black spot spoilage in dry-cured ham. Meat Sci. 2016 Dec;122:16–24.

39. Hammes WP, Knauf HJ. Starters in the processing of meat products. Meat Sci. 1994 Jan;36(1–2):155–68.

40. Delgado J, Acosta R, Rodríguez-Martín A,

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1738

Bermúdez E, Núñez F, Asensio MA. Growth inhibition and stability of PgAFP from Penicillium chrysogenum against fungi common on dry-ripened meat products. Int J Food Microbiol. 2015 Jul;205:23–9.

41. Bernáldez V, Córdoba JJ, Rodríguez M, Cordero M, Polo L, Rodríguez A. Effect of Penicillium nalgiovense as protective culture in processing of dry-fermented sausage "salchichón." Food Control. 2013 Jul;32(1):69–76.

42. Sebastian J, Dominguez KV, Brar SK, Rouissi T. Fumaric acid production using alternate fermentation mode by immobilized Rhizopus oryzae-a greener production strategy. Chemosphere. 2021 Oct;281:130858.

43. Falade AO, Adewole KE, Ekundayo TC. Aptitude of endophytic microbes for production of novel biocontrol agents and industrial enzymes towards agro-industrial sustainability. Beni Suef Univ J Basic Appl Sci. 2021 Dec 7;10(1):61.

44. Guzmán-Guzmán P, Kumar A, de los Santos-Villalobos S, Parra-Cota FI, Orozco-Mosqueda Ma del C, Fadiji AE, et al. Trichoderma Species: Our Best Fungal Allies in the Biocontrol of Plant Diseases—A Review. Plants. 2023 Jan 17;12(3):432.

45. Delgado J, Owens RA, Doyle S, Asensio MA, Núñez F. Manuscript title: antifungal proteins from moulds: analytical tools and potential application to dry-ripened foods. Appl Microbiol Biotechnol. 2016 Aug 9;100(16):6991–7000.

46. Delgado J, Peromingo B, Núñez F, Asensio MA. Use of molds and their antifungal proteins for biocontrol of toxigenic molds on dry-ripened cheese and meats. Curr Opin Food Sci. 2016 Oct;11:40–5.

47. Ferraz LP, Cunha T da, da Silva AC, Kupper KC. Biocontrol ability and putative mode of action of yeasts against Geotrichum citri-aurantii in citrus fruit. Microbiol Res. 2016 Jul;188–189:72–9.

48. Parafati L, Vitale A, Restuccia C, Cirvilleri G. Biocontrol ability and action mechanism of food-isolated yeast strains against Botrytis cinerea causing post-harvest bunch rot of table grape. Food Microbiol. 2015 May;47:85–92.

49. Rautenbach M, Troskie AM, Vosloo JA. Antifungal peptides: To be or not to be membrane active. Biochimie. 2016 Nov;130:132–45.

50. Falardeau J, Wise C, Novitsky L, Avis TJ. Ecological and Mechanistic Insights Into the Direct and Indirect Antimicrobial Properties of Bacillus subtilis Lipopeptides on Plant Pathogens. J Chem Ecol. 2013 Jul 16;39(7):869–78.

51. Di Francesco A, Martini C, Mari M. Biological control of postharvest diseases by microbial antagonists: how many mechanisms of action? Eur J Plant Pathol. 2016 Aug 21;145(4):711–7.

52. Sharma RR, Singh D, Singh R. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological Control. 2009 Sep;50(3):205–21.

53. Nally MC, Pesce VM, Maturano YP, Rodriguez Assaf LA, Toro ME, Castellanos de Figueroa LI, et al. Antifungal modes of action of Saccharomyces and other biocontrol yeasts against fungi isolated from sour and grey rots. Int J Food Microbiol. 2015 Jul;204:91–100.

54. Hua SST, Beck JJ, Sarreal SBL, Gee W. The major volatile compound 2-phenylethanol from the biocontrol yeast, Pichia anomala, inhibits growth and expression of aflatoxin biosynthetic genes of Aspergillus flavus. Mycotoxin Res. 2014 May 7;30(2):71–8.

55. Savoia D. Plant-Derived Antimicrobial Compounds: Alternatives to Antibiotics. Future Microbiol. 2012 Aug 23;7(8):979–90.

56. Kumar A, Asthana M, Gupta A, Nigam D, Mahajan S. Secondary Metabolism and Antimicrobial Metabolites of Penicillium. In: New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier; 2018. p. 47–68.

57. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. Curr Med Chem. 2014 Nov 26;22(1):132–49.

58. Ilica RA, Kloetzer L, Galaction AI, Caşcaval D. Fumaric acid: production and separation. Biotechnol Lett. 2019 Jan 1;41(1):47–57.

59. Huang W, Wang Y, Tian W, Cui X, Tu P, Li J, et

al. Biosynthesis Investigations of Terpenoid, Alkaloid, and Flavonoid Antimicrobial Agents Derived from Medicinal Plants. Antibiotics. 2022 Oct 9;11(10):1380.

60. Morath SU, Hung R, Bennett JW. Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. Fungal Biol Rev. 2012 Oct;26(2–3):73–83.

61. Atindehou MM, Adjovi YCS, Dagbozounkou GMG, Fanou LA. Identification and Characterization of the Antibacterial Activity of Fungal Metabolites on Contaminants Responsible for Foodborne Diseases [Internet]. American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS); 2020. Available from: http://asrjetsjournal.org/

62. Frisvad JC, Møller LLH, Larsen TO, Kumar R, Arnau J. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei. Appl Microbiol Biotechnol. 2018 Nov 6;102(22):9481–515.

63. Gmoser R, Ferreira JA, Lennartsson PR, Taherzadeh MJ. Filamentous ascomycetes fungi as a source of natural pigments. Fungal Biol Biotechnol. 2017 Dec 10;4(1):4.

64. Keller NP, Turner G, Bennett JW. Fungal secondary metabolism — from biochemistry to genomics. Nat Rev Microbiol. 2005 Dec;3(12):937–47.

65. Research GV. Natural Food Preservatives Market Size, Share Report, 2030 [Internet]. 2023. Available from: https://www.grandviewresearch.com/industry-analysis/natural-food-preservatives-market-report

66. Research GV. Food Preservatives Market Size And Share Report, 2030 [Internet]. 2023. Available from: https://www.grandviewresearch.com/industry-analysis/food-preservatives-market/.

67. Frisvad JC, Møller LLH, Larsen TO, Kumar R, Arnau J. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei. Appl Microbiol Biotechnol. 2018 Nov 6;102(22):9481–515.

68. Zhang Q, Jia M, Li H, Shi T, Xu Y, Zhao T, et al. Epipolythiodioxopiperazines: From Chemical Architectures to Biological Activities and Ecological Significance—A Comprehensive Review. Fermentation. 2025 Dec 17;11(12):700.

69. Wang LJ, Ma ZY, Wang XL, Wang KL, Zhang T, Han RY, et al. New Chaetoglobosins with Fungicidal Activity from Chaetomium sp. UJN-EF006 Endophytic in Vaccinium bracteatum. Journal of Fungi. 2025 Jul 7;11(7):511.

70. Huber EM. Epipolythiodioxopiperazine-Based Natural Products: Building Blocks, Biosynthesis and Biological Activities. ChemBioChem. 2022 Dec 5;23(23).

71. Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipič M, et al. Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources (Revision 1)1. EFSA Journal. 2021 Mar;19(3).

72. Stoev SD. Food Security and Foodborne Mycotoxicoses—What Should Be the Adequate Risk Assessment and Regulation? Microorganisms. 2024 Mar 14;12(3):580.

73. van Egmond HP, Schothorst RC, Jonker MA. Regulations relating to mycotoxins in food. Anal Bioanal Chem. 2007 Sep 17;389(1):147–57.

74. Davidson PM, Critzer FJ, Taylor TM. Naturally Occurring Antimicrobials for Minimally Processed Foods. Annu Rev Food Sci Technol. 2013 Feb 28;4(1):163–90.

75. Mishra B, Mishra AK, Kumar S, Mandal SK, NSV L, Kumar V, et al. Antifungal Metabolites as Food Bio-Preservative: Innovation, Outlook, and Challenges. Metabolites. 2021 Dec 23;12(1):12.

76. Yuan Q, Yang P, Liu Y, Tabl KM, Guo M, Zhang J, et al. Iturin and fengycin lipopeptides inhibit pathogenic *Fusarium* by targeting multiple components of the cell membrane and their regulative effects in wheat. J Integr Plant Biol. 2025 Aug 21;67(8):2184–97.

77. Resende LM, de Oliveira Mello É, Zeraik AE, Oliveira APBF, Souza TAM, Taveira GB, et al. Defensin-like peptides from Capsicum chinense induce increased ROS, loss of

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1738

mitochondrial functionality, and reduced growth of the fungus Colletotrichum scovillei. Pest Manag Sci. 2024 Jul 21;80(7):3567–77.

78. Marik T, Tyagi C, Balázs D, Urbán P, Szepesi Á, Bakacsy L, et al. Structural Diversity and Bioactivities of Peptaibol Compounds From the Longibrachiatum Clade of the Filamentous Fungal Genus Trichoderma. Front Microbiol. 2019 Jun 26;10.

79. Víglaš J, Dobiasová S, Viktorová J, Ruml T, Repiská V, Olejníková P, et al. Peptaibol-Containing Extracts of Trichoderma atroviride and the Fight against Resistant Microorganisms and Cancer Cells. Molecules. 2021 Oct 4;26(19):6025.

80. Muñoz IJ, Schilman PE, Barrozo RB. Impact of alkaloids in food consumption, metabolism and survival in a blood-sucking insect. Sci Rep. 2020 Jun 10;10(1):9443.

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The Dance of Life and Death: A Review of Programmed and Non-Programmed Cell Death

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ABSTRACT:

Background: *Multicellular organisms are composed of individual cells acting in tightly regulated cooperation. The health and homeostasis of multicellular organisms depend on the tight balance between cell proliferation and cell death. Cell death was divided into three main types according to morphological changes that occur: Type I cell death (apoptosis), Type II cell death (autophagy), and Type III cell death (necrosis). Recently, cell death types can be categorized into programmed and non-programmed cell death based on their signal dependency.*

Objective of the study: *This review will focus primarily on programmed regulated cell death. It will also discuss non-programmed cell death and different signaling pathways.*

KEYWORDS:

Cell death, apoptosis, necrosis, autophagy.

1. Introduction

Cell proliferation is an important intracellular process in which nearly all of the billions of cells in our body undergo in a strictly regulated manner. It allows cell populations to increase through cell growth and division. (1)

Proper regulation of the cell cycle is crucial for ensuring effective DNA repair and preventing the transmission of damaged genetic material. When cellular damage becomes irreparable, programmed cell death eliminates defective cells. Thus, multicellular organisms depend on a precise balance between cell proliferation and Death to preserve tissue integrity and overall homeostasis. (2)

While cell death is a normal physiological process, with millions of cells dying and being replaced every second, dysregulation of cell death can contribute to diseases like cancer (where cell death is suppressed) and neurodegenerative disorders (where cell death is excessive). Therefore, cell death plays a critical role in both normal function and disease pathology. (3)

2. Historical background and Classifications

Cell death historically was divided into three main types according to morphological changes that occur: Type I cell death (apoptosis), Type II cell death (autophagy), and Type III cell death (necrosis). All three can be executed through distinct, and sometimes overlapping, signaling pathways engaged in response to specific stimuli. (4) (Figure 1)

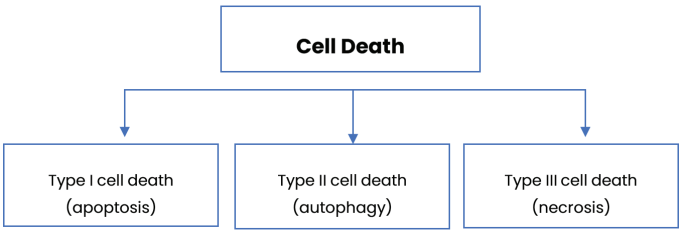

Figure 1: Classification of cell death based on its morphological dependency. (4)

The study of regulated cell death (RCD) began in 1842 when Karl Vogt observed dying cells in toads. However, research into RCD didn't really take off until the term "apoptosis" was introduced in 1972 by John Kerr, Andrew Wyllie, and Alastair Currie. (5)

Cell death can be classified into two main categories: programmed cell death (PCD) and

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non-programmed cell death. PCD is controlled by specific signals within the cell, while non-programmed cell death is caused by unexpected damage. (6) (Figure 2)

Accidental cell death is uncontrolled and occurs due to severe damage, such as from chemicals or physical injury. Regulated cell death, on the other hand, is a controlled process that can occur physiologically or in response to different types of stress. (7)

Given the morphological characteristics and molecular mechanisms, PCD can be further categorized into apoptotic cell death and nonapoptotic cell death. Apoptosis retains cell membrane integrity and occurs in a caspase-dependent manner. By contrast, nonapoptotic cell death is mostly characterized by caspase independence. (7) The recent classification is based on both morphological and signal dependency of cell death. On the other hand, the necrotic cell death can be further divided into a regulated and a non-regulated form of cell death. (8, 9) (Figure 3)

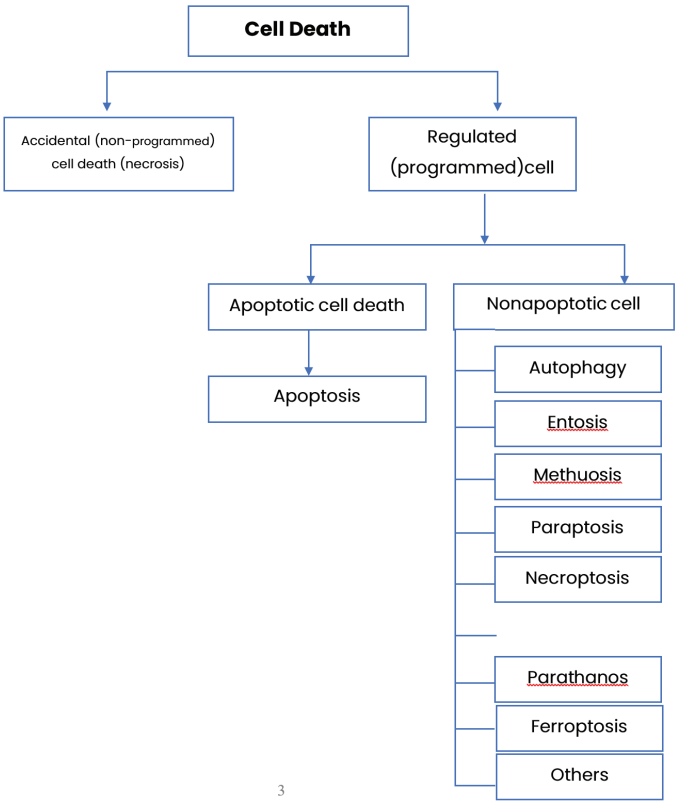

Figure 2:Classification of cell death based on its signal dependency. (10)

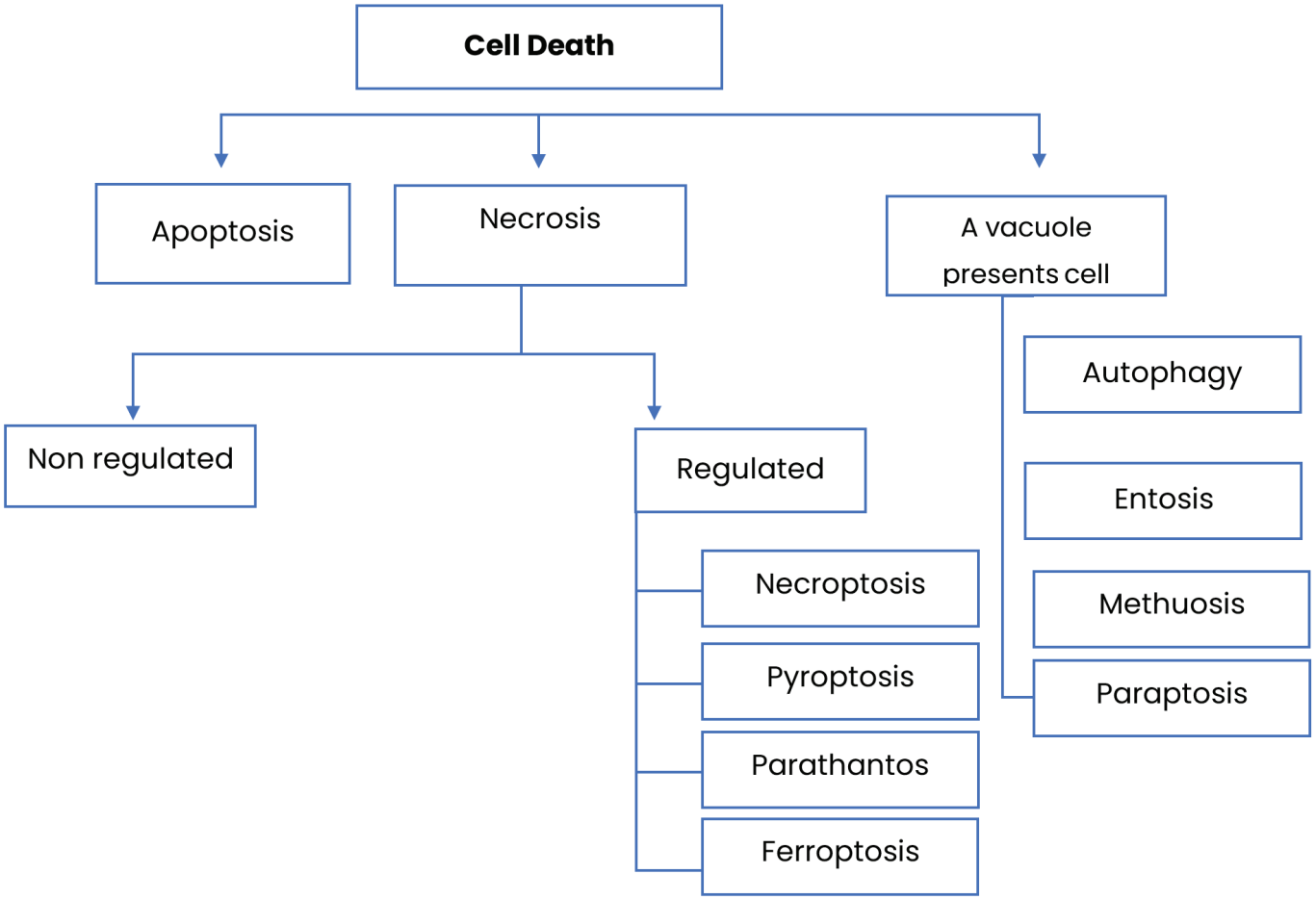

Figure 3: Classification of cell death based on its morphological and signal dependency. (9)

3. Apoptosis

Apoptosis, derived from Greek words meaning "falling off" like petals from a flower or leaves from a tree, refers to a genetically programmed form of cell death. This controlled process involves a specific sequence of cellular events and has a distinct microscopic appearance, differentiating it from other cell death mechanisms. (11)

Apoptosis is a normal biological process. All cells can undergo apoptosis, which is a way to remove unnecessary, damaged, or unwanted cells without harming nearby cells. The human body has around 100 trillion cells. Every day, billions of new cells are created through mitosis, and a similar number die through apoptosis to keep tissues healthy. (12)

Also, toxic insults, in particular by agents that damage DNA, can induce apoptosis. The low doses of stimuli can induce apoptosis, but these same stimuli can result in necrosis at higher doses. (13, 14). During apoptosis, cells maintain their outer membrane integrity and metabolic activity (to some degree) as the process proceeds to completion, which—in vivo— allows for the rapid clearance. (15)

To maintain genomic integrity, cells rigorously monitor their DNA and the machinery involved in mitosis. When damage or errors are detected at key checkpoints—such as the DNA damage checkpoint or the mitotic spindle checkpoint—the cell cycle is halted, and apoptosis is activated to prevent the propagation of defective cells. Failure of these checkpoints or the apoptotic machinery allows abnormal cells to continue dividing, contributing to tumor development. Beyond cancer, dysregulation of apoptosis has broader pathological implications: insufficient apoptosis is associated with cancer, viral infections, and autoimmune disorders, whereas excessive or premature apoptosis underlies many neurodegenerative diseases, including Alzheimer's and Parkinson's disease, as well as certain cardiovascular conditions and infections such as AIDS.(16)

Importance of apoptosis

During embryonic development, the signaling molecule Sonic hedgehog (Shh) is released from the notochord in a gradient to guide the patterning of cells in the neural tube. Cells in the neural tube express Patched 1 (Ptc1), the receptor for Shh. When Shh binds to Ptc1, it promotes the survival of the target cells. However, in the absence of Shh, cells that express Ptc1 undergo apoptosis. (17, 18)

In mammalian embryos, apoptosis occurs during cavitation and plays an essential role in shaping the developing embryo. (19) It is now widely recognized that appropriate apoptosis is crucial for normal development, as defects in the molecules that regulate apoptosis can lead to embryonic Death or severe malformations. (20) In contrast, other forms of cell death are not necessary during the developmental process. (21)

During human development, structures like the branchial arches, embryonic tails, and webbed fingers disappear before birth due to programmed cell death. (22) In a developing fetus, the hand initially appears webbed. Extra cells die through apoptosis to form fingers. Similarly, in a developing nervous system, more than half of the nerve cells die soon after they are created. (23)

1. Tissue homeostasis: The number of cells is kept relatively constant as a result of a balance between cell division and cell death. When cells are damaged or nonfunctioning, they must be replaced. Generation of new cells must be offset by cell death to maintain a stable baseline population. (23)

2. Aging can also be considered to be a physiological cell death process. For most animals, lifespan is genetically predetermined. (12)

3. It plays an important role in the immune system by removing self-reactive T cells through negative selection. Lymphocytes can induce apoptosis in target cells. (24)

4. Apoptosis happens as a cellular response to growth factors and hormones. Withdrawal of hormones results in atrophy of the hormone-dependent tissue, and apoptosis has been found to be responsible for this phenomenon in the prostate, adrenal cortex, endometrium, and mammary glands. (25, 26)

5. It may also be used to minimize the risk in cells frequently exposed to mutagenic chemicals or radiation. Diverse chemotherapeutic agents kill sensitive cells by apoptosis. (27)

6. Apoptosis prevents malignant transformation, whereas abnormal apoptosis can predispose to cancer. (28)

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2.2 Morphological and molecular events in apoptosis

Apoptosis progresses through several stages: The earliest stage recognizable by electron microscopy, apoptotic cell death is characterized by a distinct nuclear behavior involving progressive chromatin margination and compaction. Chromatin margination is first limited to thin electron-dense areas underlying the nuclear envelope. These electron-dense areas are then organized in cap-shaped compact structures. (29) These compacting areas of chromatin are rearranged due to DNA fragmentation. (30)(Figure 4)

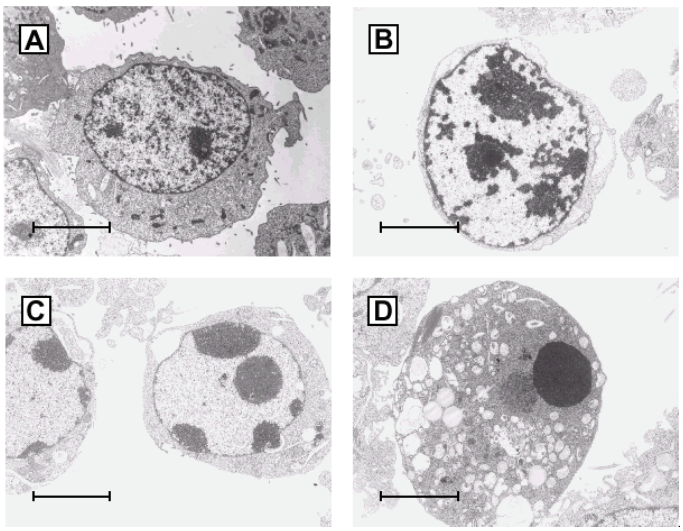

Figure 4: Electron microscopy of uninfected (A) and reovirus strain-infected (B, C, and D) A. Uninfected cells B. Initial condensation of chromatin. Margination of chromatin at the nuclear membrane. Complete condensation of the nucleus. (31)

During chromatin margination, plasma membrane blebs appear on the cell surface, and specialized surface elements such as microvilli have disappeared. (32) The membrane blebbing causes the nucleus to break (karyorrhexis). This process represents another morphological hallmark of in vitro and in vivo apoptosis, and it is controlled by actomyosin contraction. (33)

During apoptosis, nuclear fragmentation becomes evident through nuclear splitting and the frequent appearance of micronuclei, while cytoplasmic condensation leads to the breakdown of cell–cell interactions. Classically, it is understood that microtubules and intermediate filaments become disorganized at the onset of the execution phase, whereas the actin cytoskeleton drives the characteristic remodeling of the cell. In later stages, microtubules reorganize to form the apoptotic microtubule network, a specialized structure that supports apoptotic cell morphology, preserves plasma membrane integrity, and contributes to the orderly dispersion of cellular and nuclear fragments. (34, 35)

Finally, the splitting of the cellular content into distinct membrane-enclosed vesicles, termed apoptotic bodies, containing portions of the fragmented nucleus and an array of intact organelles. (32) The apoptotic cell removal by phagocytes is very rapid; therefore, the presence of apoptotic bodies (ApoBDs) is very limited in vivo. (36)

One of the critical properties of the fragmentation process is that the cytoplasm and organelles are essentially repackaged without the leakage of any potentially harmful cellular components into the extracellular space or bloodstream. (37)

Finally, the release of cell surface markers (phosphatidylserine) from the cell membrane facilitates phagocytosis by cells such as macrophages and parenchymal cells for further degradation, thereby preventing secondary necrosis. (32)

2.3 Apoptotic Bodies

Dying cells produce vesicular apoptotic bodies that are variable in size, structure, and composition. Apoptotic bodies appear after the disassembly of an apoptotic cell into subcellular fragments. The formation of ApoBDs is an important process downstream of apoptotic cell death, considered a hallmark of apoptosis. (38)

In fact, they may contain a wide variety of cellular components: micronuclei, chromatin remnants, cytosol portions, degraded proteins, DNA fragments, or even intact organelles. Reports describe that an apoptotic body contains a large amount of RNA.(39)

Apoptosis does not trigger an inflammatory reaction because apoptotic cells retain their intracellular contents and do not release them into the surrounding tissue; their apoptotic bodies are rapidly phagocytosed by neighboring cells—preventing secondary necrosis—and the cells responsible for engulfing these fragments do not produce inflammatory cytokines. (40)

Furthermore, the phagocytosis of apoptotic cells and apoptotic bodies is precisely coordinated

during the early stages of apoptosis, and a membrane lipid rearrangement occurs, which involves phosphatidylserine (PS) translocation from the inner to the outer leaflet. (41)Thus, PS, a phospholipid normally localized in the inner leaflet of the plasma membrane, is remodeled and is exposed onto the outer leaflet, which is believed to act as an "eat me" signal that facilitates the recognition and uptake of apoptotic cells by phagocytes. (42)

The formation of ApoBDs can promote efficient removal of cell debris by means of the surrounding phagocytes. Additionally, ApoBDs can harbor biomolecules, including microRNA and DNA, to regulate intercellular communication. (43)

Depending on the mechanism used by a particular cell type undergoing apoptotic cell disassembly, a different quantity and quality of ApoBDs will be generated. However, it is not yet clear why different cell types need to disassemble differently and the functional significance of such diversity. In autoimmune diseases, a defect in the clearance of ApoBD formation may contribute to the development of autoimmune disease. (44)

2.4 The clearance of dying cells (efferocytosis)

Cellular turnover is continuous in adult tissues, and rapid clearance of dying cells is essential to avoid inflammation and immune activation. This process is carried out by professional phagocytes—such as Kupffer cells, alveolar macrophages, and microglia—as well as non-professional phagocytes like epithelial cells and fibroblasts. (45)

Efferocytosis is an active, highly regulated process designed to efficiently eliminate dying cells and promote immunological tolerance. It involves a series of coordinated steps in which dying cells release "find-me" signals to attract phagocytes, which then recognize and bind to "eat-me" signals on the cell surface. The phagocyte subsequently engulfs the cellular corpse, processes and degrades it, and finally generates an appropriate immune response to the ingested material. (40)(Figure 5)

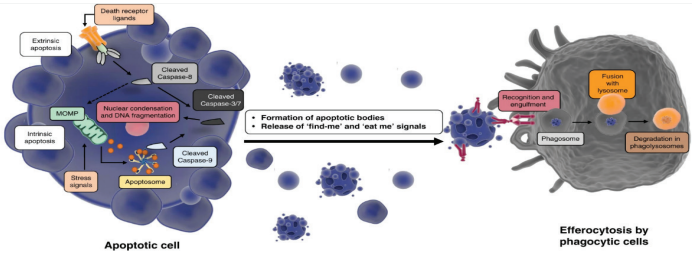

Figure 5: Clearance of dying cells. (46)

Recruitment of phagocytes to sites of cell death occurs before the completion of apoptosis, indicating that one of the first acts of a dying cell is to prepare for its own elimination. (47) During this process, apoptotic cells release 'find-me' signals, distinct molecules that establish a chemotactic gradient to attract phagocytic cells. (48)

Find-me' signals are often released in an active, caspase-dependent manner, yet these molecules are also released (in greater quantities) during other forms of cell death, such as necrosis or necroptosis. (49)

Just as dying cells must recruit phagocytes, they must also transform themselves into targets for engulfment, displaying distinct signals that differentiate them from viable cells. Thus, the simultaneous presence of 'don't eat me' signals, such as CD31, CD47, and CD61, on viable cells, may negatively regulate phagocytosis. (15)

Acidic proteases and nucleases in mature phagolysosomal compartments degrade dying cells into their basic cellular components, including fats, sterols, peptides, and nucleotides. (50)

2.5 Apoptosis pathways

Apoptosis is a multi-pathway mode of cell death that leads to cellular destruction, with the nucleus playing a crucial role in this process. (51)

Apoptosis requires energy input and thus is an active process. In more detail, apoptosis is initiated by either internal or external stimuli and mediated via two distinct pathways: the intrinsic pathway (mitochondria-mediated pathway) and the extrinsic pathway (death receptor-mediated pathway). (52)

Its induction is dependent on the presence of a wild-type p53 gene, which is a tumor suppressor protein that plays a role when DNA is damaged. P53 is a key element in apoptosis induction in cells in response to DNA damage. It becomes phosphorylated, stabilized, and activated. (53)

Cellular stress, including that induced by chemotherapy or irradiation, activates p53 either directly or indirectly. P53 can also move directly to the mitochondria, where it exerts proapoptotic activity. (54) Its transactivation can contribute to the induction of apoptosis in some cell types, and a number of genes transactivated by p53

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have been implicated in the apoptosis response. (55, 56)

Many genes involved in cell cycle regulation are also involved in the regulation of apoptosis (e.g., c-myc, c-fos, c-jun, and p53). Thus, signals that promote proliferation can also promote apoptosis. If apoptosis is blocked by survival signals, an increase in cell numbers occurs, which can manifest in cancer. For example, c-myc plus bcl-2 leads to proliferation, and c-myc plus p53 leads to apoptosis. (54)

➢ **Intrinsic apoptotic pathway (Figure 6)**

Intrinsic apoptosis is a form of RCD initiated by a variety of microenvironmental perturbations, including, but not limited to, the following:(57)

- Growth factor withdrawal.
- DNA damage (chemotherapeutic drugs).
- Endoplasmic reticulum (ER) stress.
- Reactive oxygen species (ROS) overload.
- Microtubular alterations.
- Mitotic defects.

The key to the regulation and execution of intrinsic apoptosis lies in the BCL-2 family of proteins, which includes both proapoptotic (promote apoptosis) and antiapoptotic (inhibit apoptosis) members. The careful modulation of the balance between these two groups of BCL-2 proteins can largely determine cell fate decisions between life and Death. (52) Bcl-2 contains four so-called Bcl-2 homology domains (BH1–BH4), which are absolutely required for its survival functions. (58) (Table 1)

Table 1: Bcl2 family (58)

Genes	Role of apoptosis	Functions
Bcl2 (B-cell lymphoma-2)	Inhibits apoptosis	Prevent apoptosis by inactivating (Bax and Bak proteins).
Bcl-xl (B-cell lymphoma-xl)	Inhibits apoptosis	Prevent apoptosis by rendering mitochondrial pores impermeable.
BAX (Bcl2-associated X protein)	Promotes apoptosis	Oppose Bcl2 Forms pores in the outer mitochondrial membrane (OMM). Cytochrome c release.
BAK (Bcl-2 antagonist killer	Promotes apoptosis	Same as BAX
BAD (Bcl-2 associated death promoter)	Phosphorylated BAD Antiapoptotic	No interaction with bcl2
	DE-phosphorylated BAD proapoptotic	Inactivate BCL2 so Bax is activated
BID	proapoptotic	Insertion of BAX into OMM
(Bik, Bim) other Bcl-2 death promoters		

In physiological conditions, the proapoptotic Bcl-2 protein Bax is predominantly found in the cytosol of nonapoptotic cells and is commonly thought to translocate to mitochondria following an apoptotic stimulus, initiating mitochondrial targeting and outer-membrane permeabilization. In contrast, BAK constitutively resides at the OMM.(59)

In response to apoptotic stimuli, MOMP is mediated by BAX and BAK. BAX and BAK are the only BCL2 family members characterized so far in mammalian cells for their ability to form pores across the outer mitochondrial membrane (OMM) and possibly other intracellular membranes. (60)

The critical step for intrinsic apoptosis, which is irreversible, is mitochondrial outer membrane

permeabilization (MOMP). It is controlled by proapoptotic and antiapoptotic members of the BCL2. (61)

Active BAX and BAK have also been proposed to permeabilize ER membranes, especially in response to reticular stress, resulting in the cytosolic leak of Ca2+ ions from ER and consequent mitochondrial Ca2+ uptake. (62)

This process further leads to the release of proapoptotic proteins through the intermembrane space into the cytosol. The presence of cytochrome c in the cytosol binds Apaf-1 (apoptotic protease activating factor) and caspase 9 to form a complex called "apoptosome".(63)

Activated CASP9 can catalyze the proteolytic activation of CASP3 and CASP7, which are widely perceived as the enzymes responsible for cell demolition during intrinsic (and extrinsic) apoptosis in mammalian cells. (64)

XIAP(X-linked inhibitor of apoptosis protein) is the only IAP(Inhibitor of Apoptotic protein) protein family member that counteracts the apoptotic cascade by stably binding to and hence physically blocking caspases. (65) IAPs are negatively regulated by IAP-antagonist proteins such as Smac/Diablo.(66, 67)

➢ **Extrinsic apoptotic pathway (Figure 6)**

The extrinsic pathway of apoptosis is a process whereby cells initiate programmed cell death in response to external signals, such as those from neighboring cells or the immune system. (68)

The external program that stimulates apoptosis occurs via death receptors that are members of the tumor necrosis factor receptor (TNFR) gene superfamily. Individual members of this family recognize specific ligands, but not all members of the TNF family initiate cell death. Those who initiate cell death possess a homologous cytoplasmic sequence termed death domain(DD). (68) This death domain is essential for the transmission of the death signal from the cell surface to intracellular signaling pathways. (68)

The binding of the death domain /adapter protein to the receptor ligand complex allows the binding of an initiator caspase 8 or 10 through its death effector domain (DED) to form an activated complex called the death-inducing signaling complex (DISC). Furthermore, the binding and activation allow caspase 8 to relay the death signal to an execution caspase to bring about apoptosis. (69)

Insufficient activation of caspase-3 leads to caspase-8 cleaving BID to generate its activated form: (tBID). TBID stimulates the intrinsic apoptotic pathway by binding directly to Bax/Bak, inducing MOMP.(70)

➢ **Granzyme-mediated apoptosis**

Cytotoxic lymphoid cells (predominantly NK cells and cytotoxic T cells) can induce cell death via death receptor ligands or the granzyme/perforin system. After recognition of transformed or infected cells, cytotoxic cells release secretory granules that contain perforin and granzyme B. These secreted factors are taken up by endocytosis and released into the cytosol by the perforin-dependent or -independent pathways. Once released to the cytosol, granzyme B cleaves caspases and Bid, activating apoptotic pathways. (71) (Figure 6)

The insertion of perforins into the target cell plasma membrane creates pores through which granzymes and toxins pass into the interior of the target cell. The granzymes are serine esterases that cleave and activate proteases. These enzymes have the capacity to induce DNA fragmentation and apoptosis of the target cells. (72)

2.6 Caspase family

Caspases are protease enzymes and key mediators of apoptosis, produced as inactive zymogens that are rapidly activated when needed. Fourteen caspases have been identified, with the major ones classified as initiators (caspase 2, 8, 9, 10), executioners (caspase-3, 6, 7), and inflammatory caspases (caspase 1, 4, 5). Additional members include caspase 11, involved in apoptosis and cytokine maturation during septic shock; caspase 12, linked to ER-stress-induced apoptosis; caspase 13, considered bovine-specific; and caspase 14, expressed mainly in embryonic tissues. (14, 73, 74)

Apoptosis proceeds through a caspase cascade in which initiator caspases, activated by the apoptosome (caspase 9), death receptors (caspase 8 and 10), or granzyme B (activating caspase 3 and 7), trigger downstream executioners. These caspases cleave nuclear

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and cytoplasmic substrates, activating endonucleases that fragment DNA—a hallmark of apoptosis—and promoting phosphatidylserine exposure on the cell surface. (75)

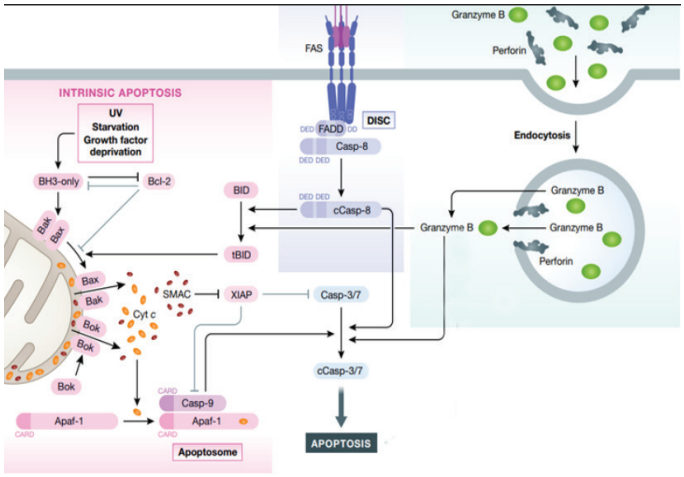

Figure 6: Apoptotic pathways and granzyme-mediated apoptosis. (70)

2.7 Anoikis

Anoikis is a programmed cell death occurring upon cell detachment from the correct extracellular matrix by disrupting integrin ligation. It is a critical mechanism in preventing dysplastic cell growth or attachment to an inappropriate matrix. Anoikis prevents detached epithelial cells from colonizing elsewhere and is thus essential for tissue homeostasis and development. (76)

It has become clear that the integrin-α6β4, a major component of hemidesmosomes, is able to transduce signals from the extracellular matrix to the interior of the cell, which critically modulates the organization of the cytoskeleton, proliferation, apoptosis, and differentiation. Maintenance of cell–cell contact is therefore important for preservation of normal epithelial structure and function. (77)

2.8 Methods of apoptosis detection

I. Morphological features

a. Light microscopy reveals the shrinking, rounding, and shedding of nuclei, chromatin morphology, and apoptotic bodies of the cells. (78)

b. Transmission electron microscopy: A common method used to assay the cellular state. It is used to detect vacuolations, chromatin condensation, and margination within the cell. Also, the cell nuclei fragmentations, apoptotic bodies, and changes in cell organelles. (78)

c. Image Cytometry: it uses a Cell Analysis Systems platform to quantify cell proliferation and apoptosis, with apoptosis measured as the percentage of epithelial cells showing characteristic apoptotic morphology under the light microscope. (79)

II. Biochemical characterization of apoptosis

a. DNA gel electrophoresis: This method is characterized by the formation of ladder-like DNA bands to detect apoptosis. (80) DNA laddering is a distinctive feature of DNA degraded by caspase-activated DNase. It can only be used for the semi-quantitative detection of apoptosis. It is also not suitable for detecting minor damage to the DNA.(81)

b. Real-time quantitative polymerase chain reaction (RT-qPCR): It is one of the most widely used methods of gene quantification. It is used to measure the mRNA expression of apoptosis-related genes. (82)

c. Analysis of mitochondrial membrane potential: Mitochondrial membrane potential, an early marker of mitochondrial apoptosis, is assessed using fluorescent cationic dyes, where high potential produces red fluorescence due to dye accumulation, and low potential yields green fluorescence as the dye fails to accumulate. (83)

III. Immunological characteristics of apoptosis

a. Western blotting: It is a laboratory technique used for the measurement of Cyto-C and the expression of apoptotic proteins. (84) The method involves using gel electrophoresis to separate the sample's proteins. The separated proteins are then exposed to an antibody specific to the target protein. The binding of the antibody is detected using a radioactive or chemical tag. This is one of the earliest detections of apoptosis. (85)

b. Immunolabeling technology: This refers to the antigen-antibody reaction of labeling antigens or antibodies with fluorescein, radioisotopes, enzymes, or electron-dense substances. (78)

c. Flow cytometry: This method is used to detect apoptotic cells after Annexin V/propidine iodine (PI) staining. Annexin V is used as a fluorescent probe to label the exposed

phosphatidylserine (PS) on the outer cell membrane. (86)This method is used to detect the level of apoptotic cells during apoptosis, as well as in necrosis. (78) Intracellular Ca2+ is labeled using fluorescent probes and then qualitatively and quantitatively analyzed by flow cytometry. (87)

d. Fluorescent ELISA: It is an immunological assay commonly used to measure the levels of apoptotic proteins in biological samples. An ELISA is a highly sensitive, quantitative method for detecting apoptosis. It is suitable for the method of detecting apoptosis at different stages. (86) The activity of caspases and Cyto-C decreases during the late stage of apoptosis. (78)

4. Necrosis

4.1 Non-regulated necrosis

Necrosis is an irreversible and unregulated form of cell death that arises from pathological injury. It is characterised by swelling of organelles, rupture of the plasma membrane, and the release of intracellular contents, resulting in tissue damage and inflammation. (88)

Necrosis occurs when cells are exposed to severe external insults and is almost always accompanied by an inflammatory response. Unlike apoptosis, it does not involve caspase activation and is not part of normal developmental processes. (89)

The main causes of necrosis include hypoxia, such as that resulting from ischaemia, shock, or respiratory failure; physical agents like trauma, extreme temperatures, radiation, electrical injury, or chemical burns; toxic chemical exposures, including poisons and harmful drugs; and biological agents such as bacteria, viruses, and fungi. (88)

➢ **Cellular mechanisms**

There are three events leading to cell rupture in necrosis:(90)(Figure 7)

1. Loss of selective plasma membrane permeability.
2. Loss of calcium homeostasis.
3. Failure of membrane ion pumps.

These three are interlinked. Any one event often leads on to another, allowing fluid and ions into the cell. This process spirals out of control: a vicious circle occurs, and the cell swells up until it finally ruptures. (90)

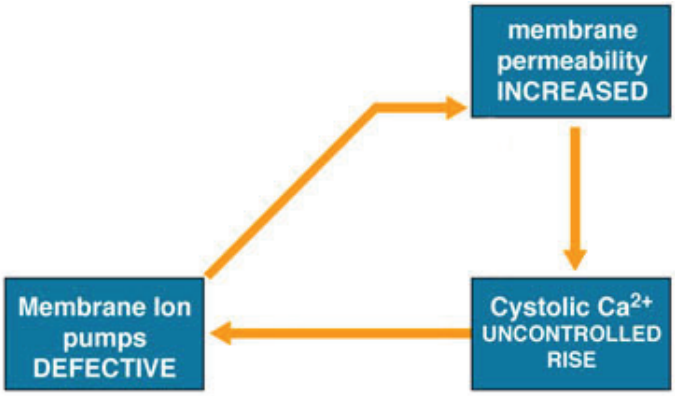

Figure 7: Events of necrosis

In necrosis, there is an important relation between ATP levels and membrane permeability. In injured cells, there is a lack of oxygen supply, leading to decreased ATP production. (91)

This lack of ATP results in the failure of the energy-dependent sodium pump in the plasma membrane, causing an influx of calcium and water, leading to cell swelling and detachment of ribosomes from the endoplasmic reticulum. Increased cytosolic calcium and oxidative stress lead to mitochondrial damage. Cytosolic calcium can also lead to the activation of several cytosolic enzymes, including phospholipases, which can attack lipids in the plasma membrane, and proteases, which lead to the breakdown of proteins. (91)

Loss of cell membrane integrity as a result of exposure to a noxious stimulus allows extracellular ions to move inside the cell, calcium ions accumulate, and flux leads to calcium matrix density formation in the mitochondria. (92)

The disruption of the lysosomal membrane leads to the release of proteolytic enzymes into the cell, such as proteases, RNAases, DNAases, and phosphatases. These, when activated in the cytosol, lead to damage to DNA, RNA, and proteins. These enzymes cause the digestion of the cellular components, causing cell destruction. These mechanisms lead to disruption of the plasma membrane, leading to the spilling of intracellular contents into the surrounding tissue. (90)

Cells undergoing necrosis release Damage-associated molecular patterns (DAMPs). DAMPs

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are molecules within living cells that are released when cell membranes are ruptured. Although DAMPs have physiological functions inside the cell, once DAMPs are released extracellularly, they elicit various biological responses, including inflammation, proliferation, tissue damage, and tissue repair, in a context-dependent manner. (93)

➢ **Primary and secondary necrosis (Figure 8)**

Unregulated cell death (usually termed "accidental" or "primary necrosis") is the response to extreme exogenous stress (e.g., burns, frost bites, strong mechanical stress), which prompts an immediate rupture of the cell membrane and release of intracellular molecules. (94)

In apoptosis, when apoptotic cells are not cleared in a timely manner, they progress to late apoptosis, which is characterized by a disrupted cell membrane and loss of cell integrity. This autolysis was termed "secondary necrosis" (95)

Progression of apoptosis to secondary necrosis occurs in vivo in physiological situations where apoptotic cells are shed into areas without phagocytes (e.g., in the gut or airways lumen) and the complete apoptotic program can fully progress. Secondary necrosis can also be observed in tissues with excessive apoptosis, which overwhelms the clearance capacity of phagocytes or when the clearance capacity itself is reduced. (96)

In the case of necrosis, intracellular damage-associated molecular patterns (DAMPs) leak out of the damaged cell, activating innate immune cells. Interestingly, microparticles released from apoptotic cells (often referred to as "apoptotic bodies" when they lose their cell membrane integrity and are quickly removed) result in a limited secretion of DAMPs into the microenvironment, which attracts phagocytes for efficient elimination. (96)

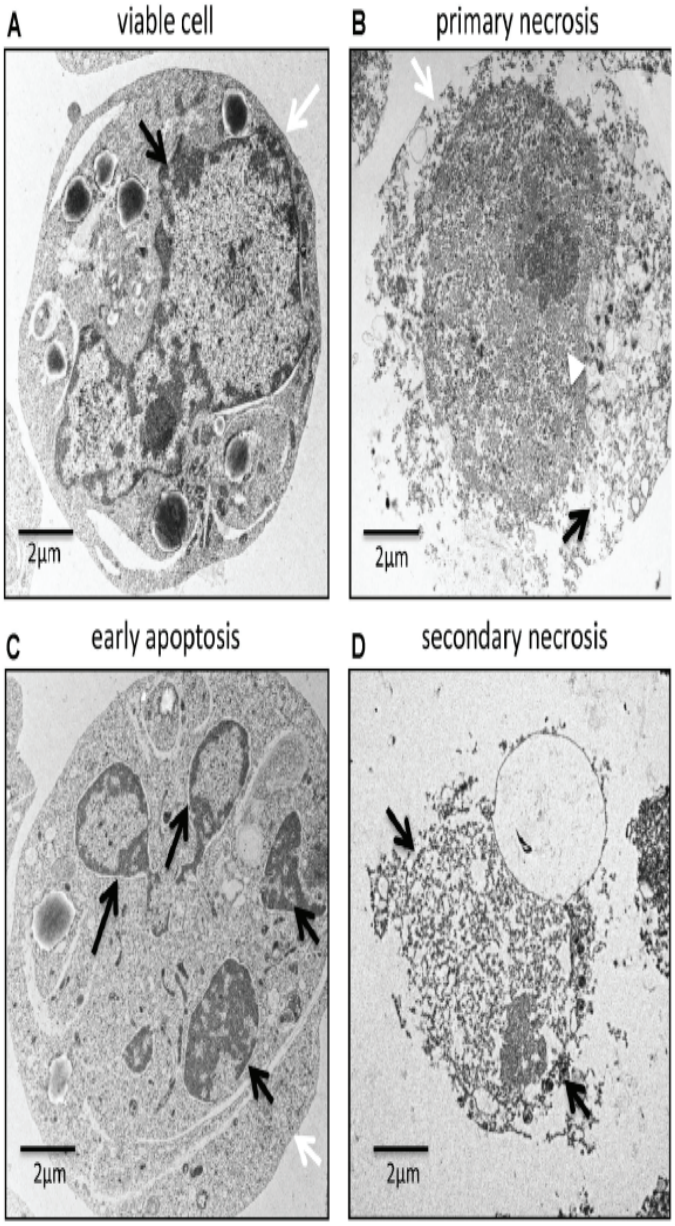

Figure 8: Transmission electron microscopic images of viable, primary necrotic, early apoptotic, and secondary necrotic cells. Human cells were cultured, and cell death (primary necrosis and apoptosis) was induced. A Viable cell with a normal morphology, including an intact cell membrane (white arrow) and nuclear membrane (black arrow). B Primary necrotic showing the loss of membrane integrity (white arrow) and low cytoplasm density (black arrow). A high DNA content can still be observed (white arrowhead). C Apoptotic cell marked by chromatin condensation and karyorrhexis (black arrows) and intact plasma membrane (white arrow). D Secondary necrosis showing a disintegrated cell membrane (black arrows) and loss of chromatin. Black bar 2 µm. (96)

4.2 Regulated necrosis

a. Necroptosis

Necroptosis has been discovered to be a novel form of programmed necrotic cell death, mechanically resembling apoptosis, while

morphologically similar to necrosis. (97)

Both apoptosis and programmed necrosis (necroptosis) are initiated by the same stimulants and activation of death receptors. However, the strength of the exposure will lead the cell toward necrosis or apoptosis. (98) The necroptosis pathway has been implicated as both an adaptive and pathogenic component of many human pathologies that involve inflammatory processes, including atherosclerosis, reperfusion injury, sepsis, inflammatory bowel disease, neurodegenerative disease, and pathological cell damage (viral infection). (99)

Necroptosis also acts as an alternative "fail-safe" cell death pathway in cases where cells are unable to undergo apoptosis, such as during viral infection, in which apoptosis signaling proteins are blocked by the virus. (100)

The most evident difference between necroptosis and apoptosis is the local inflammation caused by the release of the cell contents. The inflammation in necroptosis mainly presents as a large amount of inflammatory cell invasion and activation. (101)

The signaling pathway responsible for carrying out necroptosis is generally understood. Necroptosis is triggered by TNFR1 through interaction with the death domain (DD) that forms TRADD (Tumor Necrosis Factor Receptor Associated Death Domain). Then, RIPK1 was attracted, which recruits RIPK3, forming the necrosome. Phosphorylation of MLKL by the necrosome drives oligomerization of MLKL, allowing MLKL to insert into and permeabilize plasma membranes and organelles. (102)

Integration of MLKL leads to the inflammatory phenotype and release of damage-associated molecular patterns (DAMPs), which elicit immune responses. (102)

As in all forms of necrotic cell death, cells undergoing necroptosis rupture and leak their contents into the intercellular space. Unlike in necrosis, permeabilization of the cell membrane during necroptosis is tightly regulated. (103)

b. Pyroptosis

It is a regulated inflammatory form of programmed cell death that commonly occurs upon the recognition of intracellular pathogens. Pyroptosis can be triggered by bacteria, pathogens, or their endotoxins. (104)

The inflammation sensors of infected macrophage recognize the flagellin components of pathogens and initiate the formation of a multi-protein complex, the inflammasomes, which subsequently activate caspase1. (104) All inflammasomes require the adapter protein apoptosis-associated speck-like protein containing a CARD (ASC) for the activation of caspase 1. (105)

Caspase-1 is activated after the inflammasomes are formed. The inflammasome, which is a large supramolecular complex, subsequently mediates the maturation and secretion of interleukin-1 beta and interleukin-18. (106)

The morphological characteristics of pyroptosis include rupture of the cell membrane and release of its intracellular contents. This event provides a localized pool of potential proinflammatory molecules that include both direct activators of immune cells, such as cytokines and chemokines, as well as other signaling molecules or so-called "danger signals" able to trigger the production of inflammatory molecules from a variety of cell types. (107)

Inflammatory caspases activate Gasdermin D (GSDMD). Then, activated GSDMD translocates to the plasma membrane, where it binds to membrane phospholipids and initiates pore formation, resulting in loss of membrane integrity (as rupture of the cell membrane), cytosolic swelling, and release of cellular contents leading to cell death. The process is also accompanied by DNA condensation and fragmentation. (108)

c. Parthanatos

Parthanatos, a kind of new programmed death mode, indicates a caspase-independent cell death subroutine that critically relies on the hyper-activation of poly (ADP-ribose) polymerase 1 (PARP1). PARP-1 is important for DNA repair, genomic stability, and transcription. (109)

Cell demise from PARP-1 over-activation has been attributed to depletion of cellular energy, release of the death effector apoptosis-inducing factor (AIF) from the mitochondria, and production of excess poly-ADP ribose (PAR) polymer, a novel death signal. (109)

Upon PARP-1 overactivation, excess PAR, free or bound, shuttles outside of the nucleus and binds to specific cytosolic or mitochondrial proteins.

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PAR binding to these proteins ultimately leads to AIF release from the mitochondria. (110)

Mitochondria have been reported to contain the caspase-independent death effectors, apoptosis-inducing factor (AIF). AIF induces chromatin condensation and large-scale DNA fragmentation when released into the cytosol. (111)

Translocation of AIF into the nucleus, and not its loss from the mitochondria, kills cells. AIF translocation to the nucleus has been observed in different cell types induced to die. (109)

Parthanatos shares cytological and morphological features of apoptosis and necrosis, but is the result of a distinct molecular mechanism. Parthanatos is caspase-independent and does not involve the formation of apoptotic bodies. (112)

Nuclear mitochondrial crosstalk in parthanatos is triggered by DNA-damaging stimuli, activating PARP1. PAR, synthesized in response to DNA breaks, travels to the mitochondria and induces liberation of AIF. In turn, AIF interacts with MIF, and the latter degrades DNA.(113) AIF may recruit nucleases that induce chromatinolysis. (114)

d. Ferroptosis

Ferroptosis is an intracellular iron-dependent form of cell death that is distinct from apoptosis, necrosis, and autophagy. (115) It is characterized by the accumulation of lipid peroxide (polyunsaturated fatty acids) due to an increase in reactive oxygen species (ROS). Cellular iron is also recognized as a key factor in ferroptosis. Excess of active iron generation facilitates ROS production, which promotes lipid peroxidation and ferroptosis. (116)

Iron, which is involved in many biological functions, is a pro-oxidant agent that is able to react with hydrogen peroxide to produce reactive oxygen species (ROS). When the antioxidant system is saturated, an excess of ROS may cause cellular changes, such as damage to the plasma membrane and intracellular organelles, leading to cell death. (117) There is a role of iron in ferroptosis, but the exact mechanisms of its involvement are not clear. (118, 119)

Glutathione serves as a substrate for glutathione peroxidase (GPX4)(an antioxidant enzyme), which protects cells against oxidative stress by converting hydrogen peroxide to water. Additionally, GPX4 converts toxic lipid peroxides to nontoxic lipid alcohols. (58)

Biochemically, in ferroptosis, there is intracellular glutathione (GSH) depletion and decreased activity of glutathione peroxidase 4 (GPX4); lipid peroxides cannot be metabolized by the GPX4-catalyzed reduction reaction, and Fe^{2+} oxidizes lipids, resulting in a large amount of ROS, which promotes ferroptosis. (120)

Morphologically, ferroptosis occurs mainly in cells as reduced mitochondrial volume, increased bilayer membrane density, and reduction or disappearance of mitochondrial cristae, but the cell membrane remains intact, the nucleus is normal in size, and there is no condensation of chromatin. (116)

5. Vacuolepresenting cell death

a. Autophagy

Autophagy is an intracellular lysosomal (vacuolar) degradation process that is characterized by the formation of double-membrane vesicles (appearance of large intracellular vesicles) known as autophagosomes, which sequester in the cytoplasm. (121)

Autophagy has been described both as a means to resist starvation and as part of cellular remodeling during differentiation, metamorphosis, aging, cell transformation, physiological whole-organ changes, as well as in the removal of anomalous cellular components that accumulate following toxic insults or during cell death. (122)Autophagy is also initiated upon cellular stress as a protective response. Once the cellular stress is irreversible, the cell will be committed to Death, also through excessive levels of autophagy. (123)

Cells in the early stages of autophagy contain several autophagic vacuoles, although the nuclear structure still appears normal. Mitochondria and the endoplasmic reticulum are sometimes dilated, and the Golgi apparatus is often enlarged. The plasma membrane loses specializations such as microvilli and junctional complexes, and blebbing can occur. (121)

During late stages, both the number and size of vacuoli increase, and many of them are filled with lipids, which appear as pale gray inclusions in the cytoplasm. The nucleus of a cell undergoing

autophagic cell death can become pyknotic and identifiable. (124)

There are three defined types of autophagy: macro-autophagy, micro-autophagy, and chaperone-mediated autophagy, all of which promote proteolytic degradation of cytosolic components at the lysosome. Macro-autophagy delivers cytoplasmic cargo to the lysosome through the intermediary of a double membrane-bound vesicle, referred to as an autophagosome, that fuses with the lysosome to form an autolysosome. (125)

In micro-autophagy, by contrast, cytosolic components are directly taken up by the lysosome itself through invagination of the lysosomal membrane. Both macro-and micro-autophagy are able to engulf large structures through both selective and non-selective mechanisms. In chaperone-mediated autophagy (CMA), targeted proteins are translocated across the lysosomal membrane in a complex with chaperone proteins that are recognized by the lysosomal membrane receptor, resulting in their unfolding and degradation. (126)

➢ **The interplay of autophagy and apoptosis**

Autophagy and apoptosis often occur in the same cell, mostly in a sequence in which autophagy precedes apoptosis. This is because stress often stimulates an autophagic response, especially if the level of stress is not lethal. Apoptotic lethal programmer is activated when stress exceeds a critical duration or an intensity threshold. Nonetheless, if the cell commences apoptosis, autophagy can be inactivated, in part owing to the caspase-mediated cleavage of essential autophagy proteins, resulting in the inactivation of the autophagic programmed cell death. (127)

Autophagy induction is exacerbated if apoptosis is suppressed, for instance, by removing proapoptotic proteins such as BCL-2-associated X protein (BAX) and BCL-2 antagonist or killer (BAK) or by adding caspase inhibitors. (128)

The fact that many signal transduction pathways that are elicited by cell-intrinsic stress regulate both autophagy and apoptosis might explain the sequential activation of both processes. p53 is usually present in the cytosol but translocates to the nucleus upon DNA damage following its phosphorylation by a number of distinct stress-activated kinases. The available evidence indicates that the cytosolic pool of p53 represses autophagy and that the nuclear translocation of p53 leads to a decrease in this p53 pool, thereby facilitating the induction of autophagy. (127)

b. Entosis

It is a unique form of cell death characterized by the invasion of one living cell into another of the same type, a process that requires adhesion molecules, actin cytoskeleton remodelling, and energy expenditure. Once internalised, entotic cells may undergo regulated cell death within the entotic vacuole (entosome), via a specific autophagy-related process. (129) Interestingly, sometimes an entrapped inner cell stays viable for some time and is even able to divide inside the host cell or escape outside. (130, 131)

Entosis is believed to be triggered by integrinextracellular matrix (ECM) detachment. Subsequently, the cell produces adherens junctions with the neighboring cell and actively penetrates it, creating a cell-in-cell structure (CIC). Upon initiation of entosis, E-cadherin and β-catenin accumulate at the surface of the internalizing cells. (131)

Cytoskeleton dynamics plays a main role in entosis, linking adhesion sites and acts as an active signaling hub. A multi-molecular complex termed the mechanical ring (MR) is positioned between the invading and the engulfing cells. MR links adherens junctions with contractile actomyosin, coordinating their actions and promoting entosis. The entry of the inner cell is obviously dependent on actin filaments and actomyosin contractility. (132)

c. Methuosis

Methuosis is a distinct form of nonapoptotic cell death characterized by the massive accumulation of large fluid-filled single membrane vacuoles derived from macropinosomes. (133)

The consequent morphology resembles necrosis in the manner of cell swelling and plasma membrane integrity loss. The process begins with the abnormal fusion of nascent macropinosomes, resulting in osmotic cytoplasmic vacuolization. These enlarged vacuoles, which fail to undergo recycling or fusion with lysosomes, ultimately lead to cell death. (134)

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d. Paraptosis

The hallmark of paraptosis is the extensive cytoplasmic vacuolization originating from the dilated endoplasmic reticulum (ER) and the mitochondria. Several studies have shown that paraptosis is associated with reactive oxygen species (ROS) generation and the accumulation of misfolded proteins in the ER, as well as mitochondrial Ca2+ overload. Unlike apoptosis, Paraptosis lacks apoptotic features (e.g., DNA condensation and fragmentation, membrane blebbing, and apoptotic bodies), and unlike necrosis, cell membrane integrity is preserved in paraptosis (135, 136)

e. Ghost messages: cell death signals spread

Ghost messages refer to information transmitted from dying or dead cells to viable and healthy cells in the local or distant microenvironment, influencing their biological functions. To maintain body homeostasis, cells communicate through signaling molecules, including cell death signals. (137)

Cell death is classified as lytic or nonlytic. (138) Lytic forms—such as necrosis, necroptosis, pyroptosis, and ferroptosis—are characterized by the loss of membrane integrity and the release of damage-associated molecular patterns (DAMPs), proinflammatory cytokines, and other intracellular components into the extracellular space. (139)

In contrast, nonlytic cell death maintains membrane integrity and usually avoids triggering inflammation. Evidence indicates that dying cells can regulate inflammation, tissue repair, regeneration, and tumorigenesis through signaling to neighboring cells. (140)

Forms of ghost messages

Regardless of the death type, dying cells release various molecules and **extracellular vesicles (EVs)** that act as intercellular signals. These components influence recipient cells through receptor binding or internalization. (Figure 9)

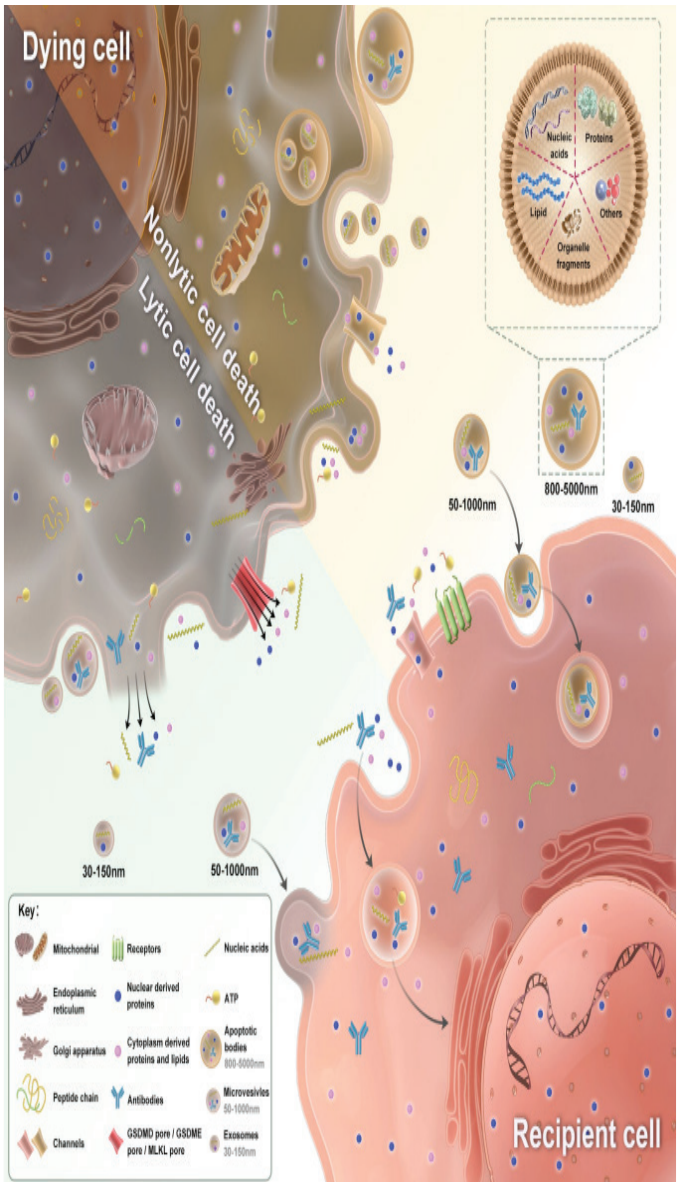

Figure 9: Ghost messages from dying cells to recipient cells(141)

1. Extracellular vesicles

Extracellular vesicles (EVs) are cell-derived lipid bilayer-enclosed membranous structures that consist of ApoBDs, exosomes, and microvesicles carrying biomolecules such as nucleic acids, proteins, and lipids. (142) It is now recognized that these vesicles can mediate intercellular communication. Vesicle secretion is a constitutive physiological process of healthy cells, but dying cells also release EVs.(143) Several studies have shown that cells undergoing nonapoptotic cell death generate a larger number of EVs compared with viable and apoptotic cells. (144)

- **Apoptotic bodies (ApoBDs)**

Large vesicles (800–5000 nm) formed during apoptosis; they are phagocytosed to prevent inflammation. Dying cells release "find-me" signals to attract phagocytes for debris clearance. (145, 146)

- **Exosomes and microvesicles**

Exosomes (30–150 nm) and microvesicles (50–1000 nm) mediate local and distant communication by transferring bioactive cargo. (146) Caspase activation during apoptosis can induce apoptotic exosome-like vesicles, which differ from classical exosomes in protein composition. (147)

The presence of microvesicles may preserve the integrity of the parent cells by eliminating complement and the risk of cytolysis. Release of microvesicles may rid the cell of toxic substances, but may also induce repair in neighboring cells. (148)

2. Soluble factors

A variety of soluble factors secreted by viable cells, such as cytokines, growth factors, receptors, hormones, and metabolites, can affect specific target cells via autocrine, paracrine, and endocrine signals. (149)Dying cells transmit messages through active secretion of some factors or passive release of intracellular contents following loss of membrane integrity. (149)

➢ **Target cells involved in ghost messages in biological processes:**

a. Immune cells

Immune cells are the first to respond to dying cells by engulfing debris and presenting antigens. Apoptosis is generally immunologically silent and promotes anti-inflammatory responses through **TGF-β** and **IL-10** secretion. (150)

TNF-α has been shown to reduce the capacity for dead cell engulfment, which exacerbates the inflammatory response. A defective clearance of apoptotic cells and dsDNAAb(double-stranded DNA antibodies)has been reported in systemic disease. (151)

During apoptosis, caspase activation opens Pannexin 1 channels, leading to (adenosine monophosphate) AMP release. As a "calm down" signal, AMP is converted to adenosine on macrophages, which activate anti-inflammatory genes. (150)

Nonapoptotic cell death released DAMPs also activate dendritic cells (DCs) and initiate adaptive T-cell immune responses. Insufficient autophagy of deteriorated organelles leads to the massive release of DAMPs, which exacerbates the inflammatory response. (152)

b. Stem and progenitor cells

Stem and progenitor cells possess self-renewal and differentiation capacity. After injury, apoptotic cells release growth factors such as **BMP**, **TGF-β**, **EGF**, **FGF**, and **IGF-1**, which recruit and activate these cells for tissue repair. (153)

PS externalization also influences cell differentiation. For example, mesenchymal stem cells (MSCs) that engulf apoptotic cells show enhanced osteogenic differentiation. (154)

c. Stromal cells and resident tissue cells

Stromal cells and resident tissue cells are a population of important functional cells that induce tissue regeneration and fibrosis. This process is essential for tissue repair after injury, but the role of ghost messages depends on the context. Active communication between apoptotic cells and healthy cells promotes surrounding cells' proliferation and maintenance of tissue homeostasis through what is commonly called apoptosis-induced compensatory proliferation. (155)

4. Conclusion

Cell death is essential for development, tissue renewal, and the preservation of homeostasis. This review highlights the major forms of programmed and non-programmed cell death, each defined by distinct molecular pathways and biological outcomes. Dysregulation of these pathways contributes to a wide range of diseases, from cancer to neurodegeneration and inflammatory disorders. Importantly, dying cells actively influence their microenvironment through signals and extracellular vesicles, affecting immunity, repair, and regeneration. A deeper understanding of these mechanisms offers promising avenues for future therapeutic strategies aimed at either promoting or preventing specific modes of cell death.

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References

1. Lee EJ. Cell Proliferation and Apoptosis in ADPKD. In: Cystogenesis. Springer Singapore; 2016. p. 25–34.

2. Kopeina GS, Efimenko AYu, Tkachuk VA, Zhivotovsky B. Programmed cell death and tissue regeneration: a link that should be resolved. Physiol Rev. 2025 Oct 1;105(4):2377–412.

3. Damiescu R, Efferth T, Dawood M. Dysregulation of different modes of programmed cell death by epigenetic modifications and their role in cancer. Cancer Lett. 2024 Mar;584:216623.

4. Yan G, Elbadawi M, Efferth T. Multiple cell death modalities and their key features (Review). World Acad Sci J. 2020 Mar 11;

5. Tang D, Kang R, Berghe T Vanden, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. Cell Res. 2019 May 4;29(5):347–64.

6. Saiyad S, Patel U. PHYSIOLOGICAL CELL DEATH: APOPTOSIS. Int J Basic Appl Physiol. 2022;11(1).

7. Tang D, Kang R. Cell death. In: Mechanisms of Cell Death and Opportunities for Therapeutic Development. Elsevier; 2022. p. 47–64.

8. Tonnus W, Belavgeni A, Beuschlein F, Eisenhofer G, Fassnacht M, Kroiss M, et al. The role of regulated necrosis in endocrine diseases. Nat Rev Endocrinol. 2021 Aug 16;17(8):497–510.

9. Yan G, Elbadawi M, Efferth T. Multiple cell death modalities and their key features (Review). World Acad Sci J. 2020 Mar 11;

10. Tang D, Kang R. Cell death. In: Mechanisms of Cell Death and Opportunities for Therapeutic Development. Elsevier; 2022. p. 47–64.

11. Lossi L. The concept of intrinsic versus extrinsic apoptosis. Biochemical Journal. 2022 Feb 11;479(3):357–84.

12. Singh N. Apoptosis in health and disease and modulation of apoptosis for therapy: An overview. Indian Journal of Clinical Biochemistry. 2007 Sep;22(2):6–16.

13. Malhi H, Gores GJ, Lemasters JJ. Apoptosis and Necrosis in the Liver: A Tale of Two Deaths? Hepatology. 2006 Feb;43(Supplement 1):S31–44.

14. Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol. 2007 Jun 1;35(4):495–516.

15. Green DR, Oguin TH, Martinez J. The clearance of dying cells: table for two. Cell Death Differ. 2016 Jun 18;23(6):915–26.

16. Song G, Liu J, Tang X, Zhong J, Zeng Y, Zhang X, et al. Cell cycle checkpoint revolution: targeted therapies in the fight against malignant tumors. Front Pharmacol. 2024 Oct 11;15.

17. Jeong J, McMahon AP. Growth and pattern of the mammalian neural tube are governed by partially overlapping feedback activities of the hedgehog antagonists patched 1 and Hhip1. Development. 2005 Jan 1;132(1):143–54.

18. Guerrero I, Ruiz i Altaba A. Longing for Ligand: Hedgehog, Patched, and Cell Death. Science (1979). 2003 Aug 8;301(5634):774–6.

19. Penaloza C, Lin L, Lockshin RA, Zakeri Z. Cell death in development: shaping the embryo. Histochem Cell Biol. 2006 Aug 1;126(2):149–58.

20. McIlwain DR, Berger T, Mak TW. Caspase Functions in Cell Death and Disease. Cold Spring Harb Perspect Biol. 2013 Apr 1;5(4):a008656–a008656.

21. Oberst A, Dillon CP, Weinlich R, McCormick LL, Fitzgerald P, Pop C, et al. Catalytic activity of the caspase-8–FLIPL complex inhibits RIPK3-dependent necrosis. Nature. 2011 Mar 17;471(7338):363–7.

22. Santos C, Marshall AR, Murray A, Metcalfe K, Narayan P, de Castro SC, et al. Spinal neural tube formation and tail development in human embryos. Elife. 2024 Dec 5;12.

23. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Programmed Cell Death (Apoptosis). Molecular Biology of the Cell 4th edition. Garland Science; 2002.

24. Gravano DM, Hoyer KK. Promotion and prevention of autoimmune disease by CD8+ T cells. J Autoimmun. 2013 Sep;45:68–79.

25. Reznikov A. Hormonal impact on tumor growth and progression. Exp Oncol. 2015 Feb;37:162–72.

26. L. Miller W, Flück CE, Breault DT, Feldman BJ. The Adrenal Cortex and Its Disorders. In: Sperling Pediatric Endocrinology. Elsevier; 2021. p. 425–90.

27. Li SY, Sun R, Wang HX, Shen S, Liu Y, Du XJ, et al. Combination therapy with epigenetic-targeted and chemotherapeutic drugs delivered by nanoparticles to enhance the chemotherapy response and overcome resistance by breast cancer stem cells. Journal of Controlled Release. 2015 May;205:7–14.

28. Wong RS. Apoptosis in cancer: from pathogenesis to treatment. Journal of Experimental & Clinical Cancer Research. 2011 Dec 26;30(1):1–14.

29. Xu X, Lai Y, Hua ZC. Apoptosis and apoptotic body: disease message and therapeutic target potentials. Biosci Rep. 2019 Jan 31;39(1).

30. Burattini S, Ferri P, Battistelli M, D'emilio A, Biagiotti L, Sestili P, et al. Apoptotic DNA fragmentation can be revealed in situ: An ultrastructural approach. Microsc Res Tech. 2009 Dec 29;72(12):913–23.

31. Clarke P, DeBiasi RL, Goody R, Hoyt CC, Richardson-Burns S, Tyler KL. Mechanisms of Reovirus-Induced Cell Death and Tissue Injury: Role of Apoptosis and Virus-Induced Perturbation of Host-Cell Signaling and Transcription Factor Activation. Viral Immunol. 2005 Mar;18(1):89–115.

32. Obeng E. Apoptosis (programmed cell death) and its signals - A review. Brazilian Journal of Biology. 2021 Dec;81(4):1133–43.

33. Charras GT, Yarrow JC, Horton MA, MahadevanL,MitchisonTJ.Non-equilibration of hydrostatic pressure in blebbing cells. Nature. 2005 May;435(7040):365–9.

34. Ávila MO, Vega AF, Maraver JG, Paz MV, De Lavera I, De La Mata M, et al. The Apoptotic Microtubule Network During the Execution Phase of Apoptosis. In: Cell Death - Autophagy, Apoptosis and Necrosis. InTech; 2015.

35. Kiraly G, Simonyi AS, Turani M, Juhasz I, Nagy G, Banfalvi G. Micronucleus formation during chromatin condensation and under apoptotic conditions. Apoptosis. 2017 Feb 25;22(2):207–19.

36. Akers JC, Gonda D, Kim R, Carter BS, Chen CC. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. J Neurooncol. 2013 May 2;113(1):1–11.

37. Zahri S, Razavi SM, Niri FH, Mohammadi S. Induction of programmed cell death by Prangos uloptera, a medicinal plant. Biol Res. 2009;42:517–22.

38. Battistelli M, Falcieri E. Apoptotic Bodies: Particular Extracellular Vesicles Involved in Intercellular Communication. Biology (Basel). 2020 Jan 20;9(1):21.

39. Bayraktar R, Van Roosbroeck K, Calin GA. Cell-to-cell communication: microRNAs as hormones. Mol Oncol. 2017 Dec 26;11(12):1673–86.

40. Poon IKH, Lucas CD, Rossi AG, Ravichandran KS. Apoptotic cell clearance: basic biology and therapeutic potential. Nat Rev Immunol. 2014 Mar 31;14(3):166–80.

41. Rysavy NM, Shimoda LMN, Dixon AM, Speck M, Stokes AJ, Turner H, et al. Beyond apoptosis: The mechanism and function of phosphatidylserine asymmetry in the membrane of activating mast cells. Bioarchitecture. 2015 Feb;4:127–37.

42. Depraetere V. "Eat me"signals of apoptotic bodies. Nat Cell Biol. 2000 Jun;2(6):E104–E104.

43. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, et al. Delivery of MicroRNA-126 by Apoptotic Bodies Induces CXCL12-Dependent Vascular Protection. Sci Signal. 2009 Dec 8;2(100).

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1809

44. Schiller M, Parcina M, Heyder P, Foermer S, Ostrop J, Leo A, et al. Induction of Type I IFN Is a Physiological Immune Reaction to Apoptotic Cell-Derived Membrane Microparticles. The Journal of Immunology. 2012 Aug 15;189(4):1747–56.

45. Arandjelovic S, Ravichandran KS. Phagocytosis of apoptotic cells in homeostasis. Nat Immunol. 2015 Sep 19;16(9):907–17.

46. Nössing C, Ryan KM. 50 years on and still very much alive: 'Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics.' Br J Cancer. 2023 Feb 2;128(3):426–31.

47. Reddien PW, Horvitz HR. CED-2/CrkII and CED-10/Rac control phagocytosis and cell migration in Caenorhabditis elegans. Nat Cell Biol. 2000 Mar 2;2(3):131–6.

48. Hochreiter-Hufford A, Ravichandran KS. Clearing the Dead: Apoptotic Cell Sensing, Recognition, Engulfment, and Digestion. Cold Spring Harb Perspect Biol. 2013 Jan 1;5(1):a008748–a008748.

49. Iyer SS, Pulskens WP, Sadler JJ, Butter LM, Teske GJ, Ulland TK, et al. Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. Proceedings of the National Academy of Sciences. 2009 Dec;106(48):20388–93.

50. Kawane K, Ohtani M, Miwa K, Kizawa T, Kanbara Y, Yoshioka Y, et al. Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. Nature. 2006 Oct 26;443(7114):998–1002.

51. Frolova AS, Chepikova OE, Deviataikina AS, Solonkina AD, Zamyatnin AA. New Perspectives on the Role of Nuclear Proteases in Cell Death Pathways. Biology (Basel). 2023 May 31;12(6):797.

52. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nat Rev Mol Cell Biol. 2019 Mar 17;20(3):175–93.

53. Yogosawa S, Yoshida K. Tumor suppressive role for kinases phosphorylating p53 in <scp>DNA</scp> damage-induced apoptosis. Cancer Sci. 2018 Nov 6;109(11):3376–82.

54. Lawen A. Apoptosis—an introduction. BioEssays. 2003 Sep 18;25(9):888–96.

55. Fridman JS, Lowe SW. Control of apoptosis by p53. Oncogene. 2003 Dec 8;22(56):9030–40.

56. Manohar S, Leung N. Cisplatin nephrotoxicity: a review of the literature. J Nephrol. 2018 Feb 5;31(1):15–25.

57. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol. 2014 Jan 20;15(1):49–63.

58. RK G, S T. Ozone: A new face of dentistry. 2009 Feb;7.

59. Schellenberg B, Wang P, Keeble JA, Rodriguez-Enriquez R, Walker S, Owens TW, et al. Bax Exists in a Dynamic Equilibrium between the Cytosol and Mitochondria to Control Apoptotic Priming. Mol Cell. 2013 Mar;49(5):959–71.

60. Moldoveanu T, Follis AV, Kriwacki RW, Green DR. Many players in BCL-2 family affairs. Trends Biochem Sci. 2014 Mar;39(3):101–11.

61. Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death. Nat Rev Mol Cell Biol. 2020 Feb 21;21(2):85–100.

62. Oakes SA, Scorrano L, Opferman JT, Bassik MC, Nishino M, Pozzan T, et al. Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. Proceedings of the National Academy of Sciences. 2005 Jan 4;102(1):105–10.

63. Xu W, Jing L, Wang Q, Lin CC, Chen X, Diao J, et al. Bax-PGAM5L-Drp1 complex is required for intrinsic apoptosis execution. Oncotarget. 2015 Oct 6;6(30):30017–34.

64. Julien O, Wells JA. Caspases and their substrates. Cell Death Differ. 2017 Aug 12;24(8):1380–9.

65. Eckelman BP, Salvesen GS. The Human Anti-apoptotic Proteins cIAP1 and cIAP2 Bind but Do Not Inhibit Caspases. Journal of Biological Chemistry. 2006 Feb;281(6):3254–60.

66. Abbas R, Larisch S. Targeting XIAP for Promoting Cancer Cell Death—The Story of ARTS and SMAC. Cells. 2020 Mar 9;9(3):663.

67. Hamacher-Brady A, Brady NR. Bax/Bak-dependent, Drp1-independent Targeting of X-linked Inhibitor of Apoptosis Protein (XIAP) into Inner Mitochondrial Compartments Counteracts Smac/DIABLO-dependent Effector Caspase Activation. Journal of Biological Chemistry. 2015 Sep;290(36):22005–18.

68. Yanumula A, Cusick JK. Biochemistry, Extrinsic Pathway of Apoptosis. StatPearls Publishing; 2020.

69. Guicciardi ME, Gores GJ. Life and death by death receptors. The FASEB Journal. 2009 Jun 13;23(6):1625–37.

70. Kist M, Vucic D. Cell death pathways: intricate connections and disease implications. EMBO J. 2021 Mar 13;40(5).

71. Voskoboinik I, Whisstock JC, Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol. 2015 Jun 22;15(6):388–400.

72. Garant PR. Oral Cells and Tissues. Quintessence Publishing (IL); 2003.

73. Yan J, Xie Y, Si J, Gan L, Li H, Sun C, et al. Crosstalk of the Caspase Family and Mammalian Target of Rapamycin Signaling. Int J Mol Sci. 2021 Jan 15;22(2):817.

74. Rai NK, Tripathi K, Sharma D, Shukla VK. Apoptosis: A Basic Physiologic Process in Wound Healing. Int J Low Extrem Wounds. 2005 Sep 1;4(3):138–44.

75. Suzuki J, Imanishi E, Nagata S. Xkr8 phospholipid scrambling complex in apoptotic phosphatidylserine exposure. Proceedings of the National Academy of Sciences. 2016 Aug 23;113(34):9509–14.

76. Taddei M, Giannoni E, Fiaschi T, Chiarugi P. Anoikis: an emerging hallmark in health and diseases. J Pathol. 2012 Jan;226(2):380–93.

77. Loro L, Vintermyr O, Johannessen A. Apoptosis in normal and diseased oral tissues. Oral Dis. 2005 Sep 25;11(5):274–87.

78. Guo M, Lu B, Gan J, Wang S, Jiang X, Li H. Apoptosis detection: a purpose-dependent approach selection. Cell Cycle. 2021 Jun 3;20(11):1033–40.

79. Sgonc R, Gruber J. Apoptosis detection: an overview. Exp Gerontol. 1998 Sep;33(6):525–33.

80. Kari S, Subramanian K, Altomonte IA, Murugesan A, Yli-Harja O, Kandhavelu M. Programmed cell death detection methods: a systematic review and a categorical comparison. Apoptosis. 2022 Aug 17;27(7–8):482–508.

81. Ansari SM, Saquib Q, Attia SM, Abdel-Salam EM, Alwathnani HA, Faisal M, et al. Pendimethalin induces oxidative stress, DNA damage, and mitochondrial dysfunction to trigger apoptosis in human lymphocytes and rat bone-marrow cells. Histochem Cell Biol. 2018 Feb 18;149(2):127–41.

82. Lugli E, Troiano L, Ferraresi R, Roat E, Prada N, Nasi M, et al. Characterization of cells with different mitochondrial membrane potential during apoptosis. Cytometry Part A. 2005 Nov 19;68A(1):28–35.

83. Heisler-Taylor T, Kim B, Reese AY, Hamadmad S, Kusibati R, Fischer AJ, et al. A new multichannel method quantitating TUNEL in detached photoreceptor nuclei. Exp Eye Res. 2018 Nov;176:121–9.

84. da Fonseca RR, Kosiol C, Vinař T, Siepel A, Nielsen R. Positive selection on apoptosis related genes. FEBS Lett. 2010 Feb 5;584(3):469–76.

85. Martinez MM, Reif RD, Pappas D. Detection of apoptosis: A review of conventional and novel techniques. Analytical Methods. 2010;2(8):996.

86. Wlodkowic D, Skommer J, Darzynkiewicz Z. Flow Cytometry-Based Apoptosis Detection. In 2009. p. 19–32.

87. Vogler M, Hamali HA, Sun XM, Bampton ETW, Dinsdale D, Snowden RT, et al. BCL2/BCL-XL inhibition induces apoptosis, disrupts cellular calcium homeostasis, and prevents platelet activation. Blood. 2011 Jun 30;117(26):7145–54.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1809

88. Khalid N, Azimpouran M. Necrosis. StatPearls Publishing; 2023.

89. Festjens N, Vanden Berghe T, Vandenabeele P. Necrosis, a well-orchestrated form of cell demise: Signalling cascades, important mediators and concomitant immune response. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 2006 Sep;1757(9–10):1371–87.

90. Sarhan M, Land WG, Tonnus W, Hugo CP, Linkermann A. Origin and Consequences of Necroinflammation. Physiol Rev. 2018 Apr 1;98(2):727–80.

91. guicciardi maria eugenia, Gores GJ. Complete lysosomal disruption: A route to necrosis, not to the inflammasome. Cell Cycle. 2013 Jul 31;12(13):1995–1995.

92. Wojtczak L, Zabłocki K. Basic Mitochondrial Physiology in Cell Viability and Death. In: Drug-Induced Mitochondrial Dysfunction. Wiley; 2008. p. 1–35.

93. Nakano H, Murai S, Moriwaki K. Regulation of the release of damage-associated molecular patterns from necroptotic cells. Biochemical Journal. 2022 Mar 18;479(5):677–85.

94. Priante G, Gianesello L, Ceol M, Del Prete D, Anglani F. Cell Death in the Kidney. Int J Mol Sci. 2019 Jul 23;20(14):3598.

95. Gao Y, Jiao Y, Gong X, Liu J, Xiao H, Zheng Q. Role of transcription factors in apoptotic cells clearance. Front Cell Dev Biol. 2023 Jan 19;11.

96. Sachet M, Liang YY, Oehler R. The immune response to secondary necrotic cells. Apoptosis. 2017 Oct 31;22(10):1189–204.

97. Ye H, Lu M, Tu C, Min L. Necroptosis in the sarcoma immune microenvironment: From biology to therapy. Int Immunopharmacol. 2023 Sep;122:110603.

98. Mozaffari S, Abdollahi M. Mitochondrial contribution to cell death. In: Mitochondrial Metabolism. Elsevier; p. 2021.

99. Choi ME, Price DR, Ryter SW, Choi AMK. Necroptosis: a crucial pathogenic mediator of human disease. JCI Insight. 2019 Aug 8;4(15).

100. Gong Y, Fan Z, Luo G, Yang C, Huang Q, Fan K, et al. The role of necroptosis in cancer biology and therapy. Mol Cancer. 2019 Dec 23;18(1):1–17.

101. Liu C, Zhang K, Shen H, Yao X, Sun Q, Chen G. Necroptosis: A novel manner of cell death, associated with stroke (Review). Int J Mol Med. 2017 Nov 22;

102. Cho S, Pronko A, Stout-Delgado H. Crosstalk between necroptosis and the inflammasome. In: Inflammasome Biology. Elsevier; 2023.

103. Purnama CA, Meiliana A, Barliana MI, Lestari K. Update of cellular responses to the efferocytosis of necroptosis and pyroptosis. Cell Div. 2023 Apr 9;18(1):5.

104. Miller DR, Cramer SD, Thorburn A. The interplay of autophagy and non-apoptotic cell death pathways. In: International review of cell and molecular biology. 2020. p. 159–87.

105. Stutz A, Horvath GL, Monks BG, Latz E. ASC Speck Formation as a Readout for Inflammasome Activation. In 2013. p. 91–101.

106. Li Y, Fan J, Ju D. Neurotoxicity concern about the brain targeting delivery systems. In: Brain Targeted Drug Delivery System. Elsevier; 2019. p. 377–408.

107. Firestein G, Budd R, Gabriel S, McInnes I, O'Dell J. Firestein & kelley's textbook of rheumatology-E-book. Elsevier Health Sciences; 2020.

108. Iorga A, Dara L. Cell death in drug-induced liver injury. In: Advances in Pharmacology. 2019. p. 31–74.

109. David KK, Andrabi SA, Dawson TM, Dawson VL. Parthanatos, a messenger of death. Frontiers in Bioscience. 2009;Volume(14):1116.

110. David KK, Andrabi SA, Dawson TM, Dawson VL. Parthanatos, a messenger of death. Frontiers in Bioscience. 2009;Volume(14):1116.

111. Nguyen TNA, Lai HT, Fernandes R, Dall'Olio FG, Blériot C, Ha-Duong T, et al. Apoptosis-inducing factor (AIF) at the crossroad of cell survival and cell death: implications for cancer and mitochondrial diseases. Cell Communication and Signaling. 2025 Jun 4;23(1):264.

112. Fatokun AA, Dawson VL, Dawson TM. Parthanatos: mitochondrial-linked mechanisms and therapeutic opportunities. Br J Pharmacol. 2014 Apr 28;171(8):2000–16.

113. Robinson N, Ganesan R, Hegedűs C, Kovács K, Kufer TA, Virág L. Programmed necrotic cell death of macrophages: Focus on pyroptosis, necroptosis, and parthanatos. Redox Biol. 2019 Sep;26:101239.

114. Novo N, Romero-Tamayo S, Marcuello C, Boneta S, Blasco-Machin I, Velázquez-Campoy A, et al. Beyond a platform protein for the degradosome assembly: The Apoptosis-Inducing Factor as an efficient nuclease involved in chromatinolysis. PNAS Nexus. 2023 Feb 3;2(2).

115. Zhang C, Liu X, Jin S, Chen Y, Guo R. Ferroptosis in cancer therapy: a novel approach to reversing drug resistance. Mol Cancer. 2022 Dec 12;21(1):47.

116. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. Cell. 2012 May;149(5):1060–72.

117. Sousa L, Oliveira MM, Pessôa MTC, Barbosa LA. Iron overload: Effects on cellular biochemistry. Clinica Chimica Acta. 2020 May;504:180–9.

118. Kim-Campbell N, Gomez H, Bayir H. Cell Death Pathways. In: Critical Care Nephrology. Elsevier; 2019. p. 113-121.e2.

119. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. Nat Rev Mol Cell Biol. 2021 Apr 25;22(4):266–82.

120. Flores-Romero H, Ros U, García-Sáez AJ. A lipid perspective on regulated cell death. In: International review of cell and molecular biology. 2020. p. 197–236.

121. Chen Y, Klionsky DJ. The regulation of autophagy – unanswered questions. J Cell Sci. 2011 Jan 15;124(2):161–70.

122. Wang L, Ye X, Zhao T. The physiological roles of autophagy in the mammalian life cycle. Biological Reviews. 2019 Apr 21;94(2):503–16.

123. Ravanan P, Srikumar IF, Talwar P. Autophagy: The spotlight for cellular stress responses. Life Sci. 2017 Nov;188:53–67.

124. Chen Y, Li X, Yang M, Liu SB. Research progress on morphology and mechanism of programmed cell death. Cell Death Dis. 2024 May 10;15(5):327.

125. Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. J Pathol. 2010 May;221(1):3–12.

126. Mizushima N. A brief history of autophagy from cell biology to physiology and disease. Nat Cell Biol. 2018 May 23;20(5):521–7.

127. Mariño G, Niso-Santano M, Baehrecke EH, Kroemer G. Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol. 2014 Feb 8;15(2):81–94.

128. Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, et al. Regulation of an ATG7- beclin 1 Program of Autophagic Cell Death by Caspase-8. Science (1979). 2004 Jun 4;304(5676):1500–2.

129. White E. Entosis: It's a Cell-Eat-Cell World. Cell. 2007 Nov;131(5):840–2.

130. Kianfar M, Balcerak A, Chmielarczyk M, Tarnowski L, Grzybowska EA. Cell Death by Entosis: Triggers, Molecular Mechanisms and Clinical Significance. Int J Mol Sci. 2022 Apr 30;23(9):4985.

131. Mlynarczuk-Bialy I, Dziuba I, Sarnecka A, Platos E, Kowalczyk M, Pels KK, et al. Entosis: From Cell Biology to Clinical Cancer Pathology. Cancers (Basel). 2020 Sep 1;12(9):2481.

132. Wan Q, Liu J, Zheng Z, Zhu H, Chu X, Dong Z, et al. Regulation of myosin activation during cell–cell contact formation by Par3-Lgl antagonism: entosis without matrix detachment. Mol Biol Cell. 2012 Jun;23(11):2076–91.

133. Maltese WA, Overmeyer JH. Methuosis. Am J Pathol. 2014 Jun;184(6):1630–42.

134. Silva-Pavez E, Villar P, Trigo C, Caamaño E, Niechi I, Pérez P, et al. CK2 inhibition with silmitasertib promotes methuosis-like cell death associated to catastrophic massive vacuolization of colorectal cancer cells. Cell Death Dis. 2019 Jan 25;10(2):73.

135. Kim E, Lee DM, Seo MJ, Lee HJ, Choi KS. Intracellular Ca2 + Imbalance Critically Contributes to Paraptosis. Front Cell Dev

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1809

Biol. 2021 Jan 12;8.

136. Hanson S, Dharan A, P. V. J, Pal S, Nair BG, Kar R, et al. Paraptosis: a unique cell death mode for targeting cancer. Front Pharmacol. 2023 Jun 15;14.

137. Zhang M, Lin Y, Chen R, Yu H, Li Y, Chen M, et al. Ghost messages: cell death signals spread. Cell Communication and Signaling. 2023 Jan 9;21(1):6.

138. Vicar T, Raudenska M, Gumulec J, Balvan J. The Quantitative-Phase Dynamics of Apoptosis and Lytic Cell Death. Sci Rep. 2020 Jan 31;10(1):1566.

139. Berghe T Vanden, Vanlangenakker N, Parthoens E, Deckers W, Devos M, Festjens N, et al. Necroptosis, necrosis and secondary necrosis converge on similar cellular disintegration features. Cell Death Differ. 2010 Jun 11;17(6):922–30.

140. Levine B, Kroemer G. Biological Functions of Autophagy Genes: A Disease Perspective. Cell. 2019 Jan;176(1–2):11–42.

141. Zhang M, Lin Y, Chen R, Yu H, Li Y, Chen M, et al. Ghost messages: cell death signals spread. Cell Communication and Signaling. 2023 Jan 9;21(1):6.

142. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018 Apr 17;19(4):213–28.

143. Li M, Liao L, Tian W. Extracellular Vesicles Derived From Apoptotic Cells: An Essential Link Between Death and Regeneration. Front Cell Dev Biol. 2020 Oct 2;8.

144. Baxter AA, Phan TK, Hanssen E, Liem M, Hulett MD, Mathivanan S, et al. Analysis of extracellular vesicles generated from monocytes under conditions of lytic cell death. Sci Rep. 2019 May 17;9(1):7538.

145. Kang M, Jordan V, Blenkiron C, Chamley LW. Biodistribution of extracellular vesicles following administration into animals: A systematic review. J Extracell Vesicles. 2021 Jun 24;10(8).

146. Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, Lazarowski ER, et al. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature. 2010 Oct 13;467(7317):863–7.

147. Liu ML, Williams KJ, Werth VP. Microvesicles in Autoimmune Diseases. In: dvances in clinical chemistry. 2016. p. 125–75.

148. Ståhl A lie, Johansson K, Mossberg M, Kahn R, Karpman D. Exosomes and microvesicles in normal physiology, pathophysiology, and renal diseases. Pediatric Nephrology. 2019 Jan 27;34(1):11–30.

149. Mitchell R, Mellows B, Sheard J, Antonioli M, Kretz O, Chambers D, et al. Secretome of adipose-derived mesenchymal stem cells promotes skeletal muscle regeneration through synergistic action of extracellular vesicle cargo and soluble proteins. Stem Cell Res Ther. 2019 Dec 5;10(1):116.

150. Yamaguchi H, Maruyama T, Urade Y, Nagata S. Immunosuppression via adenosine receptor activation by adenosine monophosphate released from apoptotic cells. Elife. 2014 Mar 25;3.

151. Schulze C, Munoz LE, Franz S, Sarter K, Chaurio RA, Gaipl US, et al. Clearance deficiency—A potential link between infections and autoimmunity. Autoimmun Rev. 2008 Oct;8(1):5–8.

152. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature. 2010 Mar;464(7285):104–7.

153. Amcheslavsky A, Wang S, Fogarty CE, Lindblad JL, Fan Y, Bergmann A. Plasma Membrane Localization of Apoptotic Caspases for Non-apoptotic Functions. Dev Cell. 2018 May;45(4):450-464.e3.

154. Verma SK, Leikina E, Melikov K, Gebert C, Kram V, Young MF, et al. Cell-surface phosphatidylserine regulates osteoclast precursor fusion. Journal of Biological Chemistry. 2018 Jan;293(1):254–70.

155. Tso GHW, Law HKW, Tu W, Chan GCF, Lau YL. Phagocytosis of Apoptotic Cells Modulates Mesenchymal Stem Cells Osteogenic Differentiation to Enhance IL-17 and RANKL Expression on CD4+ T Cells. Stem Cells. 2010 May 1;28(5):939–54.

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Phytochemical Profile and Antibacterial Activity of Stem Bark Extracts of Stereospermum kunthianum (Pink Jacaranda)

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ABSTRACT:

The global rise of antimicrobial resistance necessitates the search for novel therapeutic agents from natural sources, particularly medicinal plants. "Stereospermum kunthianum" (Pink Jacaranda) has long been employed in traditional medicine for the treatment of infectious diseases; however, its antibacterial efficacy requires scientific validation. This study investigated the phytochemical constituents and antibacterial activity of stem bark extracts of S. kunthianum. The dried stem bark powder was sequentially extracted with hexane, ethyl acetate, acetone, and methanol using a Soxhlet apparatus. Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, saponins, terpenoids, phenols, steroids, cardiac glycosides, phlobatannins, and carbohydrates. Antibacterial activity was assessed against clinical isolates of Escherichia coli and Staphylococcus aureus, using standard bioassay methods. The extracts demonstrated variable antibacterial effects, with inhibition zones ranging from 5.0 to 17.0 mm. Ethyl acetate and methanol extracts exhibited the most pronounced activity, particularly against S. aureus and E. coli. Minimum inhibitory concentration (MIC) tests revealed that the methanol extract inhibited S. aureus and E. coli at 12.5 mg/mL, while the ethyl acetate extract inhibited S. aureus at 12.5 mg/mL and E. coli at 25 mg/mL. Although less potent than chloramphenicol (Control), the findings affirm the antibacterial potential of "S. kunthianum" stem bark and provide scientific support for its ethnomedicinal use. These results highlight its promise as a source of bioactive compounds for drug discovery, warranting further investigation into its safety, mechanism of action, and potential for development into effective antimicrobial agents.

KEYWORDS:

Phytochemicals, Antibacterial activity, "Stereospermum kunthianum", Plant extracts, Bioassay, Drug discovery.

1. Introduction

Natural herbs are as old as human history; nowadays, plant products are used for many purposes, such as drugs and cosmetics (1). Natural products have been used for centuries as anti-infective agents; several antibiotics in use today were derived from plants (2). Medicinal plants are known to possess bioactive compounds that can be used in the treatment of diseases (3). Plant products can help treat disease by increasing antioxidant activity, inhibiting

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bacterial development, and modulating genetic pathways (4). More recently, the use of plants has identified different chemical compounds with many biochemical and pharmacological properties (5). These phytochemical constituents are the secondary metabolites synthesized by plants and are known to play a crucial role in plant defence mechanisms (3). An investigation of plant-originated pure compounds used as drugs in countries with WHO-Traditional Medicine Centres indicated that out of the 122 compounds so far identified, 80% were applied for the same or similar ethno-medical purposes and were obtained from 94 plant species only (6).

The plant kingdom is a rich source of biodiversity and offers a variety of plants with medicinal properties (3). "*Stereospermum kunthianum*" belongs to the Bignoniaceae family and is referred to as Pink Jacaranda in English. In Nigeria, it is designated 'Sansami' by the Hausa (Northern Nigeria), 'Ayada' by the Yoruba (Southwestern, Nigeria), and 'Alakiriti' by the Igbo (Southeastern, Nigeria) (7). According to (5), "*Stereospermum kunthianum*" is a plant that has its different parts used in traditional medicine for the treatment of different ailments and conditions, such as wounds, ulcers, gastritis, bronchitis, and other pain and inflammation-related health conditions. It is a deciduous shrub or small tree widely spread across Africa, with some species distribution in Asia. The species is well spread all over the Sahel region (8).

The leaf extracts of "*Stereospermum kunthianum*" have a promising antibacterial effect on three selected organisms, *S. aureus, S. typhi,* and *B. subtilis*, but are ineffective against *P. aeruginosa*(2). Similarly, *E. coli, S. typhi,* and *S. aureus* were susceptible to both aqueous and methanol extracts of the plant's leaf and stem bark(3). The antibacterial activity of "*S. kunthianum*" extracts could be attributed to the cellular structure of the bacteria: Gram-positive bacteria are more susceptible to the extracts than Gram-negative bacteria(3). On the other hand, the antibacterial activity of the plant is lower than that of Levofloxacin and Clotrimazole (2). This could be because of the presence of other compounds or impurities that prevent or reduce the effects of antibacterial compounds in the plants. Isolation of pure antimicrobial compounds from these extracts may result in compounds with comparable activity (2).

Infectious diseases have repeatedly been identified as one of the most serious threats to human health around the world (3). Infectious diseases accounted for 61.7 percent (5.9million) of the 9.6 million fatalities in Africa in 2013, according to the World Health Organization WHO(9). Pathogenic bacteria are capable of causing diseases in humans, which include food poisoning, gastritis, ulcers, wound infection, etc(10). The high cost of antibiotics has made the treatment of diseases very difficult. Therefore, there is a need to explore plant extracts that can act in the same biological pathways as pharmaceutical medicines and pose fewer side effects (3). Based on the aforementioned information, the study aimed to determine the phytochemical constituents of the stem bark of "*Stereospermum kunthianum*" and evaluate its antibacterial activity.

2. MATERIALS AND METHODS

Collection and Preparation of the Sample

The sample of the stem bark of *S. kunthianum* was obtained from the Yelwa Campus of Abubakar Tafawa Balewa University, Bauchi, Nigeria. Identification was carried out using standard taxonomic keys and available literature. It was dried under shade and crushed using a wooden mortar, sieved to a fine powder, and stored until used.

Extraction of the Plant Extracts

200g of the powdered sample of the stem bark of *S. kunthianum* was placed in a Soxhlet extractor. Hexane, ethyl acetate, acetone, and methanol were used sequentially for the extraction. The solvents were evaporated under reduced pressure below 50°C to give the crude solvent extracts. These were further used for phytochemical screening and antimicrobial screening.

Phytochemical Screening

Standard laboratory techniques were carried out for qualitative determination of tannins, saponins, flavonoids, cardiac glycosides, phlobatannins, terpenoids, steroids, carbohydrates, and alkaloids (11).

Collection of Bacterial isolates

Clinical isolates of *Staphylococcus aureus* and *Escherichia coli* were obtained from the

Department of Microbiology, Abubakar Tafawa Balewa University, Bauchi State, Nigeria.

Preparation of Culture Media

The culture medium used was nutrient agar (NA) for supporting the growth of microorganisms. The assay media was prepared following the manufacturer's direction by suspending 28g of the agar and dissolving it in 1 Liter of distilled water (1000ml). This gave 11.2g of the nutrient agar powder, which was dissolved in 400ml of distilled water in a 500ml conical flask. The solution was allowed to dissolve properly for 10 minutes by swirling the mixture in the flask. The flask was covered with a foil paper and autoclaved for another 15minutes at 121°C for sterilization. The prepared nutrient agar was allowed to cool before pouring it into the sterilised plates. A blue flame of the hot plate was used to sterilise the rim of the conical flask after each pouring. The plates were then covered, and the agar was left to gel. The method of bioassay employed is the Agar-disk diffusion method, and the test organisms used were *Staphylococcus aureus* and *Escherichia coli* (12).

Antimicrobial Susceptibility Test

The agar well diffusion method of (12) was used. Mueller Hinton agar was prepared as indicated by the manufacturer's directions. The media was cooled and then filled into sterile petri dishes. The plates were allowed to solidify, and then they were surface inoculated with the prepared standard inoculum containing each of the isolated organisms. A sterile 6mm diameter cork borer was used to make holes in the surface of inoculated Mueller-Hinton agar plates. Aliquots of different concentrations of the extracts were introduced into each well. The inoculated plates were kept at room temperature for 2 hours to permit the extract to diffuse into the agar. The plates were incubated at 37℃ for one day. The agar well diffusion assay was performed in triplicate (n=3 independent plates/wells per extract and organism). Zones of inhibition were measured in mm using a transparent ruler. Data are presented as mean ± standard deviation (SD). Separate plates were prepared containing the same organisms, in which standard antibiotic (Chloramphenicol) was used as a control(3).

Determination of Minimum Inhibitory Concentrations

Different concentrations of 400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL of each extract were made by two-fold serial dilutions. One hundred (100µl) each of a standardized inoculum of the test bacterium was added to mixtures of different concentrations of extracts with Mueller-Hinton broth (13). Each tube was incubated for 24 hours at 37 °C. The test tubes were examined for turbidity, indicating growth of bacteria. The MIC was recorded as the lowest concentration of extract, which hindered the growth of the organisms. After incubation, the series of dilution vessels was observed for microbial growth, usually indicated by turbidity and a pellet of microorganisms at the bottom of the vessel. The last tube in the dilution series that does not demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial agent (13). Commercial antibiotics (chloramphenicol) and DMSO were used as positive and negative (Control) reference standards to determine the sensitivity of the isolates.

Statistical Analysis

Where reported, results are significant ($p \leq 0.05$) compared to controls, with dose-response trends (e.g., increasing inhibition with concentration). Descriptive statistics were calculated using Microsoft Excel. Mean and standard deviation (SD) were computed from triplicate measurements for zones of inhibition.

3. RESULTS

Phytochemical analysis of the crude extracts (hexane, acetone, ethyl acetate, and methanol) of the stem bark of *S. kunthianum* was determined as indicated in Tables 1 and 2. Tannins, flavonoids, carbohydrates, and phlobatannins were all present in acetone, ethyl acetate, and methanol extracts. Both terpenoids and saponins were present in acetone and methanol extracts, while cardiac glycosides were present in ethyl acetate and methanol extracts. Alkaloids were present in the methanol extract, and Steroids were absent in all four crude extracts. None of the phytochemicals were detected in the hexane extract.

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The results of the antibacterial activity of the crude extracts were presented (Table 3). The crude extracts showed variable antibacterial activity against S. aureus and E. coli (Table 3). The methanol extract exhibited the strongest inhibition, with mean zones of 17.00 ± 0.50 mm (S. aureus) and 14.00 ± 0.50 mm (E. coli). Ethyl acetate followed closely (14.00 ± 0.44 mm and 12.00 ± 0.20 mm, respectively). Acetone showed moderate activity, while hexane was weakly active only against S. aureus (5.60 ± 0.53 mm) and inactive against E. coli (0.00 ± 0.00 mm). All extracts were less potent than the positive control chloramphenicol.

The minimum inhibitory concentration (MIC) for the methanol extract was 12.5 mg/mL against both *S. aureus* and *E. coli*. The MIC for the ethyl-acetate extract was 12.5 mg/mL against *S. aureus* and 25 mg/mL against *E. coli* (Table 4). Comparative analysis across studies shows aqueous extracts are less potent than methanolic ones, possibly due to better solubility of actives in methanol.

Table 1: Colour, texture, yields, and percentage recoveries of the crude extracts of Stereospermum kunthianum.

Solvent extract	Colour and Texture	Weight of sample(g)	Extract yield(g)	Percentage Recovery(%)
Hexane	Golden yellow, oily liquid	200	2.50	1.25
Ethylacetate	Dark green, oily Liquid	190	1.50	0.79
Acetone	Dark brown, oily Liquid	170	1.20	0.71
Methanol	Dirty brown Granules	150	2.00	1.30

Notes: Percentage recovery = (weight of extract/ weight of powdered sample) x 100. Hexane was used initially at 200 g; subsequent solvents were used on the remaining residue (sequential extraction).

"Crude extract yields ranged from 0.71% to 1.30% (mean = 1.01 ± 0.31%, n=4)."

Table 2: Phytochemical screening of the stem bark extracts of S. kunthianum

Phytochemicals	Names of test	Solvent extracts			
		Hexane	Ethyl-acetate	Acetone	Methanol
Alkaloids	Wagner's test	-	-	-	+
	Dragend-off's Test	-	-	-	+
Steroids	Cone. HSO-	-	-	-	-
Saponins	Frothing test	-	-	+	+
Tannins	Ferric chloride	-	+	+	+
Flavonoids	Lead acetate	-	+	+	+
Cardiac glycosides	Fehling's solution A and B	-	+	-	+
Phlobatan-nins	Hcl	-	+	+	+
Carbohy-drates	Benedict's reagent	-	+	+	+
Terpenoid	Chloroform	-	-	+	+

Key: + denotes 'Positive' (Detected); - denotes 'Negative' (Not detected)

- **Summary from text:** No phytochemicals detected in hexane extract. Tannins, flavonoids, carbohydrates, and phlobatannins are present in acetone, ethyl acetate, and methanol. Steroids are absent in all extracts.

Table 3: Antibacterial activity of the stem bark extracts of S. kunthianum

Test microorganisms	Solvent extracts and susceptibility to the test Microorganisms and zone of inhibition (mm)				Control	
	Hexane	Ethyl-acetate	Acetone	Methanol	+	-
S. aureus	5.60 ±0.53	14.00 ±0.44	10.00 ±0.50	17.00 ±0.50	19.00 ±1.00	0.0 ±0.0
E. coli	0.00 ±0.00	12.00 ±0.20	8.00 ±0.20	14.00 ±0.50	17.50 ±0.87	0.0 ±0.0

Key: +ve indicates (Chloramphenicol); -ve indicates Dimethylsulfoxide (DMSO).

By microorganism (mean across 4 extracts):

- *S. aureus*: 11.65 mm (SD of means = ±4.95 mm, range 5.60–17.00 mm)
- *E. coli*: 8.50 mm (SD of means = ±6.19 mm, range 0.00–14.00 mm)

By extract (mean across 2 bacteria, compared to chloramphenicol mean = 18.25 mm):

Extract	Mean Zone (mm)	Difference from Control (mm)	Interpretation
Hexane	2.80	−15.45	Very weak/inactive vs E. coli
Acetone	9.00	−9.25	Moderate
Ethyl-acetate	13.00	−5.25	Good
Methanol	15.50	−2.75	Strongest among the extracts
Chloram-phenicol	18.25	–	Positive Control

Values represent mean zones of inhibition (mm) from triplicate assays. Methanol extract showed the highest activity (mean 15.50 mm across organisms), followed by ethyl acetate (13.00 mm). All extracts were less potent than chloramphenicol (mean 18.25 mm).

All plant extract values represent mean ± standard deviation (SD) from triplicate (n=3) independent assays using the agar well diffusion method (6 mm well diameter, 400, 200, 100, 50, 25, 12.5 mg/mL). Assays were performed under standardized conditions at 37°C for 24 h.

Table 4: Minimum inhibitory concentration (MIC) of solvent extracts of S. kunthianum

Test Organisms	Solvent Extracts	Minimum inhibitory concentration (mg/ml)					
		400	200	100	50	25	12.5
S. aureus	Ethyl-acetate	ND	ND	ND	+	+	0+
	Methanol	+	+	+	+	+	0+
E. coli	Ethyl-acetate	ND	ND	+	+	0+	-
	Methanol	+	+	+	+	+	0+

Key: + = Inhibition; 0+ = Minimum inhibition (i.e., MIC); - = No inhibition; ND = Not determined.

- **Summary from text**:
- Methanol extract MIC = 12.5 mg/mL against both S. aureus and E. coli.
- Ethyl acetate extract MIC = 12.5 mg/mL against S. aureus; 25 mg/mL against E. coli.

MIC values (mg/mL, n=4 combinations):

Organism	Extract	MIC (mg/mL)
S. aureus	**Ethyl-acetate**	**12.5**
S. aureus	**Methanol**	**12.5**
E. coli	**Ethyl-acetate**	**25.0**
E. coli	**Methanol**	**12.5**

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By organism (mean MIC):

- *S. aureus*: 12.50 mg/mL
- *E. coli*: 18.75 mg/mL

By extract (mean MIC):

- Methanol: 12.50 mg/mL
- Ethyl-acetate: 18.75 mg/mL

The methanol extract exhibited the lowest mean MIC (12.50 mg/mL) against both organisms, indicating better potency than ethyl acetate (mean MIC 18.75 mg/mL). Overall mean MIC across tested combinations was 15.63 ± 6.25 mg/mL.

4. DISCUSSION

Preliminary phytochemical screening of the stem bark extracts of *S. kunthianum* revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, phlobatannins, and carbohydrates. The antimicrobial activity exhibited by the extracts of the stem bark of *S. kunthianum* may be due to the presence of the secondary metabolites revealed in the phytochemical screening. Secondary metabolites possess pharmacological activities that are responsible for the use of plants in traditional phytomedicine to treat diseases caused by pathogenic microorganisms (2). The finding of this study agrees with the result obtained by (14) that phenolic compounds, including alkaloids, exhibit a wide range of antimicrobial activities.

Previous findings have shown that *S. kunthianum* has no alkaloids in aqueous extract (3). However, this study showed that alkaloids were present in the methanolic extract. This may be due to geographical locations and the season. The antibacterial activity of the methanolic extract of the stem bark of *S. kunthianum* showed a marked activity compared to other solvent extracts. The extract was more potent on gram-positive *S. aureus* with a maximum zone of growth inhibition of 17.

The phytochemical profile indicates a rich diversity of bioactive compounds, predominantly polar ones (tannins, flavonoids, saponins) extractable in aqueous or methanolic solvents. This supports the plant's ethnomedicinal use, as tannins and flavonoids are known to disrupt microbial cell membranes, chelate metals, or inhibit enzymes(9). Inconsistencies (e.g., alkaloids present in some studies but absent in others) may arise from variations in extraction solvents, plant collection sites, or seasonal factors affecting metabolite accumulation. Overall, the stem bark appears to be a promising source of natural antimicrobials, but standardization of extraction methods is needed for reproducibility.

The methanol extract exhibited the lowest mean MIC (12.50 mg/mL) against both organisms, indicating better potency than ethyl acetate (mean MIC 18.75 mg/mL). Overall mean MIC across tested combinations was 15.63 ± 6.25 mg/mL.

Compared to the Control (chloramphenicol), the extracts were less potent. Indicating the crude extract is not highly potent but could be useful as a complementary therapy. Zones of inhibition (5-17 mm) suggest broad-spectrum potential, particularly against *S. aureus* and *E. coli*, which are common pathogens in traditional treatment contexts (e.g., wounds, respiratory infections).

High MIC values suggest the need for compound isolation (e.g., via chromatography) to identify potent leads(2). The antimicrobial effects validate traditional uses but highlight moderate potency, potentially enhanced by fractionation or combination with other agents.

5. CONCLUSION

The stem bark extract of *S. kunthianum* shows dose-dependent antibacterial activity against pathogenic bacteria, though it is less potent than standard antibiotics. The presence of many bioactive compounds supports its traditional use in treating infections, but high MIC values indicate the need for isolating active fractions to enhance potency. This research supports traditional applications that the plant is potent in the cure of skin infections, respiratory infections, diarrhea, venereal disease, and other infectious diseases linked to bacterial pathogens. However, clinical translation requires standardized extraction, purified compounds identification, and rigorous trials for efficacy and safety.

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References

1. Hanwa UA, Tijjani MA, Sani YM, Atiku I, Sule MI, Hassan HS, et al. Antimicrobial activity of ipolamiide isolated from the stembark of <i>Stereospermum kunthianum</i> Cham (Bignoniaceae). Journal of Pharmacy & Bioresources. 2018 Jun 14;14(2):150.

2. Adegun A. Thin Layer Chromatography Bio-autography guided identification of Antibacterial constituents of leaf extract of Stereospermum kunthianum Cham. (Bignoniaceae). The Nigerian Journal of Pharmacy. 2023 Mar 31;57(1).

3. Aliyu A, Dadah AJ, Musa FM. Antibacterial Activity of Stereospermum kunthianum against Some Bacterial Isolates. Sahel Journal of Life Sciences FUDMA. 2023 Dec 31;1(1):271–7.

4. Alzohairy MA. Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evidence-Based Complementary and Alternative Medicine. 2016 Jan;2016(1).

5. Nwinyi FC, Abdu P, Mohammed A, Saidu s. NA. Pharmacological justification for the ethnomedicinal use of stem bark extract of Stereospermum kunthianum Cham. (Family: Bignoniaceae) for wound healing effects. Journal of Medicinal Plants Studies. 2021;9(3):19–27.

6. Berhe AB. Phytochemical investigation and antimicrobial activity of leaf extracts of Stereospermum kunthianum [master's thesis]. [Haramaya, Ethiopia.]: Department of Chemistry, Haramaya University; 2018.

7. Nenge HP, Ujah FO, Ezugwu CH. Phytochemical screening and in vitro antioxidant property of ethanol extract of Stereospermum kunthianum stem bark. Chemistry Search Journal. 2021;12(1).

8. Burkill HM. The useful plants of West Tropical Africa . 2nd ed Families A–D. Vol. 1. Richmond, United Kingdom: Royal Botanic Gardens, Kew; 1985.

9. Ugboko HU, Nwinyi OC, Oranusi SU, Fatoki TH, Omonhinmin CA. Antimicrobial Importance of Medicinal Plants in Nigeria. The Scientific World Journal. 2020 Sep 22;2020:1–10.

10. Williams C, Lioyd MD. Infections and contagious diseases. . National Institute of Allergy and Infectious Diseases. 2019;

11. Ogbeba J, Iruolaje FO, Dogo BA. Antimicrobial efficacy of Guiera sengalensis and Prosopis africana leaf extract on some bacterial pathogens. European Journal of Biology and Medical Science Research.

12. Cheesbrough G, Brauner A. District laboratory practice in tropical countries . 2nd edition. New York, NY: Cambridge University Press.; 2009.

13. Andrews JM. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy. 2001;48(SUPPL. 1).

14. O. O. Olajuyigbe. In vitro pharmacological activity of the crude acetone extract of Erythrina caffra Thunb: antibacterial and antifungal assessment. Journal of Medicinal Plants Research. 2012 Mar 9;6(9).

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Protective Role of Aframomum melegueta Against Arsenic-Induced Oxidative and Neurobehavioral Toxicity in Drosophila melanogaster

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ABSTRACT:

Purpose: *This study investigated the protective effects of the ethanol extract of Aframomum melegueta against arsenic-induced oxidative stress, biochemical alterations, and neurobehavioral dysfunctions in Drosophila melanogaster. The aim was to determine whether the Extract could mitigate arsenic-mediated lipid peroxidation, antioxidant suppression, and locomotor impairment, while improving survival outcomes.*

Methodology: *Wild-type Drosophila melanogaster were divided into four groups: control, arsenic-treated, extract-treated, and combined treatment. Arsenic toxicity was induced using sodium arsenite, and the plant extract was administered through dietary inclusion. Biochemical assays (MDA, catalase, GST, thiol, protein levels), survival studies, negative geotaxis, and larval locomotion tests were performed. Data were analyzed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$).*

Findings: *Arsenic exposure significantly increased MDA and reduced catalase activity and thiol levels, indicating oxidative stress. Aframomum melegueta significantly reversed these alterations, reducing MDA and restoring catalase and thiol concentrations. GST activity increased significantly in the combined treatment. Behaviourally, arsenic impaired climbing ability and locomotor activity, while the Extract improved locomotor distance and demonstrated significant antioxidative effects and modest improvement in selected neurobehavioral parameters. Survival rates declined sharply in arsenic-treated groups but improved moderately with extract supplementation.*

Research Implications/Limitations: *The study provides preliminary evidence that Aframomum melegueta exerts antioxidative effects and modest neurobehavioral protection against arsenic-induced toxicity in Drosophila melanogaster. However, the absence of mechanistic analyses and reliance on an invertebrate model limit direct translational interpretation.*

Practical Implications: *The findings suggest that Aframomum melegueta may represent a potential source of bioactive compounds with antioxidative properties, warranting further mechanistic*

and translational investigation. Its antioxidant properties highlight its potential for incorporation into nutraceutical formulations. However, optimal dosing, long-term safety, and clinical applicability remain to be established.

KEYWORDS:

Aframomum melegueta, Antioxidants, Arsenic toxicity, Drosophila melanogaster, Lipid peroxidation, Oxidative stress, Survival assay.

1. Introduction

Arsenic, a naturally occurring metalloid, presents a considerable health hazard when found in the environment at high concentrations [1]. Arsenic is present in groundwater, soil, and air as a result of both natural processes and human activities, including mining, industrial waste discharge, and the application of arsenic-laden pesticides [2]. Prolonged exposure to arsenic, particularly via drinking water, has been linked to several detrimental health effects, including cardiovascular illnesses, dermal lesions, and multiple cancer types [3]. Arsenic enters the human body mainly by eating or inhalation, and upon absorption, it is metabolically converted into highly hazardous metabolites, including trivalent and pentavalent arsenic compounds [4]. These substances trigger a series of detrimental consequences at the cellular level, including oxidative stress, DNA damage, and disruption of essential metabolic processes [5].

Extended arsenic exposure has been widely associated with oxidative stress, cellular dysfunction, and neurological impairments across experimental models [6]. Oxidative stress is crucial in the onset and advancement of several cancer types, including arsenic-induced lung cancer [7]. Overproduction of reactive oxygen species (ROS) may lead to significant cellular damage through the oxidation of proteins, lipids, and DNA. Consequently, they may serve as a primary catalyst for carcinogenesis by fostering genetic instability, cell death, and chronic inflammation, all of which facilitate tumor development [8]. Scientific evidence has shown that arsenic exposure markedly increases ROS generation in arsenic-induced cancer, surpassing the body's antioxidant defense mechanisms [9]. This may result in lipid peroxidation, protein oxidation, and DNA strand breaks, all of which facilitate the onset and advancement of cancer [10]. *Aframomum melegueta*, referred to as grains of paradise, is a tropical plant in the Zingiberaceae family. The seeds possess a spicy, peppery flavor and are abundant in bioactive components such as gingerols, paradols, shogaols, and essential oils [11]. These compounds possess antioxidant, anti-inflammatory, and anticancer effects. In traditional and contemporary West African cuisines, *Aframomum melegueta* serves as a principal flavoring agent in soups, jollof rice, and pepper soup [12,13]. The seeds are typically subjected to maceration to facilitate the liberation of volatile aromatic constituents [14]. In Southwestern Nigeria, the seed of *Aframomum melegueta* is well-reputed in ceremonial, ritualistic, and sociocultural practices, where it may function as a masticatory or be exchanged as an emblem of hospitality and benediction [15]. The antioxidant efficacy of *Aframomum melegueta* is mostly ascribed to its elevated phenolic and flavonoid concentrations, which facilitate the neutralization of free radicals and safeguard cells from oxidative harm [16]. This study was designed as an exploratory investigation to evaluate whether the ethanol extract of *Aframomum melegueta* can attenuate arsenic-induced oxidative stress, biochemical disruptions, and neurobehavioral impairments in *Drosophila melanogaster*. We hypothesized that the Extract would mitigate arsenic toxicity primarily through modulation of oxidative stress markers and antioxidant defense enzymes. While specific molecular signaling pathways were not directly examined, the findings are intended to provide a foundation for future mechanistic studies.

2. MATERIALS

Drosophila melanogaster, Spatula, Conical flask (200ml and 1000ml), Trays, Vials, Foam (cork), Eppendorf tubes, Test tubes, Rack, Tips, Small paint brush, Refrigerator, Petri dish, Counting pad, Jars, Gloves, Animal feed, Distilled water, Centrifuge, Sensitive scale, Incubator, Hot plate, Homogenizer, Arsenic compound (26061340191), *Aframomum melegueta* seeds, Ethanol, Anesthetic CO_2.

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3. METHODS

EXPERIMENTAL DESIGN

Drosophila melanogaster was obtained and cultured in the Eureka Research Laboratory, Department of Anatomy, at Babcock University. The flies were maintained at a temperature of 25℃. After the breeding phase, the flies were divided into four treatment groups as follows:

TABLE 1: EXPERIMENTAL DESIGN

Group	Treatment	Purpose
Control	Basal diet	Serve as a baseline for comparison with other experimental groups.
Arsenic-Treated	0.005g/10g of diet	Assess the impact of arsenic exposure on oxidative stress markers and antioxidant defenses.
Plant extract	0.015g/10g of diet	Observe effects of the Extract on normal cellular processes and oxidative stress in the absence of arsenic.
Combined Treatment Group	0.005g+ 0.015g/10g of diet	Investigate the effects of the Extract against arsenic-induced oxidative stress and toxicity conditions.

Each experimental group consisted of five independent vials, with fifty flies per vial (total n = 250 flies per group). Behavioral assays were performed using randomly selected flies or larvae from each vial to ensure representative sampling. A single-dose experimental framework was employed to evaluate baseline toxic and protective effects prior to dose–response optimization.

ARSENIC EXPOSURE

Arsenic Compound: Sodium arsenite ($NaAsO_2$) was used to induce oxidative stress and lung cancer-like phenotypes in *Drosophila melanogaster*.

IDENTIFICATION AND PREPARATION OF PLANT

Aframomum melegueta was obtained from Ilishan-Remo, Ogun State, Nigeria. Fresh seeds of *A. melegueta* were air-dried (16 °C), pulverized, and soaked in absolute ethanol for 72 hours, then sieved in order to remove any non-polar compounds from the Extract. and stored in a jar.

PHYTOCHEMICAL CHARACTERIZATION BY HPLC

High-performance liquid chromatography (HPLC) analysis was conducted to characterize the phytochemical constituents of the ethanol extract of *Aframomum melegueta*. The Extract was prepared according to standard chromatographic procedures and analyzed using an HPLC system equipped with a UV–Vis detector. Chromatograms were recorded at multiple wavelengths to detect phenolic and related bioactive compounds. The resulting chromatographic profile was used to generate a chemical fingerprint of the Extract employed in this study.

DROSOPHILA MELANOGASTER STOCK AND CULTURE

The wild D. melanogaster was obtained from the Eureka *Drosophila* Laboratory, in the Department of Anatomy, Benjamin S. Carson School of Medicine, Babcock University, Ogun State, Nigeria. *D. Melanogaster* cultured on a corn meal medium containing corn meal, yeast, sugar, agar-agar and nipagin mixed with ethanol at a constant temperature and humidity (21-25°C; 60-70% relative humidity) under 12hours dark/light cycle conditions at the Eureka *drosophila* laboratory, in the department of Anatomy, Benjamin S. Carson School of Medicine, Babcock University, Ogun State, Nigeria.

MEAL PREPARATION

I. 1000ml of water was added to a pot and allowed to boil on an electric cooker.

II. A sensitive scale was used to measure 26g of yeast, and 25g of sugar was added to the boiling water, stirring this mixture for 5 minutes.

III. Using the sensitive scale again, 13g of agar-agar was measured and added gently to the boiling mixture inside the pot, and then stirred for 5 minutes.

IV. 200ml of distilled water was used to mix 90g of cornmeal in a mixing bowl. After it was mixed properly, it was added to the still-boiling mixture and stirred for 5 minutes.

V. 1 Cap of *Aframomum melegueta* and 1 cap of arsenic dissolved in ethanol were mixed with 5g of Nipagin in a petri-dish, and this was carefully combined.

VI. The Cornmeal mixture was brought down from the electric cooker and allowed to cool for some minutes.

VII. The Nipagin mixture was added to the Cornmeal mixture after being cooled and stirred.

TABLE 2: TABULAR REPRESENTATION OF MEAL PREPARATION

S/N	INGREDIENTS	FULL MEAL
1.	Distilled water	1000ml
2.	Corn meal	90g
3.	Yeast	26g
4.	Nipagin	5g
5.	Agar agar	13g
6.	Sugar	25g

DOSAGE AND ADMINISTRATION

The ethanol crude extract was solubilized in distilled water and integrated into the diet of *Drosophila melanogaster*. The arsenic dose (0.005 g sodium arsenite per 10 g of diet) was selected based on previously reported *Drosophila melanogaster* studies demonstrating consistent induction of oxidative stress and neurobehavioral impairment without causing excessive early mortality, thereby allowing meaningful biochemical and behavioral assessments.

The dose of *Aframomum melegueta* extract (0.015 g per 10 g of diet) was selected following preliminary tolerability observations, which indicated no overt toxicity or adverse behavioral effects in flies, and in alignment with doses reported for antioxidant-rich plant extracts in *Drosophila* and related in vivo models. This single-dose approach was adopted to establish initial efficacy under controlled exposure conditions.

Arsenic was delivered by dietary consumption. The exposure period was extended for 7 days, providing adequate Time for the manifestation of oxidative stress indicators. At the conclusion of the seventh day, a specific group of flies was allocated for biochemical and behavioral experiments. The remaining cohort of flies had to undergo a survival test for an additional 14 days, culminating in a total duration of 21 days.

BIOCHEMICAL ASSAY

Malondialdehyde (MDA)

The MDA concentration was analyzed as previously described by [17].

Total Thiol Content:

Total thiol concentration was described based on the method of [18].

Glutathione S-transferase

The GST activity was measured spectrophotometrically based on the method of [19]. The activity of GSH was expressed in μmol/mg protein.

Catalase Activity

Catalase activity was assessed by introducing H_2O_2 to the sample and observing its breakdown by the measurement of absorbance reduction at 240 nm. Catalase activity was quantified in units per milligram of protein [20].

Total Protein Content

Bradford Assay

The total protein content was quantified using the Bradford technique, which depended on the interaction of Coomassie Brilliant Blue dye with proteins, producing a colorimetric change measurable at 595 nm [21].

SURVIVAL TEST

The survival test was carried out to monitor the number of flies that survive for 21 days following exposure to arsenic and the plant extract. Each day, the number of active flies was counted to see the effects of the plant extract. The vials were cleaned properly, and all the dead flies were removed after 7 days consecutively for 21 days.

BEHAVIORAL ASSAY

NEGATIVE GEOTAXIS ASSAY

Negative geotaxis assay, also known as the climbing assay, was carried out to determine the locomotor activity of the flies. To carry out this

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experiment, 10 flies from the control and treated group were anesthetized and placed in labeled vials marked at 6cm. The bottom of the vials was gently tapped, and then allowed to ascend. The number of flies in each vial that ascended to the 6cm level in 10 seconds was counted, and the numbers were recorded and used to determine the climbing activity.

LARVA CRAWLING ASSAY

From each group, ten (10) third instar larvae were collected with a damp (with sucrose) paint brush and placed on a slightly wet (with sucrose) petri dish to aid the movement of the larvae. Records of larva movements were recorded for 10s, after which the videos were analyzed. Larvae's distance traveled, and number of contractions were noted down within the 10s. The speed and contraction ratio were calculated using the following formula:

Speed (s) = Distance traveled by the lava / Time taken

Contraction ratio = Distance traveled by the larva/number of contractions

STATISTICAL ANALYSIS

The data from the biochemical assays were analyzed using one-way analysis of variance (ANOVA) followed by post hoc comparisons with Tukey's test. This allowed for the comparison of means across different treatment groups and determined whether the differences were statistically significant ($p < 0.05$). Data are presented as mean ± SEM. Behavioral measurements were obtained from randomly selected flies drawn from five independent vials per group (50 flies per vial).

4. RESULTS

HPLC Phytochemical Profile of Aframomum melegueta Extract

HPLC analysis of the ethanol extract of *Aframomum melegueta* revealed the presence of multiple phytochemical constituents, evidenced by distinct peaks observed at different retention times and detection wavelengths (Figs. 1a and 1b). The chromatographic fingerprint indicates a complex mixture of phenolic and related bioactive compounds, consistent with previously reported antioxidant constituents of *Aframomum melegueta*. These phytochemicals likely contribute to the observed antioxidative and neurobehavioral effects in arsenic-exposed *Drosophila melanogaster*.

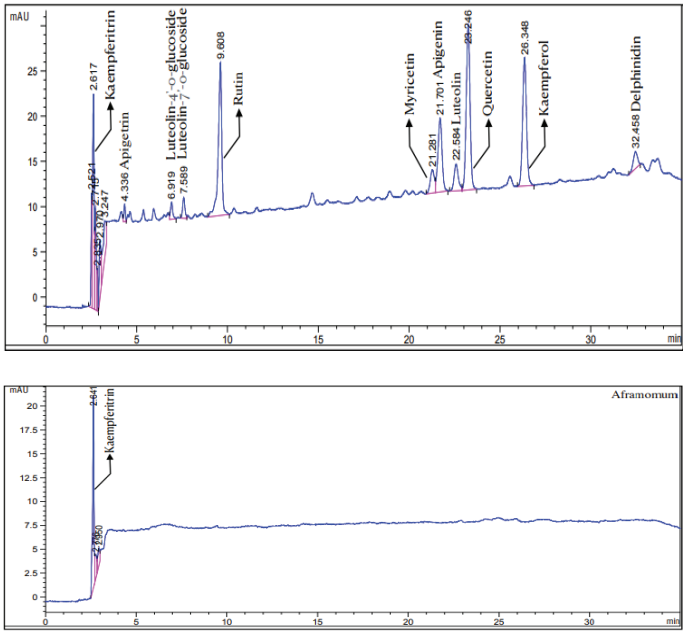

Figure 1. HPLC profiles of A. melegueta ethanol extract. (A) Separation of compounds at a fixed wavelength. (B) Detection across multiple wavelengths, confirming the presence of diverse phytochemicals with unique spectral signatures.

Legend: The results demonstrate the complex phytochemical composition of *A. melegueta*. Key bioactive compounds are indicated by numbered peaks (1, 2, 3...), corresponding to metabolites identified or characterized in subsequent analyses.

Effect of Arsenic and Aframomum melegueta on Survival Rate in Drosophila melanogaster

The survival assay revealed distinct mortality patterns across treatment groups over 21 days. Control flies maintained 100% survival until day 3, gradually declining to 78-90% by day 21. Arsenic-exposed flies showed accelerated mortality, dropping to 56-60% survival by day 21, with particularly steep declines between days 4-7 (100% to 74-84%). The *Aframomum melegueta* group exhibited intermediate survival (62-88% at day 21), while the combined treatment group (arsenic + Aframomum) demonstrated the most significant protection, maintaining 54-84% survival - comparable to arsenic-alone mortality rates but with less severe early-stage decline. These patterns suggest that while Aframomum alone provided modest protection against natural aging-

related mortality, its combination with arsenic did not prevent arsenic›s acute lethal effects, though it may have mitigated some late-stage toxicity. The differential survival trajectories highlight the time-dependent nature of arsenic toxicity and potential protective mechanisms of Aframomum that may require longer exposure periods to manifest fully in survival outcomes (Fig. 2).

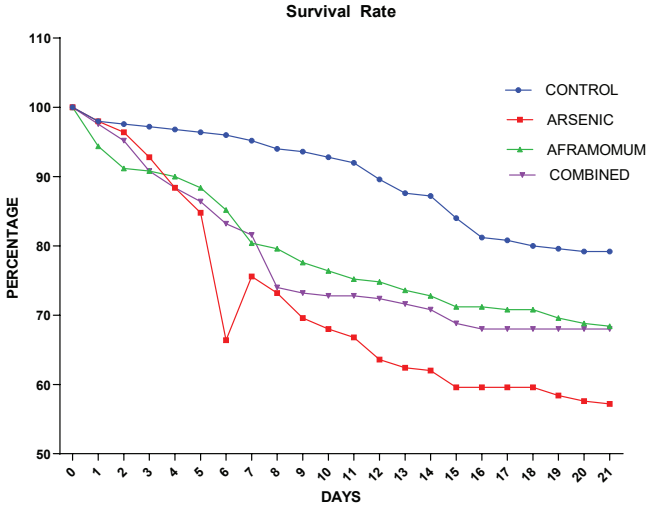

Figure 2: Effect of Aframomum melegueta extract on the survival rate of Drosophila melanogaster exposed to arsenic toxicity.

Survival percentages of flies were monitored over 21 days across treatment groups. Arsenic exposure reduced survival significantly, while *Aframomum melegueta* supplementation partially restored lifespan and mitigated late-phase mortality. Values represent mean ± SEM.

Effect of Arsenic and Aframomum melegueta on Negative Geotaxis Behavior in Drosophila melanogaster

ANOVA indicated moderate treatment effects on climbing behavior, with Tukey's tests yielding mixed significance (Fig. 3). Arsenic exposure significantly impaired geotactic performance relative to control (p = 0.0168), suggesting neurobehavioral dysfunction due to oxidative damage. *A. melegueta* alone did not differ significantly from control (p = 0.9020) or arsenic (p = 0.6793). The combined treatment showed partial improvement but did not reach statistical significance (p = 0.0628–0.9729). Although *Aframomum melegueta* treatment showed a tendency toward improved climbing performance, this effect did not reach statistical significance, indicating only partial and variable behavioral modulation.

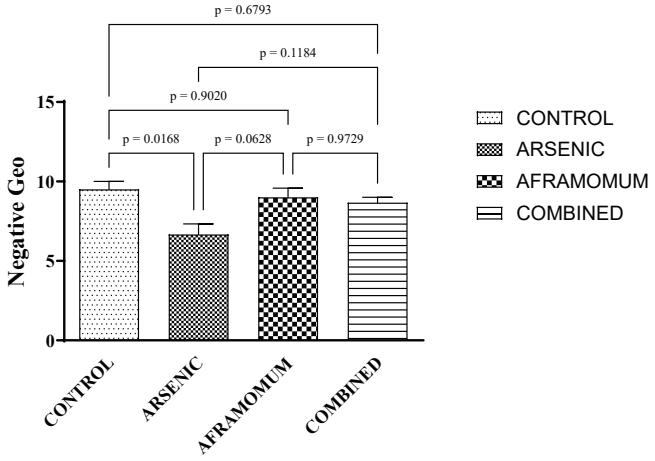

Figure 3: Effect of Aframomum melegueta extract on negative geotaxis behavior in arsenic-treated Drosophila melanogaster.

Effect of Arsenic and Aframomum melegueta on Contraction Parameters in Drosophila melanogaster

While overall ANOVA indicated treatment-dependent differences (p = 0.0408), pairwise comparisons revealed that improvements associated with *Aframomum melegueta* did not reach statistical significance, suggesting modest functional trends rather than robust effects. Tukey's test showed that arsenic exposure caused a reduction compared to control, while *Aframomum* supplementation alone or in combination partially improved contraction amplitude (Fig. 4), though these differences were not significant (p = 0.1403–0.9772). The significant ANOVA suggests at least one group differed, likely due to arsenic's depressive effect on muscular contraction, with *Aframomum melegueta* exhibiting mild protective trends against this impairment.

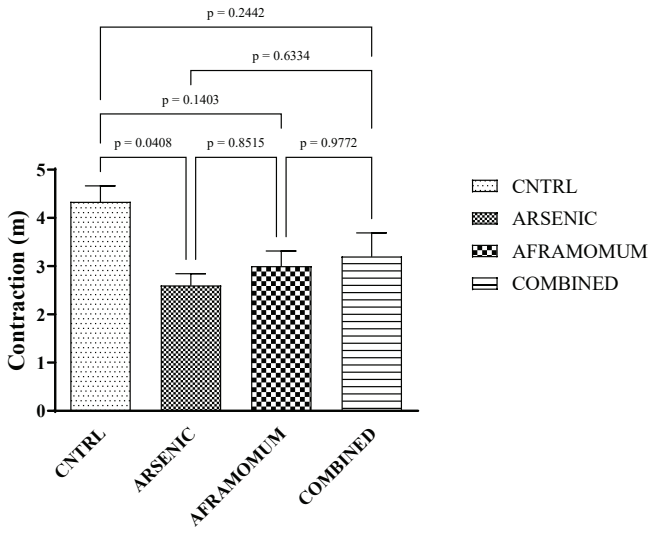

Figure 4: Effect of Aframomum melegueta extract on contraction movement in Drosophila melanogaster exposed to arsenic toxicity

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Effect of Arsenic and Aframomum melegueta on Locomotor Distance in Drosophila melanogaster

ANOVA results approached significance (p = 0.0515) with post hoc Tukey's tests indicating that *Aframomum melegueta* treatment significantly increased locomotor distance compared to arsenic exposure (p = 0.0280), indicating partial functional recovery. Control and combined groups did not differ significantly (p = 0.3973–0.9540; Fig. 5). Thus, arsenic reduced movement activity, reflecting motor impairment, whereas *Aframomum* ameliorated this reduction, highlighting its neuroprotective effect on locomotion.

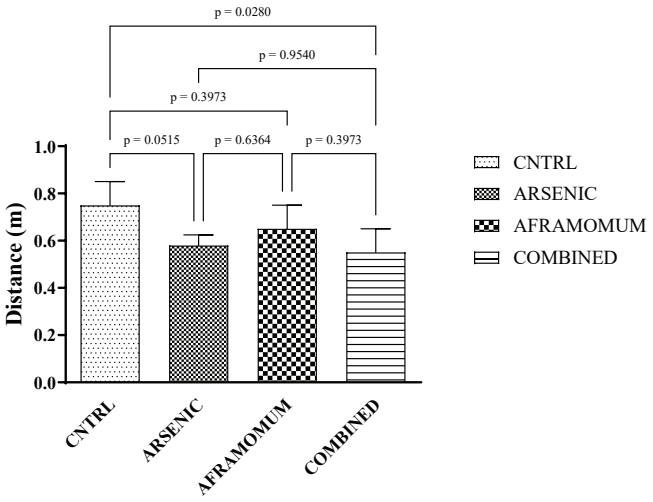

Figure 5: Effect of Aframomum melegueta extract on locomotor distance in arsenic-exposed Drosophila melanogaster

Effect of Arsenic and Aframomum melegueta on Locomotor Speed in Drosophila melanogaster

Despite a marginal overall ANOVA effect (p = 0.0400), post hoc analyses revealed no statistically significant differences between treatment groups, indicating that locomotor speed was not robustly affected (p = 0.4409–> 0.9999; Fig. 6). This suggests that while arsenic may have exerted subtle effects on movement velocity, these were not large enough to produce distinct post hoc group differences. Therefore, *Aframomum melegueta* did not significantly alter locomotor speed, though slight trends toward improvement were observed.

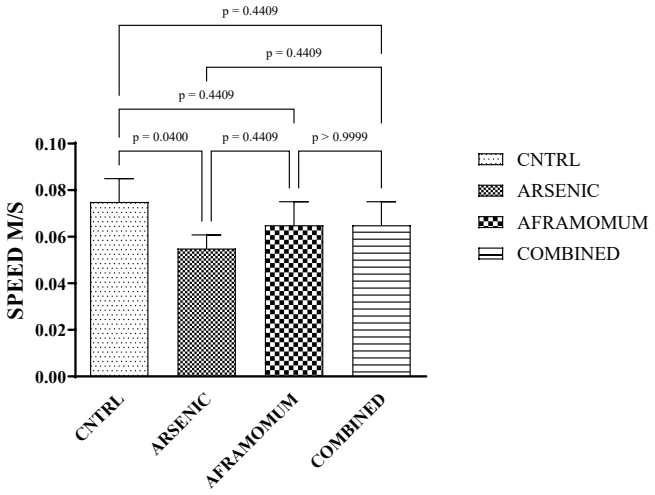

Figure 6: Effect of Aframomum melegueta extract on locomotor speed in Drosophila melanogaster exposed to arsenic toxicity

Effect of Arsenic and Aframomum melegueta on Catalase Activity in Drosophila melanogaster

ANOVA revealed a significant treatment effect on catalase activity (p = 0.0008). Post hoc Tukey's test showed that arsenic exposure caused a significant reduction in catalase levels compared with the control (p = 0.0236), indicating oxidative stress-induced enzyme inhibition (Fig. 7). Treatment with *Aframomum melegueta* significantly increased catalase activity relative to the arsenic group (p = 0.0005), while the combined treatment (arsenic + *Aframomum*) also showed a significant increase over arsenic exposure alone (p = 0.0070). These results suggest that *Aframomum melegueta* mitigated arsenic-induced oxidative suppression of catalase, restoring antioxidant capacity in *Drosophila melanogaster*.

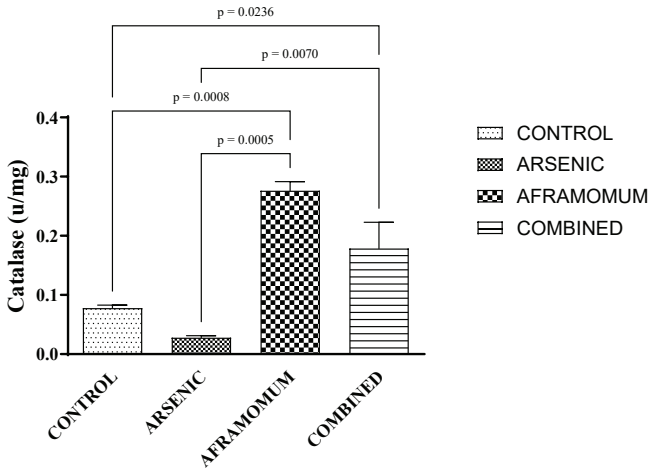

Figure 7: Effect of Aframomum melegueta extract on catalase activity in arsenic-exposed Drosophila melanogaster

Effect of Arsenic and Aframomum melegueta on Lipid Peroxidation (MDA) in Drosophila melanogaster

ANOVA results indicated a highly significant effect of treatments on MDA levels ($p = 0.0002$). Tukey's test revealed that arsenic exposure caused a marked increase in MDA compared with the control ($p = 0.0038$), reflecting lipid membrane damage due to excessive ROS generation (Fig. 8). Conversely, *Aframomum melegueta* administration significantly reduced MDA relative to the arsenic group ($p < 0.0001$), while the combined treatment also showed lower MDA compared with arsenic exposure ($p = 0.0010$). The difference between control and combined treatment was modest but significant ($p = 0.0010$). Collectively, these results demonstrate that *Aframomum melegueta* attenuated arsenic-induced lipid peroxidation, highlighting its antioxidative efficacy.

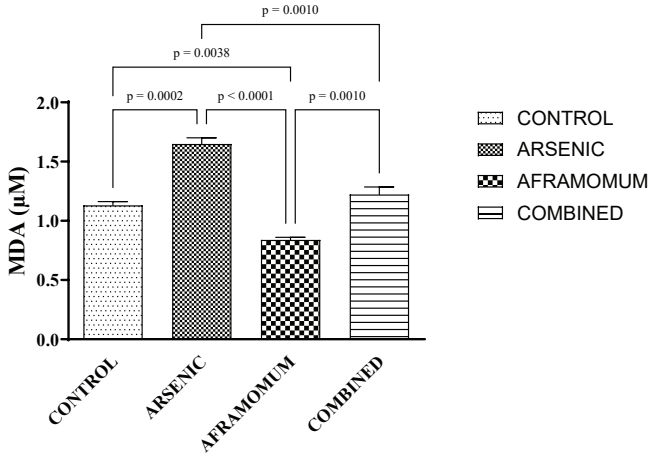

Figure 8: Effect of Aframomum melegueta extract on lipid peroxidation (MDA levels) in Drosophila melanogaster subjected to arsenic toxicity

Effect of Arsenic and Aframomum melegueta on Glutathione S-Transferase (GST) Activity in Drosophila melanogaster

ANOVA showed no significant differences across most treatment comparisons except at specific pairings. Tukey's test indicated no significant change between control and arsenic ($p = 0.8813$), or between control and *Aframomum* ($p = 0.7260$). However, the combined treatment exhibited significantly higher GST activity compared with arsenic exposure ($p = 0.0261$) and *Aframomum* alone ($p = 0.0168$). These data (Fig. 9) suggest that although individual treatments had limited impact, co-administration of *Aframomum melegueta* with arsenic enhanced detoxification enzyme activity, implying a synergistic upregulation of GST-mediated defense against oxidative insult.

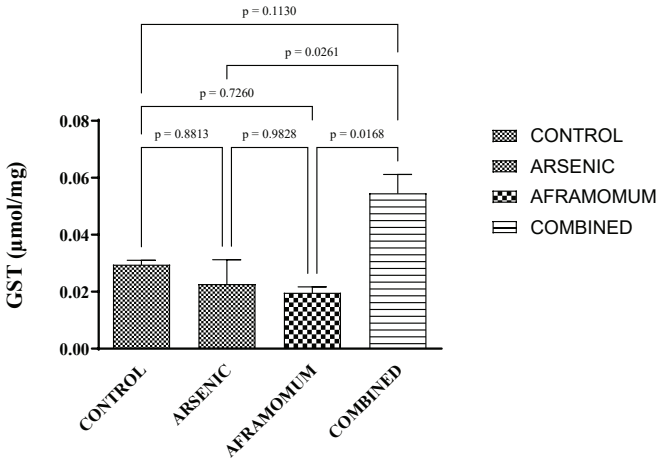

Figure 9: Effect of Aframomum melegueta extract on glutathione S-transferase (GST) activity in arsenic-treated Drosophila melanogaster

Effect of Arsenic and Aframomum melegueta on Total Thiol Levels in Drosophila melanogaster

Total thiol content showed treatment-dependent modulation. Arsenic-exposed flies exhibited decreased thiol levels compared with the control and combined treatment groups (Fig. 10), suggesting depletion of reduced thiols due to oxidative stress. *Aframomum melegueta* supplementation partially restored thiol levels, indicating antioxidant thiol preservation. Although exact p-values were not displayed, the distinct lettering denotes a statistically significant difference ($p < 0.05$) among treatments.

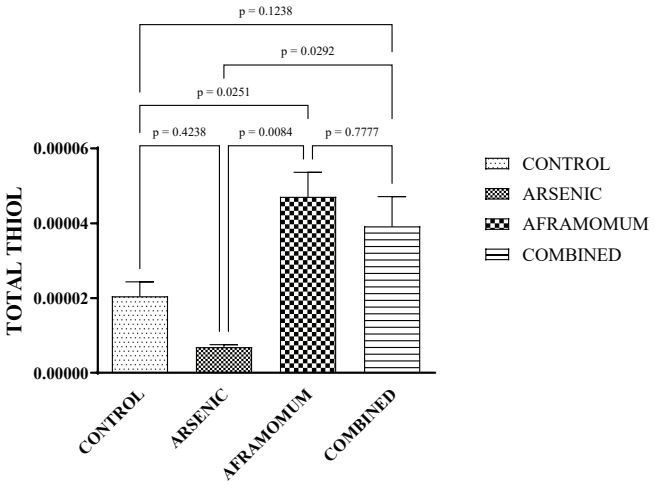

Figure 10: Effect of Aframomum melegueta extract on total thiol concentration in arsenic-exposed Drosophila melanogaster

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Effect of Arsenic and Aframomum melegueta on Total Protein Levels in Drosophila melanogaster

No significant treatment effect was observed in total protein concentration across groups. The ANOVA and Tukey's tests produced high p-values ($p = 0.9858$, $p = 0.2970$, $p = 0.6699$), showing that arsenic exposure and *Aframomum melegueta* treatment, either alone or combined, did not significantly alter overall protein content in *Drosophila melanogaster* (Fig. 11). This suggests that the interventions did not impair or stimulate general protein synthesis within the exposure period, although functional oxidative markers were altered.

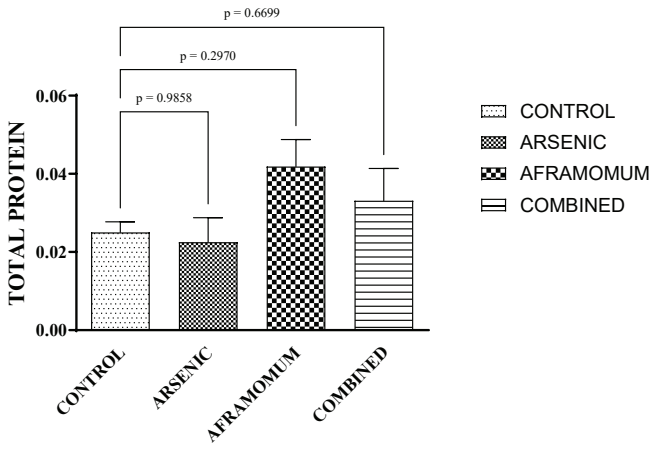

Figure 11: Effect of Aframomum melegueta extract on total protein concentration in Drosophila melanogaster under arsenic exposure

5. Discussion

The present study investigated the protective potential of *Aframomum melegueta* (grains of paradise) against arsenic-induced oxidative stress, biochemical alterations, and behavioral deficits in *Drosophila melanogaster*. The data revealed that arsenic exposure caused significant perturbations in key antioxidant and behavioral parameters [22], whereas *Aframomum melegueta* supplementation, either alone or in combination with arsenic, modulated these effects to varying degrees. Taken together, the findings highlight the phytochemical's ameliorative role against arsenic toxicity and emphasize its potential as a natural antioxidant and neuroprotective agent.

Arsenic exposure significantly suppressed catalase (CAT) activity [23] while markedly elevating lipid peroxidation levels (MDA) [24], confirming oxidative imbalance as a central mechanism of arsenic toxicity. Catalase, a primary hydrogen peroxide–scavenging enzyme, plays a vital role in cellular defense against reactive oxygen species (ROS) [25]. The significant decrease in catalase activity ($p = 0.0236$) in arsenic-exposed flies suggests enzyme inhibition via direct interaction of arsenic with sulfhydryl groups on catalase or through depletion of essential cofactors such as iron. Conversely, *Aframomum melegueta* treatment significantly restored catalase levels ($p = 0.0005$), both when administered alone and in combination with arsenic ($p = 0.0070$), demonstrating its capacity to reinforce enzymatic antioxidant defense. This observation aligns with previous reports that *Aframomum melegueta* extracts enhance catalase and superoxide dismutase (SOD) activities through their abundant phenolic and flavonoid content, including 6-gingerol, paradol, and shogaol analogs known to upregulate antioxidant gene expression and mitigate ROS accumulation [26,27].

Consistent with catalase suppression, arsenic exposure significantly increased MDA levels ($p = 0.0038$), reflecting enhanced lipid peroxidation and membrane damage [26]. Elevated MDA is a hallmark of oxidative injury resulting from peroxidation of polyunsaturated fatty acids [28]. The dramatic reduction in MDA upon *Aframomum melegueta* administration ($p < 0.0001$) indicates attenuation of lipid peroxidation, suggesting the Extract's potent ROS-scavenging activity. This is in agreement with studies by [29], who reported that *Aframomum melegueta* mitigates oxidative injury induced by heavy metals and xenobiotics in rodent tissues. The combined treatment's lowered MDA levels compared with arsenic alone ($p = 0.0010$) further supports a synergistic interaction between arsenic-induced oxidative triggers and *Aframomum*-mediated antioxidant response.

Glutathione S-transferase (GST), a critical phase II detoxification enzyme [30]the cytosolic glutathione S-transferase (GST, showed modest alterations across treatment groups. Although no significant difference was found between control and arsenic or *Aframomum* alone, the combined treatment group showed a significant elevation in GST activity compared with arsenic ($p = 0.0261$) and *Aframomum* alone ($p = 0.0168$). This suggests that co-exposure may have stimulated adaptive upregulation of GST-mediated conjugation pathways to facilitate detoxification of arsenic-derived electrophilic intermediates. The upsurge in GST could also reflect activation of the nuclear factor erythroid 2-related factor 2

(Nrf2) signaling pathway, which governs cellular redox homeostasis. The observed enhancement of GST activity may suggest involvement of redox-sensitive regulatory pathways such as Nrf2 [31]controlled production of oxidants in normal cells serves useful purposes to regulate signaling pathways. Reactive oxidants are counterbalanced by complex antioxidant defense systems regulated by a web of pathways to ensure that the response to oxidants is adequate for the body's needs. A recurrent theme in oxidant signaling and antioxidant defense is reactive cysteine thiol–based redox signaling. The nuclear factor erythroid 2–related factor 2 (Nrf2; however, this remains speculative, as direct molecular analyses were not performed in the present study.

Total thiol levels, an indirect measure of reduced glutathione (GSH) and protein thiols [32], were also modulated by treatment. Arsenic-exposed flies exhibited depleted thiol content, indicative of oxidative depletion and redox imbalance. *Aframomum melegueta* partially restored thiol concentration in the combined treatment group, consistent with its role in preserving intracellular thiol pools and reducing oxidative protein modification. These results affirm that *Aframomum melegueta* helps maintain cellular redox status by preventing excessive oxidation of cysteine residues and promoting glutathione regeneration [33].

Interestingly, total protein levels did not differ significantly across treatments ($p > 0.05$), implying that arsenic exposure and *Aframomum* intervention did not affect overall protein synthesis or degradation rates. The lack of change may reflect a selective oxidative impact restricted to specific enzymes and signaling proteins rather than a global suppression of protein metabolism.

Behavioral assays provided functional corroboration of biochemical findings. The survival rate assay revealed that arsenic exposure markedly reduced lifespan, while *Aframomum melegueta* alone or in combination modestly extended survival, particularly during later days of exposure. This partial restoration suggests that while the Extract mitigates chronic oxidative damage, it may not fully counteract arsenic's acute toxicity during early exposure phases. The observed survival improvement underscores *Aframomum's* role in delaying arsenic-induced senescence and improving overall vitality, consistent with its antioxidant action.

Arsenic exposure also caused notable impairments in neurobehavioral indices [34], including reduced climbing ability (negative geotaxis) and contraction parameters, reflecting motor dysfunction and neuromuscular weakness. The significant reduction in geotaxis performance ($p = 0.0168$) suggests neuronal toxicity in dopaminergic circuits, often implicated in motor control deficits in *Drosophila*. Although *Aframomum melegueta* did not completely restore geotaxis, the combined treatment showed partial improvement ($p = 0.0628–0.9729$), indicative of a neuroprotective trend. Similarly, contraction parameters were significantly influenced ($p = 0.0408$), where arsenic exposure impaired muscular movement amplitude, while *Aframomum* treatment showed mild recovery. These outcomes may be linked to the Extract's ability to preserve neuromuscular function through antioxidant modulation and possibly through the stabilization of acetylcholine levels, as suggested in related studies on plant-derived neuroprotectants [35]. Although oxidative stress is known to impair neuromuscular and cholinergic function [36], acetylcholinesterase activity and neurotransmitter dynamics were not evaluated in this study; therefore, the neurobehavioral improvements observed should be interpreted at a functional rather than mechanistic level.

Locomotor distance and speed further reflected arsenic-induced neurotoxicity. Arsenic significantly reduced locomotor distance, whereas *Aframomum melegueta* treatment restored movement capacity ($p = 0.0280$), indicating a functional recovery of neuromotor coordination. The partial, non-significant changes in locomotor speed ($p = 0.0400$ overall but $p > 0.44$ post hoc) suggest that while *Aframomum* improved endurance and activity span, it may not have markedly influenced velocity per se. Taken together, these behavioral findings reinforce the biochemical evidence that *Aframomum melegueta* exerts restorative effects on arsenic-induced oxidative and neuromuscular dysfunction.

Mechanistically, the ameliorative effects of *Aframomum melegueta* may be attributed to its rich repertoire of phenolic constituents and essential oils, such as 6-paradol, 6-shogaol, and 6-gingerol. These compounds are known to scavenge ROS, enhance endogenous antioxidant enzyme expression, and stabilize cellular membranes. Furthermore, the modulation of detoxification enzymes like GST and restoration of thiol levels point toward activation of the Nrf2–

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ARE signaling cascade, a key regulator of cellular defense. The protective trends observed across multiple parameters suggest that *Aframomum melegueta* acts both as a direct antioxidant and as a modulator of redox-sensitive signaling networks.

This study establishes that arsenic induces oxidative and behavioral toxicity in *Drosophila melanogaster*, characterized by elevated lipid peroxidation, suppression of antioxidant enzymes, and impaired locomotor performance. *Aframomum melegueta* effectively mitigated these effects, restoring catalase and thiol activity, reducing lipid peroxidation, enhancing GST function, and improving survival and motor performance. The findings collectively affirm the antioxidative and neuroprotective potential of *Aframomum melegueta* against heavy metal–induced toxicity. These results support its potential utility as a dietary or therapeutic agent for mitigating oxidative stress-related disorders. Further molecular studies focusing on gene expression of antioxidant enzymes, Nrf2 pathway activation, and dose–response profiling in higher models would provide deeper insights into its mechanistic efficacy and translational relevance.

Behavioral outcomes demonstrated greater variability than biochemical endpoints, which is characteristic of *Drosophila* locomotor assays. While arsenic exposure consistently impaired motor performance, the modulatory effects of Aframomum melegueta were modest and, in some assays, did not reach statistical significance. These findings suggest partial functional protection rather than complete neurobehavioral restoration.

The inclusion of HPLC-based phytochemical profiling strengthens the interpretation of the biological effects observed in this study. The chromatographic fingerprint confirms that the ethanol extract of *Aframomum melegueta* contains multiple bioactive constituents, likely including phenolic and gingerol-related compounds previously associated with antioxidant and neuroprotective activities. These compounds may collectively contribute to the attenuation of lipid peroxidation, restoration of antioxidant enzymes, and partial improvement in neurobehavioral outcomes observed following arsenic exposure. However, while HPLC profiling confirms extract complexity and reproducibility, definitive compound identification and quantification would require further targeted analyses using reference standards or mass spectrometry.

6. Study Limitations

A key limitation of this study is that the molecular mechanisms underlying the observed biochemical and behavioral effects were not examined, particularly gene expression profiles of antioxidant enzymes and the involvement of the Nrf2 signaling pathway. Nevertheless, these findings provide preliminary in vivo evidence that supports further targeted mechanistic investigations. Although HPLC profiling was performed to characterize the Extract, individual phytochemical constituents were not fully identified or quantified against reference standards. Therefore, extract standardization remains partial, and future studies should incorporate quantitative phytochemical analysis to improve translational and nutraceutical applicability. Future studies should therefore investigate these mechanistic pathways, explore dose–response relationships, and extend the findings to mammalian models to validate the translational potential of *Aframomum melegueta* as a therapeutic candidate against heavy metal–induced oxidative and neurobehavioral toxicity.

7. Conclusion

This study demonstrated that arsenic exposure induces marked oxidative and neurobehavioral disturbances in *Drosophila melanogaster*, characterized by reduced catalase activity, elevated lipid peroxidation, depletion of thiol reserves, and impaired locomotor performance. These findings establish *Aframomum melegueta* as a modulator of arsenic-induced oxidative and neurobehavioral toxicity in *Drosophila melanogaster*, providing a foundation for future mechanistic and translational studies by enhancing antioxidant enzyme activities, restoring thiol balance, and reducing lipid peroxidation, thereby protecting cellular integrity and improving behavioral outcomes. Although total protein levels remained unchanged, the modulation of catalase, GST, and thiol parameters underscores the Extract's potent redox-stabilizing capacity. Behavioral assays further confirmed partial recovery of motor coordination and survival, indicating that *Aframomum melegueta* confers both biochemical and functional protection against arsenic toxicity. These

findings highlight the therapeutic promise of *Aframomum melegueta* as a natural antioxidant and neuroprotective agent. Future studies should investigate its molecular targets, optimal dosing, and long-term safety to facilitate translational applications in metal-induced oxidative stress and neurodegenerative conditions.

Declarations

Ethical Statement:

Experiments involving *Drosophila melanogaster* are exempt from institutional ethical approval, as the species is an invertebrate model not covered under regulations governing vertebrate animal research. All experimental procedures were, however, conducted in accordance with internationally accepted standards for the humane use of invertebrate organisms in scientific research.

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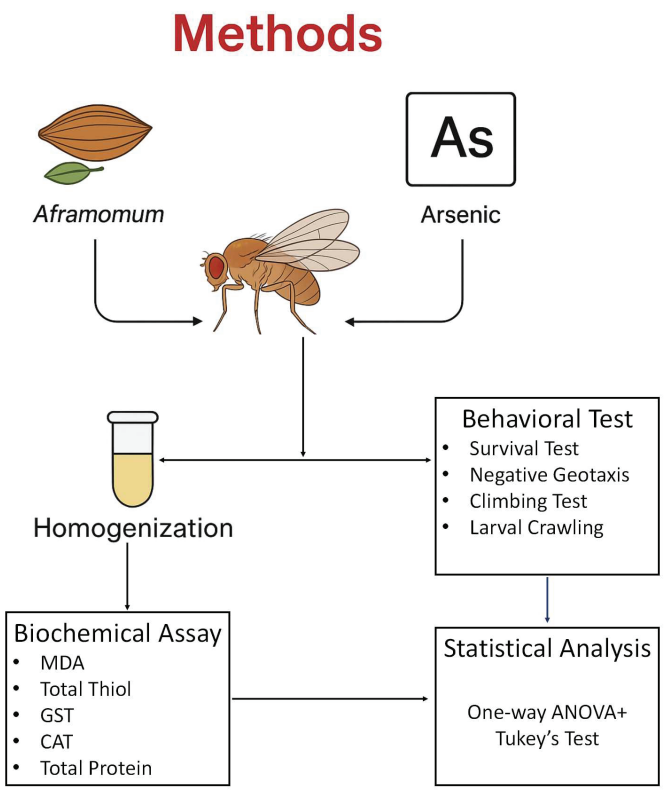
Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Graphical abstract



References

1. Liu X, Zhang R, Fan J, Chen Y, Wang H, Ge Y, et al. The role of ROS/p38 MAPK/NLRP3 inflammasome cascade in arsenic-induced depression-/anxiety-like behaviors of mice. Ecotoxicology and Environmental Safety [Internet]. 2023 Jun 7;261:115111. Available from: https://www.sciencedirect.com/science/article/pii/S0147651323006152#fig0035

2. Fatoki JO, Badmus JA. Arsenic as an environmental and human health antagonist: A review of its toxicity and disease initiation. Journal of Hazardous Materials Advances. 2022 Feb;5:100052.

3. Sultan MW, Qureshi F, Ahmed S, Kamyab H, Yusuf M. A Comprehensive Review on Arsenic Contamination in Groundwater: Sources, Detection, Mitigation Strategies and Cost Analysis. Environmental Research. 2025 Jan 15;265(16):120457.

4. Xing M, Yan D, Hai M, Zhang Y, Zhang Z, Li F. Arsenic Contamination in Sludge and Sediment and Relationship with Microbial Resistance Genes: Interactions and Remediation. Water [Internet]. 2024 Dec 17;16(24):3633–3. Available from: https://www.mdpi.com/2073-4441/16/24/3633

5. Kanel SR, Das TK, Varma RS, Kurwadkar S, Chakraborty S, Joshi TP, et al. Arsenic Contamination in Groundwater: Geochemical Basis of Treatment Technologies. ACS Environmental Au. 2023 Feb 22;3(3):135–52.

6. Ullah Z, Iqbal J, Abbasi BA, Ijaz S, Yaseen T, Murtaza G, et al. Arsenic pollution: sources, types, and effects on soil chemistry. Beneficial Elements for Remediation of Heavy Metals in Polluted Soil. 2025;55–91.

7. Hayes JD, Dinkova-Kostova AT, Tew KD.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1791

Oxidative Stress in Cancer. Cancer Cell. 2020 Aug 10;38(2):167–97.

8. Iqbal MJ, Kabeer A, Abbas Z, Siddiqui HA, Calina D, Sharifi-Rad J, et al. Interplay of oxidative stress, cellular communication and signaling pathways in cancer. Cell Communication and Signaling. 2024 Jan 2;22(1).

9. Hu Y, Li J, Lou B, Wu R, Wang G, Lu C, et al. The Role of Reactive Oxygen Species in Arsenic Toxicity. Biomolecules. 2020 Feb 5;10(2):240.

10. Cui YX, Dong L, Zhang M, Liu YN, Chen YH, Jia MZ, et al. Long-term exposure to arsenic in drinking water leads to myocardial damage by oxidative stress and reduction in NO. Toxicology. 2023 Apr 1;492:153529–9.

11. Olunkwa E, Iheanacho E, Uzoma C, Nwaogu NLA, Nkeiru J. Bioactive component analysis of aqueous seed extract of Aframomum melengueta. GSC biological and pharmaceutical sciences. 2023 Nov 30;25(2):249–72.

12. Sudeep HV, Aman K, Jestin TV, Shyamprasad K. Aframomum melegueta Seed Extract with Standardized Content of 6-Paradol Reduces Visceral Fat and Enhances Energy Expenditure in Overweight Adults – A Randomized Double-Blind, Placebo-Controlled Clinical Study. Drug Design, Development and Therapy [Internet]. 2022 Oct [cited 2022 Dec 26];16:3777–91. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/36329722/

13. Ottu PO, Babalola OO, Oluwamodupe C, Oluwatobiloba AF, Kehinde IO, Akinola OA, et al. Investigation of Aframomum melegueta compounds as ERK5 inhibitor related to breast cancer via molecular docking and dynamic simulation. In Silico Pharmacology. 2025 Jan 25;13(1).

14. Yoneshiro T, Matsushita M, Sugita J, Aita S, Kameya T, Sugie H, et al. Prolonged Treatment with Grains of Paradise (Aframomum melegueta) Extract Recruits Adaptive Thermogenesis and Reduces Body Fat in Humans with Low Brown Fat Activity. Journal of Nutritional Science and Vitaminology [Internet]. 2021;67(2):99–104. Available from: https://pubmed.ncbi.nlm.nih.gov/33952741/

15. Osuntokun OT. Aframomum Melegueta (Grains of Paradise). Annals of microbiology and infectious diseases. 2020 Jan 1;3(1):1–6.

16. Pérez-Machín R, Vega-Morales T, Elvira-Aranda C, Lledó-Rico L, Gomis-Gomis M, López-Ríos L. Aframomum melegueta Seed Extract's Effects on Anxiety, Stress, Mood, and Sleep: A Randomized, Double-Blind, Pilot Clinical Trial. Pharmaceuticals [Internet]. 2025 Feb 19;18(2):278–8. Available from: https://www.mdpi.com/1424-8247/18/2/278

17. Mohammadi AH, Balandeh E, Milajerdi A. Malondialdehyde concentrations in obsessive–compulsive disorder: a systematic review and meta-analysis. Annals of General Psychiatry. 2021 Jun 16;20(1).

18. Yu Q, Fein JB. Analysis of total thiol concentrations in environmental samples using ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS). Chemical Geology. 2023 Nov 25;644:121837–7.

19. Balcı N, Türkan F, Şakiroğlu H, Aygün A, Şen F. Purification and characterization of glutathione S-transferase from blueberry fruits (Vaccinium arctostaphylos L.) and investigated of some pesticide inhibition effects on enzyme activity. Heliyon. 2019 Apr;5(4):e01422.

20. ADEOYE OB, Iyanda AA, Daniyan MO, ADEOYE DA, Olajide O, Akinnawo OO, et al. ANTI-DYSLIPIDAEMIA AND CARDIO-PROTECTIVE EFFECTS OF NIGERIAN BITTER HONEY IN STREPTOZOTOCIN INDUCED DIABETIC RATS. Universal journal of pharmaceutical research. 2023 May 15;8(2):10–8.

21. Hayes M. Measuring Protein Content in Food: An Overview of Methods. Foods [Internet]. 2020 Sep 23;9(10):1340. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7597951/

22. Ganie SY, Javaid D, Hajam YA, Reshi MS. Arsenic toxicity: sources, pathophysiology and mechanism. Toxicology Research. 2023 Dec 8;13(1).

23. Fatoki JO, Badmus JA. Arsenic as an environmental and human health antagonist: A review of its toxicity and disease initiation. Journal of Hazardous Materials Advances. 2022 Feb;5:100052.

24. Wang L, Xu ZR. Effects of Arsenic (AsIII) on Lipid Peroxidation, Glutathione Content and Antioxidant Enzymes in Growing Pigs. Asian-Australasian Journal of Animal Sciences. 2006 Mar 29;19(5):727–33.

25. Rasheed Z. Therapeutic potentials of catalase: Mechanisms, applications, and future perspectives. International journal of health sciences [Internet]. 2024;18(2):1–6. Available from: https://pubmed.ncbi.nlm.nih.gov/38455600/

26. Onoja SO, Omeh YN, Ezeja MI, Chukwu MN. Evaluation of theIn VitroandIn VivoAntioxidant Potentials ofAframomum melleguetaMethanolic Seed Extract. Journal of Tropical Medicine [Internet]. 2014;2014:1–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4052497/

27. Khan S, Munir A, Fayyaz O, Hassan A. Phytochemical, Ethnobotanical, and Pharmacological Activities of Aframomum melegueta. Advances in bioinformatics and biomedical engineering book series [Internet]. 2024 May 7;241–78. Available from: https://www.igi-global.com/chapter/phytochemical-ethnobotanical-and-pharmacological-activities-of-aframomum-melegueta/346946

28. Girón SH, Monserrat J, Ortega MÁ, García-Montero C, Fraile-Martínez O, Gómez-Lahoz AM, et al. Prognostic Value of Malondialdehyde (MDA) in the Temporal Progression of Chronic Spinal Cord Injury. Journal of Personalized Medicine. 2023 Apr 2;13(4):626–6.

29. Al-Asady FH, AL-Mohanna MWH. Evaluation of the preventive effects of alligator pepper aframomum melegueta aqueous extract against titanium dioxide nanoparticles induced oxidative stress in adult wistar rats. International Journal of Advanced Chemistry. 2024 Oct 26;12(2):82–5.

30. Mazari AMA, Zhang L, Ye Z, Zhang J, Tew KD, Townsend DM. The Multifaceted Role of Glutathione S-Transferases in Health and Disease. Biomolecules [Internet]. 2023 Apr 18 [cited 2023 Dec 10];13(4):688–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10136111/

31. Ma Q. Role of Nrf2 in Oxidative Stress and Toxicity. Annual review of pharmacology and toxicology. 2013;53:401–26.

32. Ulrich K, Jakob U. The role of thiols in antioxidant systems. Free Radical Biology and Medicine [Internet]. 2019 Aug [cited 2019 Nov 17];140:14–27. Available from: https://www.sciencedirect.com/science/article/pii/S0891584918325425

33. El-Halawany AM, Dine RSE, El Sayed NS, Hattori M. Protective Effect of Aframomum melegueta phenolics Against CCl4-Induced Rat Hepatocytes Damage; Role of Apoptosis and Pro-inflammatory Cytokines inhibition. Scientific Reports. 2014 Jul 30;4(1).

34. Tyler CR, Allan AM. The Effects of Arsenic Exposure on Neurological and Cognitive Dysfunction in Human and Rodent Studies: A Review. Current Environmental Health Reports. 2014 Mar 21;1(2):132–47.

35. Agunloye OM, Olawuyi EA, Oboh G. Neuroprotective effects of dietary inclusions of alligator pepper (*Aframomum melegueta*) and bastard melegueta (*Aframomum danielli*) seeds on scopolamine-induced amnesia in rats. Journal of complementary & integrative medicine. 2025 Mar;22(3):427–37.

36. Zhang Y, Wang Q, Chen H, Liu X, Ke Lv, Wang T, et al. Involvement of Cholinergic Dysfunction and Oxidative Damage in the Effects of Simulated Weightlessness on Learning and Memory in Rats. BioMed research international (Print). 2018 Feb;2018:1–11.

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Serial Kinetics and Diagnostic Interrelationships of Cardiac Biomarkers in Acute Myocardial Infarction

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ABSTRACT:

Background: *Acute myocardial infarction (AMI) is a major cause of morbidity and mortality worldwide. Cardiac biomarkers are essential for the diagnosis and monitoring of AMI. Although cardiac troponin T (cTnT) is the preferred biomarker, enzymes such as creatine phosphokinase-MB (CPK-MB), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) are still widely used, especially in resource-limited settings.*

Objectives: *To evaluate the serial kinetics, absolute and percentage delta changes, and interrelationships of cTnT, CPK-MB, AST, and LDH during the first 48 hours after admission in patients with AMI, and to explore their complementary diagnostic roles in a resource-limited healthcare setting.*

Patients and Methods: *This hospital-based observational study included 50 patients with confirmed AMI and 50 age- and sex-matched healthy controls. Serial blood samples were collected from AMI patients at 0, 6, 12, 24, 36, and 48 hours after admission. Biomarker levels were measured using standard laboratory methods. Absolute and percentage delta changes were calculated relative to controls. Pearson's correlation coefficient was used to assess relationships between biomarkers at each time point.*

Results: *All biomarkers were significantly elevated in AMI patients compared with controls. cTnT showed a progressive and sustained rise, reaching over 1100% above control values at 48 hours. CPK-MB and AST demonstrated earlier peaks around 24 hours, followed by a partial decline, while LDH showed a delayed but marked rise. Most biomarker pairs showed no significant linear correlation at any time point; weak negative correlations observed at 12 hours were small in magnitude and likely of limited clinical significance.*

Conclusion: *Cardiac biomarkers in AMI exhibit distinct temporal kinetics with minimal linear correlation. Lack of correlation should not be interpreted as lack of diagnostic utility; rather, it highlights that biomarkers reflect different pathophysiological phases of myocardial injury. cTnT remains the most reliable biomarker, while CPK-MB, AST, and LDH should be interpreted as complementary rather than interchangeable markers.*

KEYWORDS:

Acute myocardial infarction; Cardiac biomarkers; Troponin T; CPK-MB; AST; LDH.

1. Introduction

Acute myocardial infarction (AMI) is one of the leading causes of morbidity and mortality worldwide and remains a major public health challenge, particularly in low- and middle-income countries.[1] Rapid urbanization, sedentary lifestyle, tobacco use, unhealthy diet, and rising prevalence of diabetes and hypertension have all contributed to an increasing burden of ischemic heart disease. In the setting of AMI, time is a critical determinant of outcome: early diagnosis and prompt reperfusion therapy significantly reduce infarct size, preserve left ventricular function, and improve short- and long-term survival. Therefore, the availability of accurate, sensitive, and timely diagnostic tools is central to effective

management of patients presenting with chest pain suggestive of myocardial ischemia.[2]

Cardiac biomarkers have become indispensable in the evaluation of suspected AMI. They provide objective biochemical evidence of myocardial injury, complementing clinical assessment and electrocardiographic changes. Among these, cardiac troponins (including cardiac troponin T, cTnT) are currently considered the gold standard for detecting myocardial necrosis. cTnT is highly specific to cardiac myocytes and is released into the circulation following irreversible damage to the myocardial cell membrane. Its concentration begins to rise within a few hours after the onset of ischemia, typically peaks over 12–24 hours, and remains elevated for several days. This characteristic profile makes cTnT extremely useful not only for confirming the diagnosis of AMI but also for identifying patients who present late, when other markers may already have normalized.[3]

Before the widespread adoption of troponins, enzymes such as creatine phosphokinase-MB (CPK-MB), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were widely used as markers of myocardial injury. CPK-MB is relatively more specific for cardiac muscle compared to AST and LDH, and classically shows an early rise, peaking around 12–24 hours and returning to baseline within 2–3 days. This early peaking Pattern makes CPK-MB useful in detecting reinfarction or assessing very early changes after reperfusion. AST and LDH, although less specific to cardiac tissue, are released from multiple organs in response to cell injury and necrosis. Their elevations in AMI reflect the extent of tissue damage but can be influenced by concomitant hepatic, skeletal muscle, or systemic conditions, which may limit their specificity.

Despite these limitations, AST and LDH continue to be measured in many clinical laboratories, especially in resource-constrained settings where high-sensitivity troponin assays may not be universally available or may be reserved for selected patients. In such environments, clinicians often rely on a combination of biomarkers to support the diagnosis and to understand the temporal sequence of myocardial injury. Thus, understanding the dynamic behavior of both specific (cTnT, CPK-MB) and non-specific (AST, LDH) markers is important, particularly in settings where patterns over time may provide additional diagnostic and prognostic information.[3]

Although the individual kinetic profiles of these biomarkers are well described, limited data are available on their serial interrelationships and relative delta changes within the same patient population over predefined early time intervals. Understanding these patterns may aid clinical interpretation, particularly in settings where multiple biomarkers are used simultaneously.[4]

Rationale of the Study: Despite widespread guideline endorsement of troponin-based diagnosis, practical constraints in resource-limited environments necessitate continued reliance on traditional cardiac enzymes. Clinicians frequently encounter discordant biomarker results, particularly when patients present at varying times after symptom onset.

This study was designed as an exploratory observational analysis to provide population-specific data on serial biomarker kinetics, absolute and percentage delta changes, and correlation patterns. The aim was not to redefine diagnostic thresholds but to better understand the complementary roles and limitations of commonly used cardiac biomarkers in routine clinical practice.

Aim

To evaluate the serial Pattern and interrelationship of cardiac biomarkers (cTnT, CPK-MB, AST, and LDH) in patients with acute myocardial infarction as compared to healthy controls over the first 48 hours after admission.

Objectives

1. **To measure and compare** the mean levels of cTnT, CPK-MB, AST, and LDH in AMI patients and age- and sex-matched healthy controls at predefined time intervals (0, 6, 12, 24, 36, and 48 hours after admission).
2. **To calculate** the absolute delta (Δ = Cases - Control) and percentage delta (%Δ) for each biomarker at each time point to assess the magnitude and evolution of biochemical changes over time.
3. **To analyze** the correlation between pairs of biomarkers (cTnT vs CPK-MB, cTnT vs AST, cTnT vs LDH, CPK-MB vs AST, CPK-MB vs LDH, and AST vs LDH) at 6, 12, 24, 36, and 48 hours using Pearson's correlation coefficient (r).
4. To assess whether correlation patterns

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support complementary rather than interchangeable clinical use of these biomarkers.

2. MATERIALS AND METHODS

Study Design

A hospital-based observational study with serial biomarker measurements.The study design involved:

- Prospective enrollment of patients presenting with suspected AMI who fulfilled predefined inclusion and exclusion criteria.
- Serial sampling of blood at standardized time intervals after admission.
- Parallel comparison with an age- and sex-matched control group.
- Statistical analysis focusing on temporal trends, delta changes, and correlations between biomarkers.

Study Population

The study included two groups:

- **Cases:**

- Patients diagnosed clinically and biochemically with acute myocardial infarction (**n = 50**). Diagnosis was made by the treating physician based on typical clinical presentation, characteristic ECG changes, and elevated cardiac markers consistent with AMI.

- **Controls:**

- Age- and sex-matched healthy individuals (**n = 50**). with no history of cardiovascular disease, recruited from hospital staff, patient attendants, or individuals attending routine health check-ups. Controls had no acute illness at the time of sampling and no known chronic systemic disease that could influence cardiac biomarker levels.

- Patients were included if they presented within 6 hours of symptom onset to capture early biomarker kinetics.

Inclusion Criteria (Cases)

- Adults aged **≥ 18 years**.
- Confirmed AMI based on:
  - Typical clinical presentation (e.g., chest pain, dyspnea, diaphoresis),
  - ECG changes suggestive of myocardial infarction (e.g., ST elevation/depression, new-onset LBBB, T-wave inversion), and
  - Elevated cardiac markers, as interpreted by the treating physician.

- Presentation within **6 hours of the onset of symptoms**, to ensure the ability to capture the early kinetics of biomarker release.

Exclusion Criteria

Patients were excluded if they had any of the following conditions known to alter biomarker levels independently of acute coronary occlusion:

- **Chronic kidney disease** (which may cause persistently elevated troponin and affect the clearance of markers).

- **Chronic liver disease or skeletal muscle disorders** (which can elevate AST, LDH, and CPK-MB independently of cardiac injury).

- **Known myocarditis or heart failure**, which may cause chronic or intermittent elevations of cardiac biomarkers.

- **Recent trauma or surgery**, or any acute condition (e.g., severe myopathy, hemolysis), is likely to influence biomarker levels.

- **Hemolysed or inadequate blood samples** can spuriously alter enzyme measurements and compromise assay reliability.

Sample Collection and Biomarker Measurement

Timing of Sample Collection

Blood samples were collected from cases at predefined time intervals to capture the dynamic changes in biomarker levels:

- **Baseline (0 hours):** At admission, as soon as possible after presentation.
- **6 hours** after admission.
- **12 hours** after admission.
- **24 hours** after admission.
- **36 hours** after admission.
- **48 hours** after admission.

These time points were selected to cover the

early, peak, and declining phases of biomarker release following AMI.

For the control group, blood samples were obtained once, at a corresponding baseline time point for comparison of mean values.

Pre-analytical Procedures

- Venous blood samples were collected using standard aseptic techniques.
- Samples were drawn into appropriate vacutainers (e.g., plain or serum-separating tubes) and allowed to clot, then centrifuged to separate serum.
- All samples were processed promptly according to laboratory protocol to minimize pre-analytical variability.
- Samples showing visible hemolysis or inadequate volume were discarded, and re-sampling was done wherever feasible.

Biomarkers and Analytical Methods

The following biomarkers were measured using standard laboratory techniques:

1. **Cardiac Troponin T (cTnT)**

- Measured by an **immunoassay (chemiluminescence-based)** on an automated analyzer.
- The assay used manufacturer-recommended reagents, calibration standards, and internal quality control procedures.

2. **Creatine Phosphokinase-MB (CPK-MB)**

- Estimated by an **enzymatic immunoinhibition method**, which selectively measures the MB isoenzyme fraction of creatine kinase.

3. **Aspartate Aminotransferase (AST)**

- Determined by the **kinetic UV method**, measuring the rate of change in absorbance due to the enzymatic reaction.

4. **Lactate Dehydrogenase (LDH)**

- Measured by an **enzymatic spectrophotometric method**, based on the catalytic conversion of lactate to pyruvate (or vice versa) with associated change in absorbance.

Quality control was maintained by running appropriate internal control sera with each batch of tests and following standard operating procedures for instrument maintenance and calibration.

3. Data Analysis

1. Biomarker Comparison

- For each biomarker (cTnT, CPK-MB, AST, LDH), **mean ± standard deviation (SD)** values were calculated separately for cases and controls.
- For cases, mean values were calculated at each time point (0, 6, 12, 24, 36, and 48 hours).
- **Absolute delta (Δ)** and **percentage delta (%Δ)** were calculated using control values as reference:

- **Absolute delta (Δ):**

Δ=Mean value in cases-Mean value in controls

- **Percentage delta (%Δ):**

%Δ=(Mean value in cases-Mean value in controlsMean value in controls)×100. Where appropriate, inferential statistical tests (e.g., Student's t-test for normally distributed data or non-parametric equivalents) could be used to compare means between cases and controls at each time point (you can specify these exactly in your thesis depending on what you actually used).

2. Correlation Analysis

To evaluate the linear relationships between different biomarkers at various time points, **Pearson's correlation coefficient (r)** was calculated for the following pairs:

- cTnT vs CPK-MB
- cTnT vs AST
- cTnT vs LDH
- CPK-MB vs AST
- CPK-MB vs LDH
- AST vs LDH

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Correlation analysis was performed at **6, 12, 24, 36, and 48 hours** after admission (time points at which substantial biomarker changes are expected).

The strength of correlation was interpreted as:

- **r = ±0.00–0.19** → No correlation
- **r = ±0.20–0.39** → Weak correlation
- **r = ±0.40–0.59** → Moderate correlation

A **p-value < 0.05** was considered statistically significant.

3. Graphical Representation

To visually assess relationships between biomarkers:

- **Scatter plots** were generated for each biomarker pair at each time interval.
- The plots allowed visualization of:
  - Direction of association (positive/negative),
  - Degree of scatter around any apparent line of best fit,
  - Presence or absence of linear trends and potential outliers.

These graphical representations support the numerical correlation analysis and help in an intuitive understanding of how biomarker levels move together over time.

4. Statistical Software

Data entry and analysis were performed using standard statistical software such as **SPSS**, **R**, or **Microsoft Excel**.

- Descriptive statistics were used to summarize biomarker levels. Absolute and percentage delta values were calculated using mean control values as a fixed reference.
- Pearson's correlation coefficient was applied as an exploratory method to assess linear relationships between biomarkers. Formal normality testing and data transformation were not performed, which is acknowledged as a limitation.
- Multiple correlation analyses were conducted without adjustment for multiple comparisons; therefore, isolated statistically significant findings were interpreted cautiously.

Table 1: Cardiac Biomarkers – Control vs Cases with Delta (Cases - Control)

Group	Marker	Time	Value	Δ (Cases - Control)
Control	cTnT	0 h	0.4734	
Cases	cTnT	6 h	1.3872	1.3872 - 0.4734 = **0.9138**
Cases	cTnT	12 h	2.1962	2.1962 - 0.4734 = **1.7228**
Cases	cTnT	24 h	3.5166	3.5166 - 0.4734 = **3.0432**
Cases	cTnT	36 h	5.09	5.09 - 0.4734 = **4.6166**
Cases	cTnT	48 h	5.748	5.748 - 0.4734 = **5.2746**
Control	CPK-MB	6 h	29.9452	
Cases	CPK-MB	6 h	100.0588	100.0588 - 29.9452 = **70.1136**
Cases	CPK-MB	12 h	110.4162	110.4162 - 29.9452 = **80.4710**
Cases	CPK-MB	24 h	193.4552	193.4552 - 29.9452 = **163.5100**
Cases	CPK-MB	36 h	104.6802	104.6802 - 29.9452 = **74.7350**
Cases	CPK-MB	48 h	124.656	124.656 - 29.9452 = **94.7108**
Control	AST	0 h	38.242	
Cases	AST	6 h	75.4474	75.4474 - 38.242 = **37.2054**
Cases	AST	12 h	89.8944	89.8944 - 38.242 = **51.6524**
Cases	AST	24 h	180.3238	180.3238 - 38.242 = **142.0818**
Cases	AST	36 h	104.2618	104.2618 - 38.242 = **66.0198**
Cases	AST	48 h	128.6026	128.6026 - 38.242 = **90.3606**
Control	LDH	0 h	184.5122	
Cases	LDH	6 h	512.4012	512.4012 - 184.5122 = **327.8890**
Cases	LDH	12 h	602.5992	602.5992 - 184.5122 = **418.0870**
Cases	LDH	24 h	1019.8852	1019.8852 - 184.5122 = **835.3730**
Cases	LDH	36 h	1559.2512	1559.2512 - 184.5122 = **1374.7390**
Cases	LDH	48 h	1837.2454	1837.2454 - 184.5122 = **1652.7332**

Table 1 highlights distinct temporal patterns of biomarker elevation in AMI. While all biomarkers showed significant increases compared with controls, cTnT and LDH exhibited sustained and progressively increasing delta values, whereas CPK-MB and AST peaked earlier and showed partial declines. These findings underscore the differing release kinetics and clinical utility of individual cardiac biomarkers in the early evolution of acute myocardial infarction.

Table 2: Cardiac Biomarkers – Control vs Cases with Absolute and Percentage Delta Over Time

Group	Marker	Time	Value	Δ (Cases - Control)	%Δ vs Control
Control	cTnT	0 h	0.4734		
Cases	cTnT	6 h	1.3872	0.9138	193.0%
Cases	cTnT	12 h	2.1962	1.7228	363.9%
Cases	cTnT	24 h	3.5166	3.0432	642.8%
Cases	cTnT	36 h	5.09	4.6166	975.2%
Cases	cTnT	48 h	5.748	5.2746	1114.2%
Control	CPK-MB	6 h	29.9452		
Cases	CPK-MB	6 h	100.0588	70.1136	234.1%
Cases	CPK-MB	12 h	110.4162	80.4710	268.7%
Cases	CPK-MB	24 h	193.4552	163.5100	546.0%
Cases	CPK-MB	36 h	104.6802	74.7350	249.6%
Cases	CPK-MB	48 h	124.656	94.7108	316.3%
Control	AST	0 h	38.242		
Cases	AST	6 h	75.4474	37.2054	97.3%
Cases	AST	12 h	89.8944	51.6524	135.1%
Cases	AST	24 h	180.3238	142.0818	371.5%
Cases	AST	36 h	104.2618	66.0198	172.6%
Cases	AST	48 h	128.6026	90.3606	236.3%
Control	LDH	0 h	184.5122		
Cases	LDH	6 h	512.4012	327.8890	177.7%
Cases	LDH	12 h	602.5992	418.0870	226.6%
Cases	LDH	24 h	1019.8852	835.3730	452.7%
Cases	LDH	36 h	1559.2512	1374.7390	745.1%
Cases	LDH	48 h	1837.2454	1652.7332	895.7%

Table 2 demonstrates distinct temporal and quantitative differences in biomarker behavior following AMI. cTnT and LDH showed the greatest and most sustained percentage increases over time, whereas CPK-MB and AST exhibited earlier peaks followed by partial declines. These findings emphasize the differing release kinetics and complementary clinical roles of cardiac biomarkers in the early evolution of acute myocardial infarction.

Figure 1: Correlation Heat Map Analysis of cardiac Biomarkers

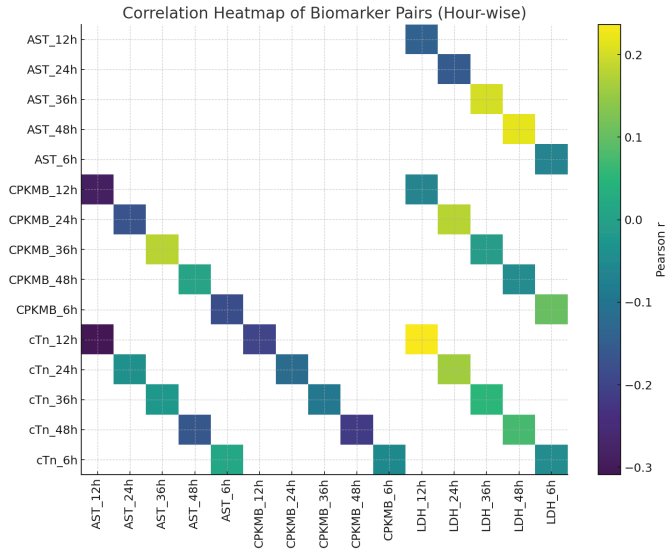

Figure 1: The correlation heat map depicting pairwise relationships among cardiac biomarkers at different time points demonstrated an overall lack of meaningful linear association between the studied parameters. Across all time intervals (6, 12, 24, 36, and 48 hours), cardiac troponin T (cTnT) showed negligible correlation with CPK-MB, with correlation coefficients consistently close to zero and no statistically significant associations (all $p > 0.05$). This Pattern indicates that variations in CPK-MB levels are largely independent of cTnT concentrations throughout the first 48 hours following myocardial injury.

Similarly, the heat map revealed weak to near-zero correlations between CPK-MB and AST at all measured time points. The correlation coefficients were small and inconsistent in direction, with no time point demonstrating statistical significance. Notably, at 48 hours, the correlation between CPK-MB and AST was virtually absent ($r \approx 0.01$, $p = 0.05$), reinforcing the lack of a linear relationship.

Overall, the heat map visualization confirms that while these biomarkers are all associated with myocardial injury, they exhibit **distinct temporal release kinetics and biological behavior**, resulting in minimal linear correlation with one another. This supports the concept that cTnT, CPK-MB, and AST provide **complementary rather than interchangeable diagnostic information** during the evolution of acute myocardial infarction.

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Table 3: Pearson Correlation Between Myocardial Infarction Biomarkers (cTnT, CPK-MB, AST, LDH) at Different Time Intervals (6h, 12h, 24h, 36h, 48h)

Time	Bio-marker 1	Bio-marker 2	n	r (Correlation)	p-value	Interpretation
6h	cTnT	CPK-MB	50	-0.055	0.704	No correlation
6h	cTnT	AST	50	0.017	0.905	No correlation
6h	cTnT	LDH	50	-0.046	0.751	No correlation
6h	CPK-MB	AST	50	-0.179	0.213	No correlation
6h	CPK-MB	LDH	50	0.103	0.479	No correlation
6h	AST	LDH	50	-0.066	0.651	No correlation
12h	cTnT	CPK-MB	50	-0.196	0.172	No correlation
12h	cTnT	AST	50	-0.309	**0.029**	Weak negative correlation (significant)
12h	cTnTT	LDH	50	0.237	0.098	Trend, not significant
12h	CPK-MB	AST	50	-0.288	**0.043**	Weak negative correlation
12h	CPK-MB	LDH	50	-0.063	0.666	No correlation
12h	AST	LDH	50	-0.142	0.326	No correlation
24h	cTnT	CPK-MB	50	-0.118	0.415	No correlation
24h	cTnT	AST	50	-0.037	0.801	No correlation
24h	cTnT	LDH	50	0.159	0.270	No correlation
24h	CPK-MB	AST	50	-0.170	0.237	No correlation
24h	CPK-MB	LDH	50	0.177	0.218	No correlation
24h	AST	LDH	50	-0.155	0.283	No correlation
36h	cTnT	CPK-MB	50	-0.094	0.516	No correlation
36h	cTnT	AST	50	-0.022	0.879	No correlation
36h	cTnT	LDH	50	0.053	0.713	No correlation
36h	CPK-MB	AST	50	0.180	0.211	No correlation
36h	CPK-MB	LDH	50	-0.007	0.962	No correlation
36h	AST	LDH	50	0.201	0.162	No correlation
48h	cTnT	CPK-MB	50	-0.217	0.130	No correlation
48h	cTnT	AST	50	-0.163	0.259	No correlation
48h	cTnT	LDH	50	0.073	0.614	No correlation
48h	CPK-MB	AST	50	0.009	0.951	No correlation
48h	CPK-MB	LDH	50	-0.048	0.740	No correlation
48h	AST	LDH	50	0.217	0.130	No correlation

Table 3 Correlation analysis revealed no consistent linear association between most biomarker pairs. Weak negative correlations observed at 12 hours were small in magnitude and not sustained at later time points.

4. DISCUSSION

The present hospital-based observational study evaluated the serial behavior and interrelationship of four cardiac biomarkers—cTnT, CPK-MB, AST, and LDH—in patients with acute myocardial infarction, compared with age- and sex-matched healthy controls. The analysis focused both on **absolute and percentage delta changes** over time and on **correlation patterns** between biomarkers at 6, 12, 24, 36, and 48 hours after admission.

Overall, three key findings emerged:

1. All four biomarkers showed substantial elevation in AMI cases compared with controls, but with distinct temporal kinetics, consistent with their known biological behavior.

2. cTnT and LDH exhibited the greatest and most sustained percentage increases over 48 hours, while CPK-MB and AST showed earlier peaks followed by partial declines.

3. Despite clear rises in all markers, there was no significant linear correlation between most biomarker pairs at any time point, except for a weak negative correlation between cTnT and AST and between CPK-MB and AST at 12 hours.

These findings have important implications for understanding biomarker dynamics and for their practical use in clinical settings, particularly where resources are limited.

Serial Pattern of cTnT and Comparison with Literature

In this study, cTnT showed a marked and progressive rise over time**,** from approximately 193% above control at 6 hours to more than 1100% above control at 48 hours. The absolute delta increased from 0.91 ng/mL at 6 hours to 5.27 ng/mL at 48 hours, indicating a sustained and steadily increasing release of troponin into the circulation. This Pattern reflects ongoing or evolving myocardial necrosis and the prolonged release and slow clearance of cTnT from injured cardiomyocytes.

This temporal profile is consistent with established literature, which shows that cardiac troponin levels rise within a few hours of symptom onset, peak around 12–24 hours, and remain elevated for up to 7–10 days following AMI.[2] The Third Universal Definition of MI emphasizes the importance of detecting a rise and/or fall of troponin, with at least one value above the 99th percentile, to diagnose AMI.[6] Our findings align with this concept of dynamic change: the progressive increase over 48 hours demonstrates clear biochemical evidence of acute myocardial injury.

Modern reviews have consistently shown that troponin, especially high-sensitivity assays, outperforms older markers (CK-MB, AST, LDH) in both sensitivity and specificity for AMI diagnosis. [7] The large percentage deltas observed in our study reinforce the central role of cTnT as the primary diagnostic and monitoring biomarker in AMI and support guideline recommendations that troponin be used as the preferred marker, with other enzymes relegated to supportive roles. [6]

Behavior of CPK-MB: Early Peak and Partial Decline

CPK-MB in our study showed a pattern typical of an early peaking biomarker. Relative to control values at 6 hours, CPK-MB increased by about 234–269% at 6–12 hours, reached a maximum percentage delta of ~546% at 24 hours, and then declined slightly to 249–316% above control by 36–48 hours. This Pattern reflects the earlier release and faster clearance of CPK-MB, which historically made it useful for detecting early infarction and reinfarction.

These findings are in line with classic descriptions that CPK-MB rises within 3–6 hours, peaks at around 12–24 hours, and returns toward baseline within 2–3 days. [6,10] Previous comparative studies have shown that although CK-MB can be diagnostically useful, troponin generally has equal or superior diagnostic accuracy and better prognostic value for infarct size and adverse outcomes. [8]

Our data demonstrate that while CPK-MB does rise substantially in AMI, its profile differs from the sustained rise of cTnT. The partial decline by 36–48 hours means that late presenters might have near-normal or falling CK-MB despite still markedly elevated cTnT. This supports current practice where CK-MB is considered adjunctive at best and primarily useful when evaluation of reinfarction or very early dynamic changes is needed, rather than as a standalone diagnostic marker.[9]

AST and LDH: Supportive, Less Specific, and Late Markers

AST in this study showed a **moderate early rise** (~97–135% above control at 6–12 hours), peaking at **~371% above control at 24 hours**, followed by a partial decline at 36–48 hours (172–236% above control). **LDH**, by contrast, showed a **delayed but very large rise**, from ~178% at 6 hours to almost **900% above control at 48 hours**, with especially large deltas from 24 hours onward.

Historically, AST and LDH were the first enzymes used for AMI diagnosis, with AST introduced in the 1950s and LDH soon after.[11] AST typically rises within 3–4 hours of infarction, peaks around 15–28 hours, and then declines, while LDH levels

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increase within 6–12 hours, peak at 1–3 days, and return to baseline within 8–14 days.[12] Our findings mirror these classical kinetics, with the prominent late rise in LDH reflecting its role as a **late marker** of tissue injury.

However, both AST and LDH are **non-specific**, being released from the liver, skeletal muscle, and other tissues, and modern guidelines no longer recommend them as primary diagnostic markers for AMI.[2] In our study, although they clearly increased in AMI cases, their interpretive value is limited by this lack of specificity and by their delayed peaks. The patterns we observed support their role, at most, as **supportive or historical markers** rather than as first-line tests.

Lack of Significant Correlation Between Biomarkers

One of the most striking findings of this study is that, despite clear elevations in all biomarkers, **most pairwise correlations were not statistically significant** at any time point. Specifically:

- At **6 hours**, all correlations among cTnT, CPK-MB, AST, and LDH were in the "no correlation" range ($|r| < 0.20$, $p > 0.05$).
- At **12 hours**, there was a **weak but statistically significant negative correlation** between cTnT and AST ($r = -0.309$, $p = 0.029$) and between CPK-MB and AST ($r = -0.288$, $p = 0.043$). All other correlations at this time point were non-significant.
- At **24, 36, and 48 hours**, none of the biomarker pairs showed significant correlation ($p > 0.05$), with most r values close to zero.

Thus, even though each marker rises in AMI, **they do not move in a parallel or linearly related fashion** in individual patients across the time points studied. The absence of strong correlations does not imply a lack of clinical usefulness. Instead, it reflects differing biological behavior, timing of release, and specificity of each biomarker. Weak correlations observed at isolated time points are likely incidental and should not be over-interpreted.

Comparison with Other Studies

Several studies have documented **moderate to strong correlations** between peak CPK-MB and peak troponin values or infarct size in AMI, especially when peak values or area-under-the-curve (AUC) measures are used.[13] In contrast, more recent or differently designed studies (e.g., single-time-point measurements, different populations, or small sample sizes) have reported **weak or non-significant correlations** between troponin and CK-MB, suggesting that these biomarkers may reflect partly distinct physiological aspects of myocardial injury and may not always behave in parallel.[11,13]

Our findings fall closer to the latter group, showing **the absence of meaningful linear correlation** between cTnT and CPK-MB at all studied time points (r ranging from -0.055 to -0.217, all $p > 0.05$). This suggests that, in our cohort:

- Individual variability in infarct size, timing of presentation (even within the ≤6 h inclusion window), reperfusion status, and comorbidities may have led to **asynchronous release and clearance** of different biomarkers.
- Non-cardiac factors (e.g., subclinical hepatic or muscle involvement) may have influenced AST and LDH levels independently of the degree of myocardial necrosis, weakening correlations with cardiac-specific markers.
- The relatively smaller sample size (n = 50) may have limited the power to detect modest correlations.

The weak negative correlation between cTnT and AST at 12 hours, although statistically significant, is **small in magnitude** and unlikely to have major clinical implications. It may reflect random variability, differential timing of peak release, or confounding by extracardiac AST sources. A similar argument applies to the weak negative correlation between CPK-MB and AST at 12 hours.

Overall, the lack of robust correlation in this study reinforces the idea that:

- **Each biomarker has its own kinetic profile and influencing factors**, and
- Absence of correlation does not negate their individual diagnostic value, particularly for cTnT.

Pathophysiological and Clinical Implications

1. **Troponin as the Central Marker**

2. The very large and sustained percentage increases in cTnT compared with controls, coupled with its well-established cardiac specificity, support its role as the **primary biomarker** for diagnosis and risk stratification in AMI.[2]

3. **Timing Matters More Than "Panel Parallelism"**

4. The absence of strong correlations between markers suggests that clinicians should interpret them **in the context of timing** rather than expecting them to rise and fall together. For example:

- In the early hours, CPK-MB may rise earlier while cTnT is still rising.
- In late presenters, CK-MB may have started to decline while cTnT and LDH remain markedly elevated.

5. **Limited Interchangeability of Non-troponin Markers**

6. Our correlation data suggest that **CPK-MB, AST and LDH cannot reliably substitute for cTnT** at any time point, since their values do not show consistent linear relationships with troponin. This aligns with current guideline recommendations to use non-troponin markers only as adjuncts or for specific purposes (e.g., suspected reinfarction, historical comparison). [9]

7. **Resource-limited Settings**

8. In settings where only some markers are available, our findings highlight that:

- CPK-MB may still help in early diagnosis, but its interpretation must consider its early peak and decline.
- AST and LDH elevations should be interpreted with caution, given their poor specificity and lack of reliable correlation with cTnT.
- Whenever possible, **troponin assays should be prioritized**, even if the frequency of sampling has to be reduced, as the most informative test.

Strengths and Limitations

Strengths

- Serial measurement of four biomarkers at multiple time points (0, 6, 12, 24, 36, 48 hours) allowed a detailed description of **dynamic changes** and **delta values** over the first 48 hours of AMI.
- Inclusion of a control group enabled calculation of **absolute and percentage deltas**, providing a more meaningful context than raw values alone.
- Relevance to real-world, resource-limited settings

Limitations

- The modest sample size may have limited statistical power to detect moderate correlations.
- Patients were not stratified by STEMI/NSTEMI, infarct size, or reperfusion status.
- Use of a single control mean may exaggerate percentage delta values, especially for low-baseline biomarkers such as cTnT.
- Lack of adjustment for multiple comparisons increases the risk of type I error.

Conclusion: This study highlights that cardiac biomarkers in AMI show distinct and time-dependent kinetic patterns with minimal linear correlation. cTnT remains the cornerstone biomarker, while CPK-MB, AST, and LDH provide complementary information. Correlation alone should not be used to judge clinical interchangeability; timing, specificity, and biological behavior are more important determinants of diagnostic value.

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References

1. Patibandla S, Gupta K, Alsayouri K. Cardiac biomarkers. Treasure Island (FL): StatPearls Publishing; 2025.

2. Aydin S, Ugur K, Aydin S, Sahin İ, Yardim M. Biomarkers in acute myocardial infarction: current perspectives. Vasc Health Risk Manag. 2019 Jan;Volume 15:1–10.

3. Basit H, Huecker MR. Myocardial infarction serum markers [Internet]. StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532966/

4. Khalil H. Traditional and novel diagnostic biomarkers for acute myocardial infarction. Egypt J Intern Med. 2022 Dec 9;34(1):87.

5. Netala VR, Hou T, Wang Y, Zhang Z, Teertam SK. Cardiovascular Biomarkers: Tools for Precision Diagnosis and Prognosis. Int J Mol Sci. 2025 Mar 30;26(7):3218.

6. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD. Third universal definition of myocardial infarction. Nat Rev Cardiol. 2012 Nov 25;9(11):620–33.

7. Lazar DR, Lazar FL, Homorodean C, Cainap C, Focsan M, Cainap S, et al. High-Sensitivity Troponin: A Review on Characteristics, Assessment, and Clinical Implications. Dis Markers. 2022 Mar 28;2022:1–13.

8. Tzivoni D, Koukoui D, Guetta V, Novack L, Cowing G. Comparison of Troponin T to Creatine Kinase and to Radionuclide Cardiac Imaging Infarct Size in Patients With ST-Elevation Myocardial Infarction Undergoing Primary Angioplasty. Am J Cardiol. 2008 Mar;101(6):753–7.

9. Lippi G, Plebani M. Understanding cardiac troponin biology: all other cardiac biomarkers shall rest in peace? J Lab Precis Med. 2019 Mar;4:9–9.

10. Gnaneswar G, Chandra BS, Priyanka P, Gopinath C. Diagnosis of Myocardial Infarction by Biomarkers-A Review. Scholars Academic Journal of Pharmacy. 2017;6(10).

11. Sridhar C, Krithika C, Sanjana M, Nivedha A. Biomarkers in acute myocardial infarction. International Journal of Early Childhood Special Education. 2022;14(1):4400–3.

12. Costa TN, Cassaro Strunz CM, Nicolau JC, Gutierrez PS. Comparison of MB Fraction of Creatine Kinase Mass and Troponin I Serum Levels With Necropsy Findings in Acute Myocardial Infarction. Am J Cardiol. 2008 Feb;101(3):311–4.

13. Ali S, Usama Abid, Tasra Bibi, M. Saim Qasim, Shahid Sultan, Hafiz Ayaz Ahmad, et al. DIFFERENTIATING TROPONIN I AND CK-MB IN MYOCARDIAL INFARCTION DIAGNOSIS: A NOVEL APPROACH TO BIOMARKER COMPARISON. Insights-Journal of Health and Rehabilitation. 2025 May 25;3(3 (Health & Rehabilitation)):457–63.

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Natural Inhibitors from Polyalthia longifolia Target NDM-1 and DNA Gyrase B in Multidrug-Resistant Klebsiella pneumoniae and Acinetobacter baumannii: An Integrated Experimental and Computational Study

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ABSTRACT:

Background: *Acinetobacter baumannii and Klebsiella pneumoniae are WHO critical-priority pathogens with widespread multidrug resistance, posing a significant threat to global health. This study evaluated the antibacterial activity of solvent fractions of Polyalthia longifolia leaves and examined the molecular docking, pharmacokinetic, and toxicity profiles of its phytochemicals against NDM-1 and DNA gyrase B.*

Results: *All fractions exhibited antibacterial activity, with zones of inhibition ranging from 18.33 ± 0.33 to 23.00 ± 0.58 mm. The aqueous fraction was most active (A. baumannii: 22.33 ± 0.33 mm; K. pneumoniae: 23.00 ± 0.58 mm) and showed the lowest minimum inhibitory concentration (6.25 mg/mL). High-performance liquid chromatography identified Quercetin, Apigenin, and catechin as major bioactive constituents. Docking revealed that mangiferin (-9.263 kcal/mol), myricetin (-8.703 kcal/mol), and Baicalin (-8.411 kcal/mol) bound NDM-1 more strongly than reference drugs, while Quercetin (-7.400 kcal/mol), catechin (-7.262 kcal/mol), and Apigenin (-7.216 kcal/mol) showed high affinity for DNA gyrase B. ADME profiling indicated good oral bioavailability, moderate lipophilicity, and minimal CYP450 interference. Toxicity predictions confirmed low hepatotoxicity, cardiotoxicity, and mutagenic risks.*

Conclusion: *P. longifolia leaves possess potent antibacterial metabolites. Mangiferin, Quercetin, and myricetin showed strong inhibitory potential with favorable pharmacokinetic and safety profiles, supporting their promise as leads against multidrug-resistant K. pneumoniae and A. baumannii.*

KEYWORDS:

Polyalthia longifolia, antibiotic resistance, Klebsiella pneumoniae, Acinetobacter baumannii, NDM-1, DNA gyrase B, phytomedicine.

1. Introduction

Antimicrobial resistance (AMR) is widely recognized as one of the greatest threats to global health, with the World Health Organization describing it as a "silent pandemic" projected to cause 10 million deaths annually by 2050 if left unaddressed (1). Multidrug-resistant (MDR) bacteria, defined as organisms resistant to at least one agent in three or more antimicrobial classes, pose particular challenges to modern medicine (2). Among these, *Klebsiella pneumoniae* and *Acinetobacter baumannii* are classified as critical priority pathogens due to their high resistance levels and their association with severe clinical infections, including pneumonia, bloodstream infections, urinary tract infections, and wound sepsis (3–8).

Understanding the factors that drive resistance in these pathogens is essential for developing effective interventions. Mechanisms such as indiscriminate antibiotic use, horizontal gene transfer, biofilm formation, and efflux pump activity collectively limit the effectiveness of conventional therapies (5,9,10). Consequently, the rise of MDR pathogens has placed substantial strain on healthcare systems worldwide, particularly in low- and middle-income countries where access to novel antimicrobials is limited (11). The slowdown in new antibiotic development, due to high costs, long development timelines, and low returns on investment, further underscores the urgent need

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for alternative therapeutic strategies (12).

Medicinal plants offer a promising source of structurally diverse bioactive compounds with antimicrobial potential. Historically, natural products have provided the foundation for many frontline antibiotics, and contemporary research continues to validate their importance in drug discovery (13–15). Phytochemicals such as alkaloids, flavonoids, terpenoids, phenolics, and tannins exhibit antibacterial, antifungal, anti-inflammatory, and antioxidant properties, highlighting their potential as templates for novel antimicrobial agents (16,17). These characteristics make plant-derived compounds particularly attractive for combating MDR pathogens and addressing the limitations of conventional antibiotics.

Among the many medicinal plants with antimicrobial potential, *Polyalthia longifolia* (family Annonaceae), commonly known as the Ashoka tree, has emerged as a particularly promising candidate. Its selection is based on both its rich phytochemical composition and its historical use in managing infections. Traditionally used across Asia and Africa, various plant parts—including leaves, bark, and seeds—have been employed to treat infections, fever, wound healing, and gastrointestinal disorders (18,19). Ethnobotanical surveys further highlight that leaf decoctions are widely used to treat bacterial infections (20), reflecting both cultural relevance and practical accessibility in resource-limited settings.

2. Materials and Methods

Test Microorganisms

Multidrug-resistant clinical strains of *Klebsiella pneumoniae* and *Acinetobacter baumannii* were used, selected due to their classification as critical-priority pathogens by the World Health Organization. Bacterial inocula were standardized to ~1 × 10^6 CFU/mL using a 0.5 McFarland turbidity standard prepared by mixing 0.05 mL of 1% barium chloride dihydrate with 9.95 mL of 1% sulfuric acid. Strains were subcultured on nutrient agar, incubated at 37 °C for 18 hours, and suspended in sterile saline to match the McFarland standard visually (21).

Plant Material, Extraction, and Fractionation

Air-dried leaves of *Polyalthia longifolia* (syn. *Monoon longifolium*), Annonaceae, were collected and authenticated, and a voucher specimen was prepared. Crude ethanolic extraction was performed using maceration (22). Briefly, 520 g of powdered leaves were soaked in 3 L of ethanol for three days with occasional shaking, filtered through muslin and Whatman No. 1 paper, concentrated, and stored at −20 °C. Fractionation was conducted via liquid–liquid extraction (23). The crude Extract was dissolved in water (1:10, w/v) and sequentially partitioned with n-hexane, a 1:1 n-hexane: ethyl acetate mixture, ethyl acetate, and water to yield four fractions: F1 (n-hexane), F2 (n-hexane: ethyl acetate), F3 (ethyl acetate), and F4 (aqueous). All fractions were concentrated, freeze-dried, and stored at 4 °C for further analysis. This polarity-gradient approach facilitates the enrichment of metabolites according to their solubility and chemical characteristics, enabling targeted evaluation of bioactivity.

Antibacterial Assays

The agar well diffusion method was used to evaluate antibacterial activity (23). Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions. Wells (6 mm) were loaded with 100 µL of Extract (100 mg/mL prepared in 5% DMSO), while amoxicillin or Ofloxacin served as positive controls based on the confirmed susceptibility of the multidrug-resistant (MDR) isolates to these antibiotics. Plates were allowed to pre-diffuse for 15 minutes at room temperature and subsequently incubated at 37 °C for 24 hours. Zones of inhibition were measured in millimeters. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth dilution method followed by subculturing. Serial dilutions of the extracts (100–3.125 mg/mL) were prepared in Mueller–Hinton broth, inoculated with 0.5 mL of standardized bacterial suspension, and incubated at 37 °C for 24 hours. The MIC was defined as the lowest concentration showing no visible bacterial growth, while the MBC was defined as the lowest concentration yielding no growth upon subculture.

HPLC Profiling

Approximately 2 g of Extract was mixed with 20 mL of 1:1 acetonitrile–methanol, agitated for 30 minutes, and the organic phase was transferred to a 25 mL volumetric flask. Analysis was performed on an Agilent 1200 RP-HPLC system using a Hypersil BDS C18 column (250 mm × 4.0 mm i.d.) with a mobile phase of 0.1% formic acid in water (A) and acetonitrile (B), gradient elution, 0.6 mL/min flow rate, 20 µL injection, and 280 nm detection. Compounds were identified by comparing retention times and UV spectra with standards (23).

Generation and Preparation of Compound Library

Twelve (12) phytochemical compounds identified from Polyalthia longifolia by HPLC analysis were selected for *in silico* screening. The corresponding chemical structures of these HPLC-identified compounds, along with that of the reference drug, were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) in Structure Data File (SDF) format. All molecules were imported into the Schrödinger workspace (Schrödinger, 2021) and prepared using the LigPrep module to optimize molecular geometry, assign appropriate protonation states at physiological pH, and generate relevant tautomers and stereoisomers for subsequent computational analyses.

Protein Retrieval and Preparation

The X-ray crystallographic structures of the target proteins—*Klebsiella pneumoniae* metallo-β-lactamase-1 (NDM-1, PDB ID: 4EYB) and *Acinetobacter baumannii* DNA gyrase B (PDB ID: 7PQI)—were obtained from the RCSB Protein Data Bank. The selection of reference ligands for *in silico* screening was based on their mechanistic relevance to the chosen protein targets and known resistance pathways rather than phenotypic susceptibility profiles. Accordingly, Ofloxacin and novobiocin were used as standard inhibitors due to their established interactions with DNA gyrase B and ATP-binding enzymes, respectively. Protein preparation and grid generation were performed using the Schrödinger Protein Preparation Wizard with the OPLS4 force field.

Structure-based Virtual Screening

Prepared compounds from *P. longifolia*, along with the standard ligand, were docked against the target proteins using the extra precision (XP) Glide protocol in Maestro (Schrödinger Suite, 2021). XP docking was employed due to its high accuracy in ranking ligand binding affinities, albeit with longer computational time (23).

Binding Energy Calculations

Docked protein-ligand complexes were further refined using the local optimization feature in Prime. The binding free energy (△_bind) of each complex was estimated through MM/GBSA calculations with the OPLS4 force field, providing quantitative insights into the stability and affinity of ligand interactions.

Statistical Analysis

All experimental data were processed using SPSS v22. One-way ANOVA was performed to compare group means, followed by Duncan's New Multiple Range Test for post hoc analysis. Statistical significance was considered at $p \leq 0.05$.

3. Results

Antibacterial Activity of P. longifolia

The antibacterial activity of the different solvent fractions of *P. longifolia* against multidrug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* is presented in Table 1. All fractions demonstrated inhibitory effects, with inhibition zones ranging between 18.33 ± 0.33 mm and 23.00 ± 0.58 mm. The aqueous fraction (F4) produced the highest zones of inhibition against both test organisms (22.33 ± 0.33 mm for *A. baumannii* and 23.00 ± 0.58 mm for *K. pneumoniae*), followed by the n-hexane fraction (F1). The ethyl acetate fraction (F3) exhibited the lowest activity, particularly against *A. baumannii*. The aqueous fraction exhibited the lowest MIC (6.25 mg/mL) against both *A. baumannii* and *K. pneumoniae*, suggesting the greatest potency. The ethyl acetate and n-hexane: ethyl acetate fractions recorded higher MICs of 25 mg/mL against *A. baumannii*. In contrast, all fractions except Fraction 4 required 12.5 mg/mL to inhibit *K. pneumoniae*. The aqueous fraction again demonstrated the lowest MBC against *A. baumannii* (12.5 mg/mL), whereas the ethyl acetate fraction was most effective against *K. pneumoniae* (12.5 mg/mL). Fractions 1 and 2 exhibited higher MBCs (25–50 mg/mL).

Phytochemical Profile by HPLC

The HPLC chromatographic analysis (Table 2, Figure 1) revealed the presence of several bioactive compounds in the *P. longifolia* leaf extracts, notably Quercetin, Apigenin, catechin, and kaempferol.

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Table 1: Antibacterial Activity of P. longifolia Extracts

Test organisms	Zone of Inhibition (mm)				MIC (mg/mL)				MBC (mg/mL)			
	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
A. baumannii	21.33 ± 0.67	19.33 ± 0.58	18.33± 0.33	22.33± 0.33	12.5	25	25	6.25	50	50	25	12.5
K. pneumoniae	22.67 ±0.67	21.67 ±0.58	21.33± 0.67	23.00± 0.58	12.5	12.5	12.5	6.25	25	25	12.5	25

Legend: F1- n-hexane fraction, F2- n-hexane/ethyl acetate fraction, F3- ethyl acetate fraction, and F4- aqueous fraction.

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
1.317	VV E	20.25056	1.00097e-1	2.02702		Caffeic Acid
1.567	VV E	28.07921	4.69604e-3	1.31861e-1		Ferulic Acid
2.454	VV E	40.72340	3.20764e-2	1.30626		Maleic Acid
3.227	VV E	36.74120	1.7158e-2	1.13671		Catechin
4.051	VB	1399.65674	6.70379e-3	9.38301		Magiferin
4.598	BB	142.88849	4.25662e-2	6.08223		Naringin
5.845	BB	80.81638	1.42089e-2	1.14831		Myricetin
8.147	VV X	1.19665e-1	7.75186	9.27626e-1		Baicalin
9.273	MM	20.02323	2.00894e-2	4.02254e-1		Quercetin
10.875	VV X	1.47642e-2	19.68298	2.90603e-1		Phloretin
11.063	MM	10.13986	8.18996e-2	8.30450e-1		Apigenin
12.257	MM	20.87885	3.63575e-2	7.59102e-1		Kaempferol

Table 2: HPLC-Identified Compounds of Polyalthia longifolia Leaf Extracts

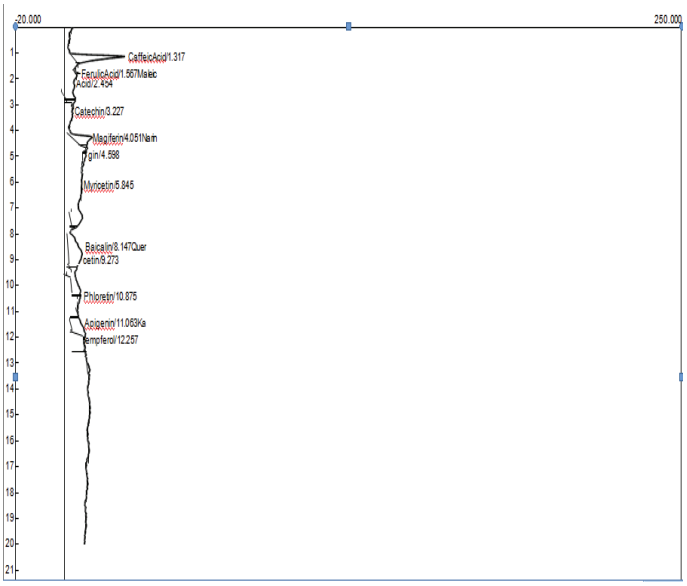


Figure 1: Chromatograph of Polyalthia longifolia Leaf Extract

Molecular Docking Interaction of P. longifolia with K. pneumoniae NDM-1

The binding affinities of the identified phytochemicals toward NDM-1 were compared with standard drugs (Ofloxacin and novobiocin). Among the tested compounds, mangiferin (-9.263 kcal/mol), myricetin (-8.703 kcal/mol), and Baicalin (-8.411 kcal/mol) exhibited stronger binding affinities than Ofloxacin (-6.327 kcal/mol) and oxacillin (-6.286 kcal/mol) (Figure 2). These top-ranking ligands showed multiple hydrogen bonds with key active site residues, including GLN123, ASN220, and ASP124 (Figure 4, Table 3). Hydrophobic interactions were primarily observed with residues such as MET67, LEU65, ILE35, and TRP93, while mangiferin and Baicalin displayed π–π stacking with PHE70. The high binding affinities suggest potential inhibition of the bacterial β-lactamase enzyme comparable to or greater than that of the reference drug.

Molecular Docking Interaction of P. longifolia with A. baumannii DNA Gyrase B

For *A. baumannii*, Quercetin (-7.4 kcal/mol), catechin (-7.262 kcal/mol), and Apigenin (-7.216 kcal/mol) demonstrated the highest binding affinities compared to the standard novobiocin (-6.111 kcal/mol) (Figure 3). These compounds formed multiple hydrogen bonds and hydrophobic interactions with key residues such as ASP87, THR279, and VAL57 (Figure 5, Table 4). Kaempferol and myricetin also interacted favorably, forming π–cation or hydrogen bonds within the catalytic pocket, suggesting competitive inhibition potential against DNA gyrase B.

ADME and Drug-Likeness Properties

The ADME profiles of the top bioactive compounds are presented in Table 5. Most phytochemicals complied with Lipinski's rule of five, indicating good oral bioavailability and pharmacokinetic potential. Compounds such as Quercetin, myricetin, and kaempferol displayed moderate lipophilicity (iLOGP ≈ 1.1–2.0) and acceptable polar surface areas (TPSA ≤ 150 Å²).

High gastrointestinal absorption was predicted for most compounds, particularly Apigenin, kaempferol, and catechin. The compounds

exhibited variable CYP enzyme inhibition, with limited interactions with CYP2C9 and CYP2C19, suggesting low metabolic interference.

Toxicity Prediction

The toxicity profiles (Table 6) revealed that all tested compounds were non-carcinogenic and exhibited low hepatotoxicity and cardiotoxicity risks (low hERG inhibition). None of the lead compounds showed significant Ames mutagenicity or acute oral toxicity, indicating favorable safety margins. Among all, mangiferin, Quercetin, and myricetin presented the most promising safety–efficacy balance, supporting their potential as lead candidates for further development against multidrug-resistant *K. pneumoniae* and *A. baumannii*.

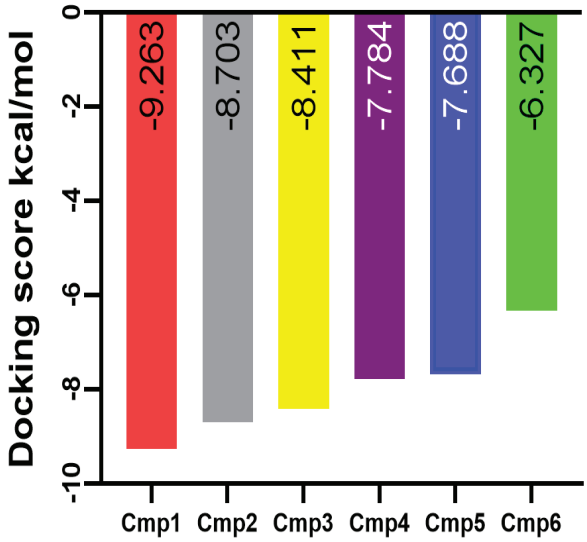


Figure 2: Graphical representation of the binding affinity calculation of the lead compounds from P. longifolia against K. pneumoniae NDM-1

Legend: cmp 1= Mangiferin cmp 2= Myricetin cmp 3= Baicalin cmp 4= Quercetin cmp 5 = Maleic acid cmp 6 = Ofloxacin

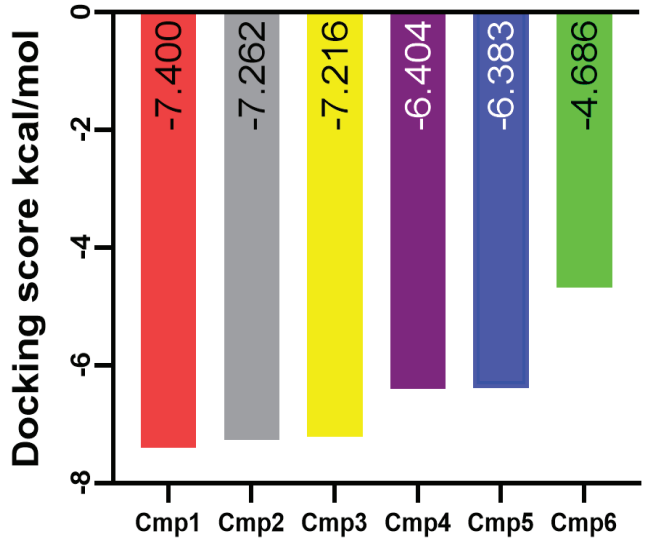
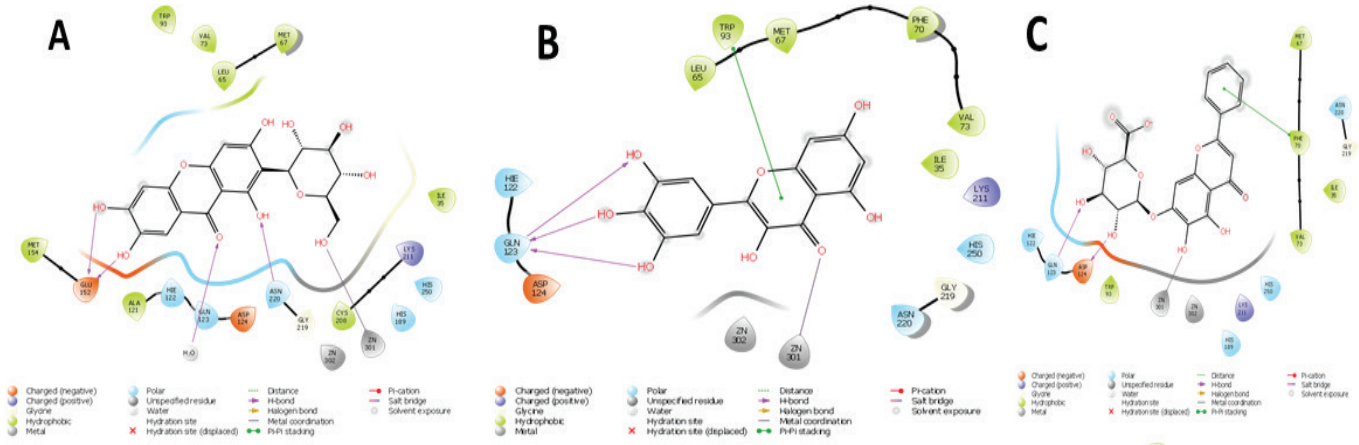


Figure 3: Graphical representation of the binding affinity calculation of the lead compounds from P. longifolia against A. baumannii DNA gyrase B

Legend: cmp 1= Quercetin cmp 2= Catechin cmp 3= Apigenin cmp 4= Kaempferol cmp 5 = Myricetin cmp 6 = Novobiocin



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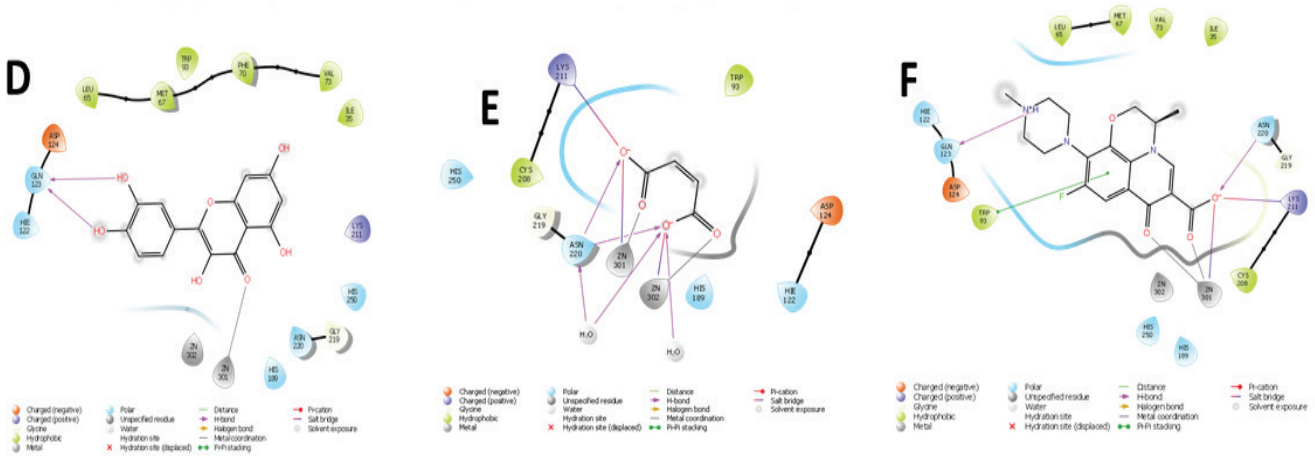

Figure 4: 2D Interaction between P. longifolia lead compounds and Klebsiella pneumoniae protein. Legend : (A) Mangiferin; (B) Myricetin; (C) Baicalin; (D) Quercetin; (E) Maleic acid; (F) Ofloxacin (standard).

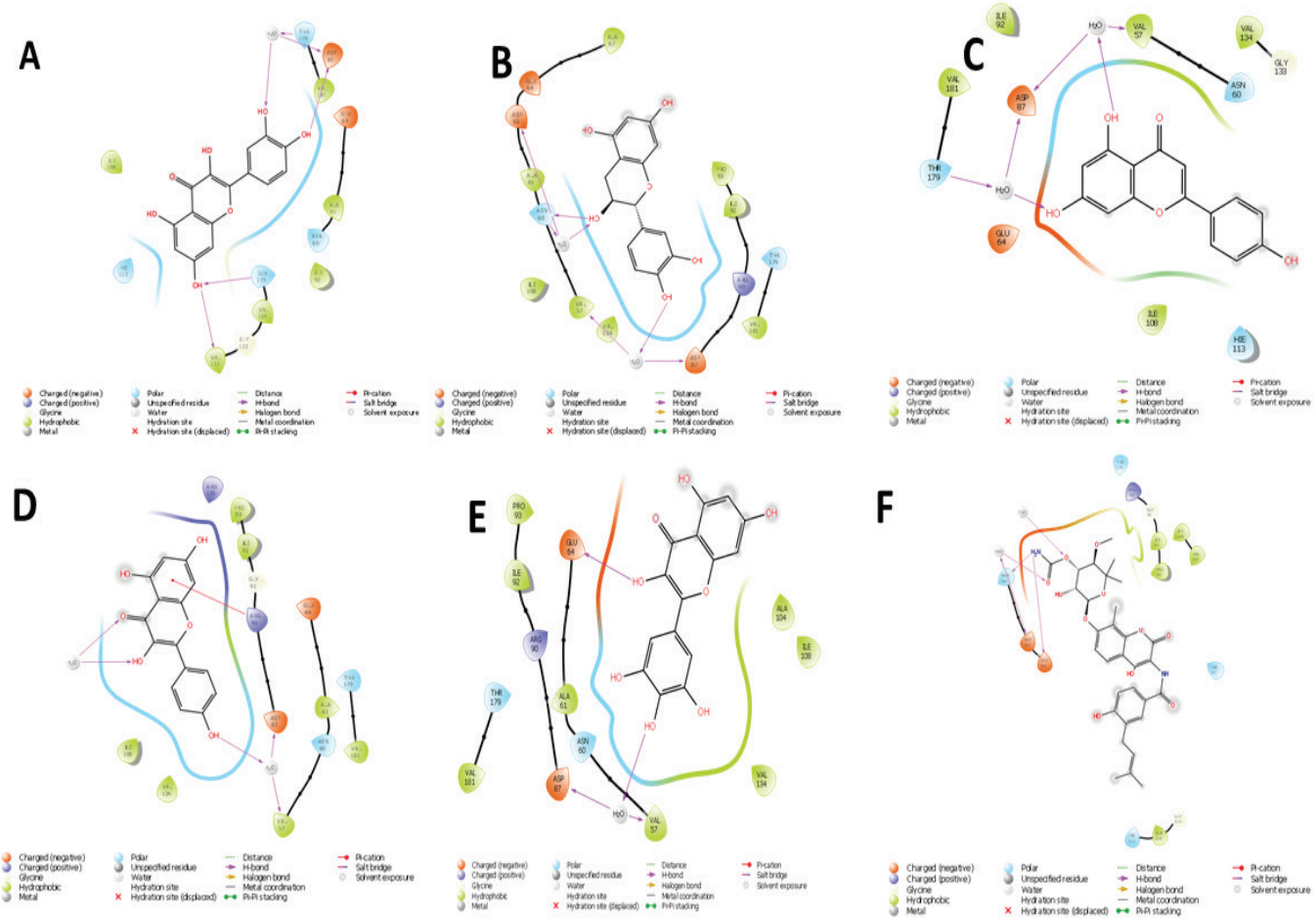

Figure 5: 2D interaction between lead compounds and Acinetobacter baumannii protein
Legend: (A) Quercetin; (B) Catechin; (C) Apigenin; (D) Kaempferol; (E) Myricetin; (F) Novobiocin (standard).

Table 3: Hydrogen Bonds and Hydrophobic Interactions of the Lead Phytochemicals from P. longifolia with K. pneumoniae

Compound Name	H-Bond	Hydrophobic interactions	Other Interactions
Mangiferin	GLU152, ASN220	MET154, ALA121, CYS208, ILE35, LEU65, MET67, VAL73, TRP93	NONE
Myricetin	GLN123	TRP93, LEU65, MET67, PHE70, VAL73, ILE35	Pi-Pi stacking: TRP93
Baicalin	GLN123, ASP124	MET67, PHE70, VAL73, ILE35	Pi-Pi stacking: PHE70
Quercetin	GLN123	LEU65, MET67, TRP93, PHE70, VAL73, ILE35	NONE
Maleic acid	ASN220	TRP93, CYS208	Salt bridge: LYS211, ZN302, ZN302
Ofloxacin	GLN123, ASN220	LEU65, MET67, VAL73, ILE35, TRP93, CYS208	Salt bridge: LYS211, ZN301, Pi-Pi stacking: TRP93

Table 4: Hydrogen Bonds and Hydrophobic Interactions of the Lead Phytochemicals from P. longifolia with A. baumannii

Compound Name	H-Bond	Hydrophobic interactions	Other Interactions
Quercetin	THR279, ASP87, SER135, VAL132	VAL181, ALA61, ILE92, VAL134, VAL132, ILE108	NONE
Catechin	ASP63, ASN60, ASP87, VAL57	ALA67, PRO93, ILE92, ALA61, ILE108, VAL57, VAL134, VAL181	NONE
Apigenin	ASP87, THR179, VAL57,	VAL57, ILE92, VAL134, VAL181, ILE108	NONE
Kaempferol	ASP87, VAL57,	PRO93, ILE92, ALA61, ILE108, VAL134, VAL57, VAL181	Pi cation: ARG90
Myricetin	GLU64, ASP87, VAL57	ALA61, VAL57, VAL134, ILE108, ALA104, ILE92, PRO93, VAL181	NONE
Novobiocin	ASN60, GLU64	ILE92, ILE108, VAL107, PRO93, ALA114	NONE

Table 5: Drug likeness and ADME profile of the top bioactive compounds of P. longifolia

Compound Name	MW	HBA	HBD	TPSA	iLOGP	ROV	ESOL Log S	GIA	CYP2C19 inhibitor	CYP2C9 inhibitor
Quercetin	302.04	7	5	131.36	1.447712	0	-3.72159	0.133589	0.005855004	0.432249367
Ofloxacin	361.14	7	1	75.01	-0.11273	0	-1.97932	3.46E-06	2.59E-05	5.96E-06
Novobiocin	612.23	13	6	200.01	1.790881	1	-4.47123	0.122134	0.711179674	0.007009505
Myricetin	318.04	8	6	151.59	1.115212	0	-3.44294	0.31806	0.002221007	0.995218813
Mangiferin	422.08	11	8	201.28	0.059778	1	-3.15526	0.751839	1.91E-08	0.000311245
Maleic acid	116.01	4	2	74.6	-0.02442	0	0.404371	0.949129	3.26E-10	5.12E-05
Kaempferol	286.05	6	4	111.13	1.965317	0	-3.64796	0.015143	0.132401496	0.79935813
Catechin	290.08	6	5	110.38	1.172588	0	-2.58137	0.016103	9.82E-06	2.47E-06
Baicalin	446.08	11	6	187.12	1.300846	1	-2.30448	0.008194	6.57E-06	7.80E-07
Apigenin	270.05	5	3	90.9	2.980917	0	-4.21599	0.001684	0.112379447	0.002128024

Legend: MW= molecular weight, HBA= Hydrogen bond acceptor , HBD= Hydrogen bond donor, ROV=, GIA= Gastrointestinal absorption, BA= bioavailability

Table 6: Toxicity profile of the top bioactive compounds of P. longifolia

Compounds	Ames	hERG	Hepatoxicity	Carcinogenecity	LD50(mg/kg)
Quercetin	0.586042	0.052722	0.337382	0.600177	['-']
Ofloxacin	0.920446	0.405136	0.988498	0.76824	[(9, 8, 7, 6, 5, 4, 25, 16, 15, 13, 12, 11)]
Novobiocin	0.748943	0.141221	0.88874	0.34689	['-']
Myricetin	0.657129	0.040223	0.325381	0.501712	['-']

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Mangiferin	0.845841	0.015823	0.498865	0.282849	['–']
Maleic acid	0.207986	0.036178	0.242399	0.021734	['–']
Kaempferol	0.545969	0.069277	0.386179	0.715984	['–']
Catechin	0.458893	0.1343	0.556749	0.226083	['–']
Baicalin	0.499818	0.009341	0.428817	0.045225	['–']
Apigenin	0.617563	0.099782	0.435063	0.793352	['–']

Legend: Ames = Mutagenecity potential, hERG= Cardiotoxicity, LD50= Acute oral toxicity

- **Abbreviations**
- **ADME** – Absorption, Distribution, Metabolism, and Excretion
- **AMES** – Mutagenicity potential (Ames test)
- **ATP** – Adenosine Triphosphate
- **CYP** – Cytochrome P450 enzymes
- **DMSO** – Dimethyl sulfoxide
- **DNA** – Deoxyribonucleic acid
- **F1–F4** – Extract fractions (F1: n-hexane, F2: n-hexane:ethyl acetate, F3: ethyl acetate, F4: aqueous)
- **HPLC** – High-Performance Liquid Chromatography
- **hERG** – Human Ether-à-go-go-Related Gene (Cardiotoxicity potential)
- **iLOGP** – Lipophilicity prediction index
- **LD50** – Median lethal dose (Acute oral toxicity)
- **MBC** – Minimum Bactericidal Concentration
- **MIC** – Minimum Inhibitory Concentration
- **MDR** – Multidrug-resistant
- **NDM-1** – New Delhi Metallo-β-lactamase-1
- **PDB** – Protein Data Bank
- **TPSA** – Topological Polar Surface Area
- **XP Glide** – Extra Precision Glide docking protocol

β-lactam – Beta-lactam antibiotics

4. DISCUSSION

This study evaluated the antibacterial activity of different solvent fractions of *Polyalthia longifolia* leaves against multidrug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*, two critical-priority nosocomial pathogens (8,24). The rising prevalence of these resistant bacteria underscores the urgent need for alternative therapeutics, particularly natural products with established bioactive efficacy.

Antibacterial screening revealed variable inhibitory potencies among the solvent fractions. The aqueous fraction consistently produced the largest zones of inhibition and the lowest MIC and MBC values against both pathogens, indicating a higher concentration of polar antibacterial constituents. HPLC profiling confirmed the presence of Quercetin, rutin, and other phenolic compounds, which are known to act synergistically to enhance antibacterial activity. These findings align with previous reports demonstrating that polar extracts of *Polyalthia longifolia* exhibit broad-spectrum antibacterial activity largely attributed to flavonoids, phenolics, and glycosides (25–27). Flavonoids such as Quercetin have been reported to disrupt bacterial membrane integrity, inhibit nucleic acid synthesis, and interfere with energy metabolism (27), thereby supporting the observed potency of the aqueous fraction.

In addition to polar phytochemicals, non-polar and moderately polar fractions of *P. longifolia* in the present study also exhibited antibacterial activity, albeit to a lesser extent. The n-hexane fraction showed appreciable inhibitory effects, particularly against *Klebsiella pneumoniae*, suggesting the involvement of lipophilic constituents such as terpenoids and fatty acids with membrane-disrupting and quorum-sensing inhibitory properties (28,29). Notably, clerodane-type diterpenoids previously isolated from *P. longifolia* have been reported to possess significant antibacterial activity**,** supporting the bioactivity observed in non-polar fractions. Faizi et al. (30) demonstrated that clerodane diterpenoids isolated from *P. longifolia* exhibited marked antimicrobial effects against pathogenic bacteria, highlighting the contribution of diterpenoid scaffolds to the plant's antibacterial profile.

The ethyl acetate fraction also demonstrated notable antibacterial activity and recorded the lowest MBC value against *K. pneumoniae*, emphasizing the bactericidal potential of moderately polar metabolites, including terpenoids, phenolic acids, and other semi-polar compounds. Collectively, these results underscore the influence of solvent polarity on phytochemical extraction efficiency and antibacterial efficacy, and further corroborate earlier reports that both polar phenolics and non-

polar diterpenoids contribute to the antimicrobial potential of P. longifolia (31).

Variability in bacterial susceptibility was evident, with *K. pneumoniae* being more sensitive than *A. baumannii*, consistent with the latter's robust efflux pumps and biofilm-mediated resistance (7,32). Fractionation preserved antibacterial efficacy, with the aqueous fraction achieving inhibition zones of 22.3 mm (*A. baumannii*) and 23.0 mm (*K. pneumoniae*), MICs of 6.25 mg/mL, and MBCs as low as 12.5 mg/mL, comparable to previous studies using crude extracts (26,33). The strong performance of the aqueous fraction likely reflects synergistic interactions among diverse metabolites (15).

At the molecular level, resistance in *K. pneumoniae* and *A. baumannii* is largely mediated by β-lactamases and DNA gyrase enzymes (34,35). The metallo-β-lactamase NDM-1 hydrolyzes β-lactam antibiotics (36,37), while Ofloxacin inhibits bacterial replication via stabilization of the DNA–DNA gyrase complex. DNA gyrase B in *A. baumannii* catalyzes ATP-dependent negative supercoiling of DNA, and Novobiocin competes at its ATP-binding site (38-40). Docking of *P. longifolia* phytochemicals against these targets provides mechanistic insight into their observed antibacterial activity.

Docking analyses revealed that Mangiferin, Myricetin, and Baicalin exhibited stronger binding affinities toward NDM-1 than Ofloxacin. Mangiferin (-9.263 kcal/mol) formed key hydrogen bonds with GLU152 and ASN220 and hydrophobic contacts with MET154, ALA121, CYS208, ILE35, LEU65, MET67, VAL73, and TRP93, stabilizing the complex. Myricetin (-8.703 kcal/mol) and Baicalin (-8.411 kcal/mol) showed robust interactions via hydrogen bonds, hydrophobic contacts, and π–π stacking, consistent with previous findings that hydroxyl-rich flavonoids enhance binding to target enzymes (41). Quercetin (-7.784 kcal/mol) and Maleic acid (-7.688 kcal/mol) demonstrated moderate binding, while reference antibiotics Oxacillin (-6.286 kcal/mol) and Ofloxacin (-6.327 kcal/mol) displayed weaker affinities. These findings indicate that natural compounds may demonstrate superior inhibitory interactions compared to standard drugs, in agreement with previous studies (22,42).

For *A. baumannii*, Quercetin exhibited the highest binding affinity (-7.400 kcal/mol), forming multiple hydrogen bonds with THR279, ASP87, SER135, VAL132, and ILE108, complemented by hydrophobic interactions. These results suggest that the natural compounds adopt favorable conformations within the receptor's active site, enabling stable and meaningful interactions, consistent with previous findings (43,44). Catechin (-7.262 kcal/mol) and Apigenin (-7.216 kcal/mol) displayed comparable stabilizing interactions, while Kaempferol (-6.404 kcal/mol) and Myricetin (-6.383 kcal/mol) engaged in hydrogen bonding, hydrophobic contacts, and π–cation interactions with ARG90. By contrast, reference antibiotics Novobiocin (-4.686 kcal/mol) and Ofloxacin (-4.415 kcal/mol) showed lower docking scores, reflecting weaker binding tendencies and highlighting the potential of these natural compounds as effective inhibitors.

The correlation between *in silico* binding affinities and *in vitro* antibacterial outcomes reinforces the predictive accuracy of docking results. The aqueous fraction's superior potency is likely attributable to hydrophilic flavonoids, including Quercetin and Mangiferin, which interact favorably with both NDM-1 and DNA gyrase B, supporting dual inhibitory mechanisms that impair β-lactam hydrolysis and DNA replication.

ADMET profiling confirmed the pharmacological potential of the lead phytochemicals. Most compounds complied with Lipinski's Rule of Five, indicating favorable oral bioavailability and membrane permeability (45). Moderate lipophilicity (iLOGP: -0.024 to 2.98) supported membrane transport while maintaining solubility. Gastrointestinal absorption was high, and limited CYP450 inhibition suggested minimal metabolic interference. Notably, Myricetin and Catechin exhibit significant antioxidant and antibacterial activity with negligible CYP450 inhibition (46,47), consistent with the present study's findings. In contrast, Baicalin and Mangiferin showed restricted bioavailability due to high TPSA and numerous hydrogen bond donors, reflecting pharmacokinetic limitations typical of glycosylated polyphenols (41,48). Toxicity predictions indicated low mutagenic, hepatotoxic, and cardiotoxic risks for the natural compounds, whereas reference antibiotics Ofloxacin and Novobiocin displayed higher hepatotoxicity and Ames values, corroborating clinical reports of dose-related hepatocellular injury (49).

Overall, the combined *in silico* and *in vitro* evidence indicates that *P. longifolia* bioactive compounds exhibit dual antibacterial mechanisms—targeting β-lactamase and DNA gyrase B enzymes—similar to Novobiocin and Ofloxacin, while maintaining superior safety and pharmacokinetic profiles.

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5. Conclusion

Polyalthia longifolia leaf fractions exhibit significant antibacterial activity against multidrug-resistant *A. baumannii* and *K. pneumoniae*, with the aqueous fraction showing the highest efficacy. The n-hexane fraction also demonstrated notable activity, highlighting contributions from both polar and non-polar phytochemicals. HPLC analysis identified flavonoids, phenolic acids, and alkaloids—including Mangiferin, Myricetin, Baicalin, Catechin, and Quercetin—as key bioactive compounds likely acting synergistically.

Molecular docking revealed strong binding affinities of these phytochemicals to clinically relevant bacterial targets (NDM-1 and DNA gyrase B), surpassing or matching standard antibiotics and supporting dual inhibitory potential observed *in vitro*. ADMET profiling confirmed favorable pharmacokinetic and safety profiles, with good oral bioavailability, low CYP450 inhibition, and minimal toxicity, though some polyhydroxylated flavonoids showed limited gastrointestinal absorption.

These findings underscore the therapeutic potential of *P. longifolia* as a source of bioactive compounds with dual antibacterial mechanisms, combining cell wall disruption and DNA replication inhibition. Further *in vitro* and *in vivo* validation, along with optimization for solubility and absorption, is essential to translate these findings into clinically useful antimicrobial therapies.

References

1. World Health Organization. Antimicrobial resistance: Global report on surveillance Report: 2021 [Internet]. www.who.int. 2021. Available from: https://www.who.int/publications/i/item/9789240027336

2. Geng Y, Liu Z, Ma X, Pan T, Chen M, Dang J, et al. Infection prevention and control measures for multidrug-resistant organisms: a systematic review and network meta-analysis. Infection. 2025 Mar 17;53.

3. Abebe AA, Birhanu AG, Tessema TS. Antibiotic Resistance and Virulence Mechanisms in Klebsiella pneumoniae: Understanding for Better Interventions. Bacteria. 2026 Feb 2;5(1):9.

4. Hao SY, Lin HY, Yeh PI, Hsiao HC, Lin CY, Huang WH, et al. Multidrug-Resistant Acinetobacter baumannii Pneumonia in Clinical Therapy: A Systematic Review and Network Meta-analysis on Effectiveness and Safety of Different Antibiotic Combination Regimens. International Journal of Antimicrobial Agents. 2026 Apr;107815.

5. Kulaç Ö, Başkan C, Koşar N, Balcı PÖ, Havuz SG, Sırıken B. Klebsiella pneumoniae clinical isolates: extended spectrum β-lactamase production, biofilm formation, and virulence factors. Biologia. 2024 Aug 6;79(10):3209–17.

6. Mukhopadhyay H, Bairagi A, Mukherjee A, Prasad AK, Roy AD, Nayak A. Multidrug resistant Acinetobacter baumannii: A study on its pathogenesis and therapeutics. Current Research in Microbial Sciences. 2024 Dec 6;8:100331.

7. Oluyele O, Olaoluwa SF, Akanji V, Egunjobi G, Eweola E. Fractionation-Guided Evaluation of Justicia secunda and Kalanchoe pinnata as Inhibitors of DNA Gyrase and Penicillin-Binding Proteins in Multidrug-Resistant Acinetobacter baumannii and Klebsiella pneumoniae. Journal of Natural Products Discovery. 2026;4(4).

8. World Health Organization. WHO Bacterial Priority Pathogens List, 2024 [Internet]. 2024. Available from: https://iris.who.int/bitstream/handle/10665/376776/9789240093461-eng.pdf

9. Patra M, Gupta A, Kumar D, Kumar B. Antimicrobial Resistance: A Rising Global Threat to Public Health. Infection and Drug Resistance. 2025 Oct;Volume 18:5419–37.

10. Asmare Z, Tamrat E, Erkihun M, Endalamaw K, Alelign D, Getie M, et al. Antimicrobial resistance pattern of Acinetobacter baumannii clinical isolate in Ethiopia. A systematic review and meta-analysis. BMC Infectious Diseases. 2025 Apr 12;25(1).

11. Totaro V, Guido G, Cotugno S, De Vita E, Asaduzzaman M, Patti G, et al. Antimicrobial Resistance in Sub-Saharan Africa: A Comprehensive Landscape Review. The American journal of tropical medicine and hygiene. 2025;tpmd250035.

12. Gargate N, Laws M, Rahman KM. Current economic and regulatory challenges in developing antibiotics for Gram-negative bacteria. npj Antimicrobials and Resistance. 2025 Jun 11;3(1).

13. Oluyele O, Bimboye PT. Harnessing Blighia sapida as a Source of Novel Antimicrobials: Activity of Crude and Fractionated Extracts Against MDR Pathogens. Biology, Medicine, & Natural Product Chemistry. 2026;15(1):221–7.

14. Akorful RAA, Asafo-Adjei K, Kotey FCN, Donkor ES. Effectiveness of African Herbal Preparations against Multidrug-Resistant Klebsiella pneumoniae: A Systematic Review. Scientific African. 2025;28(2).

15. OLUYELE O. HPLC PROFILING AND ANTI-CANDIDAL ACTIVITY OF CRUDE AND FRACTIONATED EXTRACTS OF TITHONIA DIVERSIFOLIA. Quantum Journal of Medical and Health Sciences. 2025 Jun 28;4(3):48–55.

16. Goswami C, Pawase PA, Shams R, Pandey VK, Tripathi A, Rustagi S, et al. A Conceptual Review on Classification, Extraction, Bioactive Potential and Role of Phytochemicals in Human Health. Future foods. 2024 Feb 1;9:100313–3.

17. Dattatray TV, Baburao SP, Shivaji CS. A comprehensive review on Polyalthia longifolia. Traditional Medicine Research. 2021;6(2).

18. Musa N, Ayisa T. Medicinal properties of Polyalthia longifolia (Masquerade tree). International Journal of Agriculture and Earth Science. 2024;10(5):140–9.

19. Ismaila M, Dharmaraj S, Ong E, Jun TG, Sasidharan S. Polyalthia longifolia var. angustifolia thw.: a review of ethnobotany, phytochemistry, pharmacology applications and future prospects as a green anti-aging agent. Advances in Traditional Medicine. 2025 Jun 6;25(4).

20. Firdous SM, Ahmed SN, Hossain SM, Ganguli S, Fayed MAA. Polyalthia longifolia: phytochemistry, ethnomedicinal importance, nutritive value, and pharmacological activities review. Medicinal Chemistry Research. 2022 Jun 10;31(8):1252–64.

21. Oluyele O, Oladunmoye M, Ogundare A, Onifade A, Okunnuga N. Microbial spectrum and susceptibility profile of opportunistic pathogens isolated from cancer patients attending a tertiary healthcare centre in Akure, Nigeria. Microbes, Infection and Chemotherapy. 2023;3:1–10.

22. Oluyele O, Oladunmoye M. Susceptibility Patterns of Staphylococcus aureus Isolated from Wounds Swabs to Extracts of Vernonia amygdalina. Journal of Advances in Medical and Pharmaceutical Sciences. 2017 Jan 10;13(4):1–11.

23. Oluyele O, Omoboyowa DA, Aderogba AE, Osei KA. Piper guineense (Swhan and Thon) Inhibits Lanosterol-14α-demethylase in Multi-Drug Resistant Non-albicans Candida Species: In vitro and In silico Studies. Advances in Medical, Pharmaceutical and Dental Research. 2025 Mar 15;5(1):10.

24. Olaniyan TO, Martínez-Vázquez AV, Escobedo-Bonilla CM, López-Rodríguez C, Huerta-Luévano P, Castrejón-Sánchez O, et al. The Prevalence of ESKAPE Pathogens and Their Drug Resistance Profiles in Aquatic Environments Around the World. Microbiology Research. 2025 Sep 4;16(9):201.

25. Chioma D, Reminus O. Phytochemical Analysis of Polyalthia Longifolia (Fresh Leaves). Direct Research Journal of Chemistry and Material Science. 2023 Sep;11(7):49.

26. Isamotu BA, Dikwa KB, Appah J. Antibacterial activity of aqueous and methanolic extracts of *Polyalthia longifolia* and *Hyptis suaveolens* against clinically relevant isolates from Nigerian Defence Academy Hospital, Kaduna, Nigeria. Science World Journal. 2025 Aug 20;20(2):815–23.

27. Liu Y, Zhu J, Liu Z, Zhi Y, Mei C, Wang H. Flavonoids as Promising Natural Compounds for Combating Bacterial Infections. International Journal of Molecular Sciences. 2025 Mar 10;26(6):2455.

28. Masyita A, Mustika Sari R, Dwi Astuti A, Yasir B, Rahma Rumata N, Emran TB, et al. Terpenes and Terpenoids as Main Bioactive Compounds of Essential oils, Their Roles in Human Health and Potential Application as Natural Food Preservatives. Food Chemistry: X. 2022 Mar;13(100217):100217.

29. Câmara JS, Perestrelo R, Ferreira R, Berenguer CV, Jorge, Castilho PC. Plant-Derived Terpenoids: A Plethora of Bioactive Compounds with Several Health Functions and Industrial Applications—A Comprehensive Overview. Molecules. 2024 Aug 15;29(16):3861–1.

30. Faizi S, Khan RA, Mughal NR, Malik MS, Sajjadi KS, Ahmad A. Antimicrobial activity of various parts of *Polyalthia longifolia* var. *pendula*: isolation of active principles from the leaves and the berries. Phytotherapy Research. 2008 Apr 3;22(7):907–12.

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31. Wakeel A, Jan SA, Ullah I, Shinwari ZK, Xu M. Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. PeerJ. 2019 Oct 9;7:e7857.

32. Abdi SN, Ghotaslou R, Ganbarov K, Mobed A, Tanomand A, Yousefi M, et al. Acinetobacter baumannii Efflux Pumps and Antibiotic Resistance. Infection and Drug Resistance. 2020 Feb 12;13:423–34.

33. Umar FJ, Idris FT, Usman A, Balarabe FT, Adamu A. Antibacterial Activity of Polyalthia longifolia Leaf Extracts against Staphylococcus aureus and Escherichia coli. UMYU Journal of Microbiology Research (UJMR). 2024 Jun 9;9(3):8–12.

34. Li Y, Kumar S, Zhang L. Mechanisms of Antibiotic Resistance and Developments in Therapeutic Strategies to Combat Klebsiella pneumoniae Infection. Infection and Drug Resistance. 2024 Mar;17:1107–19.

35. Ramachandran S, Deepak K, Shrikumaran M, et al. Unveiling meropenem resistance and co-resistance patterns in *Klebsiella pneumoniae and Acinetobacter baumannii*: A global genome analysis using ML/DL and association mining. *BMC Microbiol.* 2025;25:699. https://doi.org/10.1186/s12866-025-04394-6

36. Raghunath D. New metallo β-lactamase NDM-1. The Indian journal of medical research. 2010 Nov;132(5):478–81.

37. King DT, Worrall LJ, Gruninger R, Strynadka NCJ. New Delhi Metallo-β-Lactamase: Structural Insights into β-Lactam Recognition and Inhibition. Journal of the American Chemical Society. 2012 Jul 5;134(28):11362–5.

38. Dighe SN, Collet TA. Recent advances in DNA gyrase-targeted antimicrobial agents. European Journal of Medicinal Chemistry. 2020 Aug;199:112326.

39. Cotman AE, Durcik M, Benedetto Tiz D, Fulgheri F, Secci D, Sterle M, et al. Discovery and Hit-to-Lead Optimization of Benzothiazole Scaffold-Based DNA Gyrase Inhibitors with Potent Activity against *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Journal of Medicinal Chemistry. 2023 Jan 12;66(2):1380–425.

40. Rajakumari K, Aravind K, Balamugundhan M, Jagadeesan M, Somasundaram A, Brindha Devi P, et al. Comprehensive review of DNA gyrase as enzymatic target for drug discovery and development. European Journal of Medicinal Chemistry Reports. 2024 Dec;12:100233.

41. Huang T, Liu Y, Zhang C. Pharmacokinetics and Bioavailability Enhancement of Baicalin: A Review. European Journal of Drug Metabolism and Pharmacokinetics. 2019 Apr 1;44(2):159–68.

42. Pancu DF, Scurtu A, Macasoi IG, Marti D, Mioc M, Soica C, et al. Antibiotics: Conventional Therapy and Natural Compounds with Antibacterial Activity—A Pharmaco-Toxicological Screening. Antibiotics. 2021 Apr 7;10(4):401.

43. Ali M, Hassan M, Ansari SA, Alkahtani HM, Al-Rasheed LS, Shoeb Anwar Ansari. Quercetin and Kaempferol as Multi-Targeting Antidiabetic Agents against Mouse Model of Chemically Induced Type 2 Diabetes. Pharmaceuticals. 2024 Jun 8;17(6):757–7.

44. Boo HJ, Yoon D, Choi Y, Kim Y, Cha JS, Yoo J. Quercetin: Molecular Insights into Its Biological Roles. Biomolecules. 2025 Feb 20;15(3):313.

45. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews. 2001 Mar;46(1-3):3–26.

46. Almatroodi SA, Rahmani AH. Unlocking the Pharmacological Potential of Myricetin Against Various Pathogenesis. International Journal of Molecular Sciences. 2025 Apr 28;26(9):4188–8.

47. Nadalin P, Kim JK, Park SU. Recent studies on myricetin and its biological and pharmacological activities. EXCLI Journal. 2023 Nov 28;22:1223.

48. Imran M, Arshad MS, Butt MS, Kwon JH, Arshad MU, Sultan MT. Mangiferin: a natural miracle bioactive compound against lifestyle related disorders. Lipids in Health and Disease. 2017 May 2;16(1).

49. Andrade RJ, Tulkens PM. Hepatic safety of antibiotics used in primary care. Journal of Antimicrobial Chemotherapy. 2011 Jul 1;66(7):1431–46.

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Correlation of SCORAD Score With Absolute Eosinophil Count in Atopic Dermatitis: Experience From a Tertiary Care Center

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ABSTRACT:

Background: *Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disorder commonly associated with Th2-mediated immune dysregulation and peripheral eosinophilia. Clinical severity is most often assessed using the SCORing Atopic Dermatitis (SCORAD) index; however, its routine application may be limited in high-volume clinical settings. Absolute eosinophil count (AEC) is an inexpensive and widely available laboratory parameter that may serve as an objective marker of disease severity.*

Objectives: *To assess the correlation between SCORAD score and absolute eosinophil count in patients with atopic dermatitis and to evaluate the potential of AEC as an objective biomarker for clinical severity.*

Patients and Methods: *This hospital-based, cross-sectional study included 100 consecutive patients of all age groups with clinically diagnosed atopic dermatitis attending a tertiary care center. Diagnosis was established using the Hanifin and Rajka criteria. Disease severity was assessed using the SCORAD index and classified as mild (<25), moderate (25–50), or severe (>50). Absolute eosinophil count was measured from peripheral blood samples using an automated hematology analyzer. Statistical analysis included descriptive statistics and Pearson's correlation coefficient to evaluate the relationship between SCORAD and AEC, with $p < 0.05$ considered statistically significant.*

Results: *The mean age of patients was 12.8 ± 6.4 years, with a male predominance (56%). A statistically significant moderate–to–strong positive correlation was observed between SCORAD score and absolute eosinophil count ($r = 0.57$; $p < 0.0001$). Absolute eosinophil counts increased progressively with increasing disease severity across SCORAD-defined categories.*

Conclusion: *Absolute eosinophil count demonstrates a significant positive correlation with clinical severity of atopic dermatitis. Given its simplicity, affordability, and widespread availability, AEC may serve as a useful objective adjunct to SCORAD for assessing disease severity, particularly in resource-limited clinical settings.*

KEYWORDS:

Atopic dermatitis; SCORAD; Absolute eosinophil count; Disease severity; Biomarker.

1. Introduction

Atopic dermatitis (AD), also known as atopic eczema, is a chronic, relapsing inflammatory skin disorder characterized by intense pruritus, eczematous lesions, and xerosis.[1] It is one of the most common dermatological conditions in childhood, though it may occur at any age. The global prevalence of AD has been increasing over the last few decades, imposing a substantial burden on patients, families, and healthcare systems through direct medical costs, impaired quality of life, and sleep disturbances.[2]

The pathogenesis of AD is multifactorial, involving a complex interaction between genetic predisposition, immune dysregulation,

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environmental triggers, and epidermal barrier dysfunction.[3] The hallmark immunological feature of AD is Th2-mediated inflammation, leading to elevated serum IgE levels and increased circulating eosinophils in many patients.[4] Absolute eosinophil count (AEC) is therefore widely regarded as an accessible surrogate marker of allergic inflammation and immune activation in atopic disorders.[5]

Clinical assessment of AD severity is commonly performed using the SCORing Atopic Dermatitis (SCORAD) index, a validated composite tool that incorporates the extent of involvement, intensity of clinical signs, and subjective symptoms such as pruritus and sleep loss. SCORAD is extensively used in both clinical practice and research.[6]

While both SCORAD and AEC independently reflect aspects of disease severity, the degree of correlation between these parameters remains variable across studies. Understanding this relationship is important, as it can help determine whether AEC can serve as an accessible, objective biomarker for assessing AD severity—especially in resource-constrained settings where structured scoring systems may be underutilized.

Rationale of the Study

Although SCORAD is a comprehensive and widely accepted tool for assessing AD severity, its routine use in many clinical settings—particularly high-volume outpatient departments and peripheral centers—may be limited by time constraints, need for training, and reliance on subjective patient-reported symptoms. In contrast, the Absolute Eosinophil Count is an inexpensive, easily obtainable laboratory parameter available even in basic healthcare facilities.

Previous studies have reported inconsistent correlations between SCORAD and AEC, ranging from weak to moderate associations. These discrepancies may be due to differences in study design, sample size, age distribution, AD phenotype (intrinsic vs. extrinsic), and regional variations in eosinophil-modulating conditions such as parasitic infections and environmental allergens. Given these inconsistencies, there is a need for locally derived evidence to understand whether AEC can reliably reflect clinical severity in the population served by a tertiary care center.

Establishing a clear relationship between SCORAD and AEC would allow clinicians to use AEC as a supportive, objective marker in the assessment of AD severity, especially when standardized scoring systems are difficult to apply. Such evidence would also aid in early identification of patients with potentially severe disease, guide treatment decisions, and facilitate monitoring of disease progression.

This study aims to evaluate the correlation between SCORAD score and AEC in patients with atopic dermatitis and to assess whether AEC can reliably reflect clinical severity.

AIM

To assess the correlation between SCORAD score and Absolute Eosinophil Count (AEC) in patients diagnosed with atopic dermatitis.

OBJECTIVES

1. To determine the SCORAD score of patients with clinically diagnosed atopic dermatitis.
2. To measure the Absolute Eosinophil Count (AEC) in the study population.
3. To analyze the correlation between SCORAD score and AEC using appropriate statistical methods.
4. To compare AEC levels across AD severity categories (mild, moderate, severe) based on SCORAD.
5. To evaluate the potential of AEC as an objective biomarker for predicting clinical severity in atopic dermatitis.

2. MATERIALS AND METHODS

Study Design and Setting

This was a hospital-based, cross-sectional observational study conducted in the Department of Dermatology, N.R.S. Medical College & Hospital, a tertiary care teaching institution. The study aims to assess clinical patterns and relevant hematological parameters among patients diagnosed with atopic dermatitis.

Study Duration

The study was conducted over a period of 12 months, from March 2017 to February 2018.

Study Population

The study population consisted of 100 patients of both sexes and all age groups who were clinically diagnosed with Atopic Dermatitis based on the Hanifin and Rajka diagnostic criteria. These patients were recruited from those attending the Dermatology Outpatient Department (OPD) of N.R.S. Medical College & Hospital during the study period.

Sample Size

The sample size for the present study was determined based on the requirement to detect a statistically significant correlation between the SCORAD index and Absolute Eosinophil Count (AEC). Using Fisher's Z-transformation method for correlation analysis, and assuming an expected moderate correlation coefficient of r = 0.30, with a 95% confidence level ($Z\alpha/2 = 1.96$) and 80% power ($Z\beta = 0.84$), the minimum required sample size was calculated to be approximately 85. To ensure adequate precision, compensate for possible missing or incomplete data, and improve the robustness of subgroup analyses, the sample size was increased. Thus, a total of 100 consecutive patients fulfilling the inclusion and exclusion criteria were finally enrolled in the study.

Inclusion Criteria

Patients were included in the study if they fulfilled all of the following criteria:

1. Clinical diagnosis of atopic dermatitis based on Hanifin and Rajka diagnostic criteria, with the presence of at least three major and three minor criteria.
2. Newly diagnosed cases of atopic dermatitis who had not received any form of treatment (topical or systemic) for AD in the preceding 2 months.
3. Patients of any age group and either sex attending the Dermatology OPD during the study period.
4. Patients (or parents/guardians, in the case of minors) who were willing to adhere to the study protocol and agreed to undergo all necessary investigations, and who provided written informed consent.

Exclusion Criteria

1. Patients were excluded from the study if any of the following were present:
2. Refusal to give consent by the patient and/or legal guardian (for minors), or unwillingness to participate in the study.
3. History of being on treatment for atopic dermatitis (topical or systemic) during the last 2 months prior to enrollment.
4. Current use of any immunosuppressive drug (such as systemic corticosteroids, cyclosporine, methotrexate, biologics, etc.) for any indication.
5. Patients with clinical evidence or history suggestive of parasitic infestations or helminthic infections were excluded to avoid confounding effects on eosinophil counts.
6. Patients who are unwilling or unable to provide informed consent.

Sampling Technique

A non-probability consecutive sampling method was used. All eligible patients presenting to the Dermatology OPD during the study period and meeting the inclusion criteria were invited to participate until the required sample size was achieved.

Data Collection Procedure

Clinical Evaluation and SCORAD Assessment

History and Clinical Examination

A detailed history was obtained regarding age, sex, duration of disease, age of onset, family history of atopy, associated atopic conditions (asthma, allergic rhinitis), triggering or aggravating factors, and treatment history.

A thorough dermatological examination was conducted to document the distribution and morphology of lesions.

SCORAD Scoring: Disease severity was assessed using the SCORing Atopic Dermatitis (SCORAD) index. All SCORAD assessments were performed by a single dermatologist trained in standardized SCORAD scoring, thereby minimizing

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interobserver variability. SCORAD was selected over indices such as EASI because it incorporates both objective signs and subjective symptoms (pruritus and sleep loss) and is widely validated and commonly used in routine clinical practice, particularly in resource-limited settings.

Bottom of Form

The SCORAD was calculated using three components.

Extent (A): The percentage of body surface area (BSA) involved was estimated using the "rule of nines" or the Lund–Browder chart and scored from 0 to 100.

Intensity (B): Six clinical signs (erythema, edema/papulation, oozing/crusts, excoriation, lichenification, and dryness of non-lesional skin) were rated on a 4-point scale from 0 (absent) to 3 (severe). The sum of these six items (maximum score of 18) constituted the intensity score.

Subjective Symptoms (C): Pruritus and sleep disturbance over the preceding 3 days and nights were each scored by the patient or parent using a visual analogue scale (VAS) ranging from 0 (none) to 10 (worst possible).

The SCORAD index was then calculated using the standard formula:

SCORAD = A/5 + 7B/2 + C

Classification of Disease Severity Based on the total SCORAD score, patients were categorized into:

Mild AD: SCORAD < 25

Moderate AD: SCORAD 25–50

Severe AD: SCORAD > 50

Laboratory Evaluation: Absolute Eosinophil Count (AEC)

Sample Collection

On the same day as the clinical assessment, approximately 2–3 mL of venous blood was collected from each patient under aseptic precautions into an EDTA tube.

Measurement of AEC

A complete blood count (CBC), including differential leukocyte count, was performed using an automated hematology analyzer available in the central laboratory.

Absolute Eosinophil Count (cells/µL) was obtained directly from the analyzer output or calculated as:

AEC=Total leukocyte count×% eosinophils /100

Internal quality control procedures of the laboratory were followed as per institutional norms.

Timing of Testing

Wherever possible, blood samples were collected in the morning, and analysis was completed on the same day to minimize pre-analytical variability.

Study Variables

Primary variables

1. SCORAD score (continuous)
2. Absolute Eosinophil Count (AEC; cells/µL; continuous)

Secondary variables

1. Age, sex, duration of disease, age of onset
2. Family history of atopy
3. Presence of associated atopic conditions (asthma, allergic rhinitis)
4. AD severity categories (mild, moderate, severe) based on SCORAD

Data Management

All data was recorded in a pre-designed case record form (CRF) and subsequently entered into a spreadsheet (e.g., Microsoft Excel). Data was checked for completeness, consistency, and outliers. Any discrepancies were resolved by referring to the original CRFs.

Statistical analysis was performed using SPSS. For the demographic profile, descriptive statistical methods were applied. Age, duration of disease, and other continuous variables were summarized using mean and standard deviation, whereas categorical variables such as sex distribution, age categories, family history

of atopy, and associated atopic conditions were expressed as frequencies and percentages. Continuous variables such as SCORAD and Absolute Eosinophil Count (AEC) were expressed as mean ± standard deviation, while categorical variables were summarized as frequencies and percentages. The distribution of continuous variables was assessed using the Shapiro–Wilk test and visual inspection of Q–Q plots, and both SCORAD and AEC were found to follow an approximately normal distribution; therefore, parametric tests were applied. The primary analysis examining the relationship between SCORAD and AEC was conducted using the Pearson correlation coefficient (r), with a p-value <0.05 considered statistically significant. A scatter plot with a regression line and a correlation heatmap was generated to visually depict the linear association between the two variables.

3. Results

Table 1: Demographic profile of the study population (n = 100)

Variable	Category	Frequency	Percentage
Age (years)	Mean ± SD	12.8 ± 6.4	—
	0–5	22	22%
	6–12	38	38%
	13–18	26	26%
	>18	14	14%
Sex	Male	56	56%
	Female	44	44%
Duration of disease	<1 year	18	18%
	1–5 years	52	52%
	>5 years	30	30%
Family history of atopy	Present	41	41%
	Absent	59	59%
Associated atopic conditions	Asthma	19	19%
	Allergic rhinitis	28	28%
	Both	11	11%
	None	42	42%

A moderate–to–strong correlation was observed between SCORAD and AEC (r = 0.57).

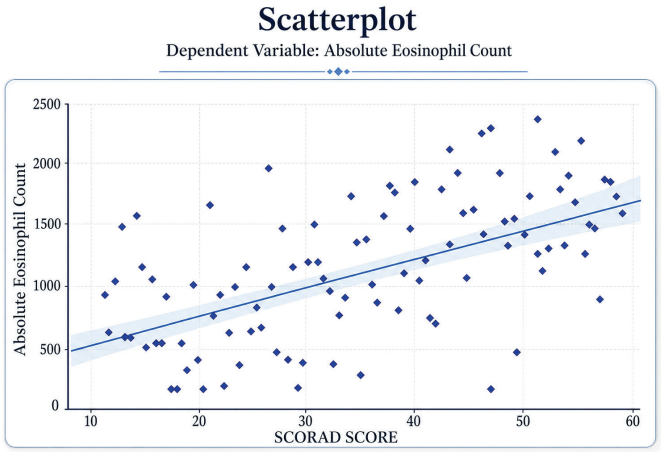

Figure 1: Scatter Plot Showing Correlation between SCORAD Score and Absolute Eosinophil Count

The Pearson correlation coefficient between SCORAD and AEC was r = 0.57 (**p < 0.0001**), indicating a **moderate to strong positive linear relationship** between the two variables. This means that as one variable increases, the other tends to increase in a proportional manner. The associated **p-value (<0.0001)** demonstrates that this correlation is **highly statistically significant**, implying that the likelihood of observing such a relationship by random chance is extremely low. Therefore, the analysis provides strong statistical evidence that SCORAD and AEC are positively correlated in the study population.

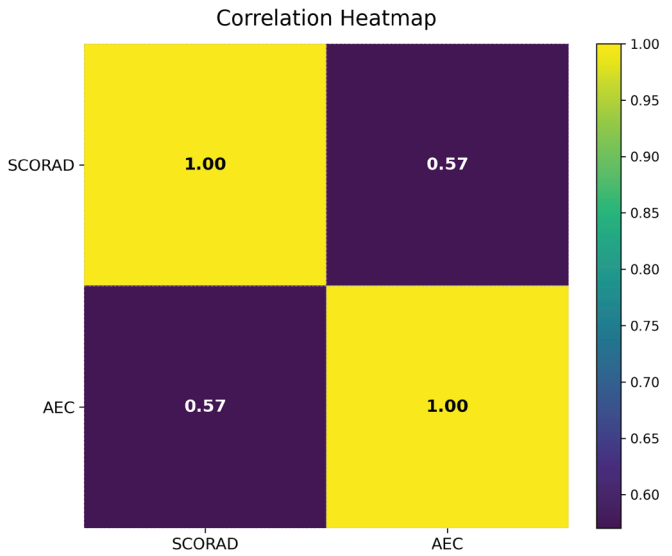

Figure 2: Correlation Heatmap of SCORAD Score and Absolute Eosinophil Count

4. DISCUSSION

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease in which clinical severity reflects a complex interaction of epidermal barrier dysfunction, Th2-skewed immunity, and environmental triggers.

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Comparison of Demographic Profile With Other Studies

The demographic characteristics of the present study population (n = 100) show that atopic dermatitis was predominantly observed in children and adolescents, with a mean age of **12.8 ± 6.4 years**, and **60% of cases occurred below 18 years of age**. This age pattern aligns with the classical epidemiology of atopic dermatitis described by Dhar et al.[5], who also reported a higher burden in the pediatric age group. Similarly, Kumar MK et al. [7] found that the majority of affected individuals in Eastern India were younger children, supporting the early-onset nature of the disease. In our study, **males constituted 56%** of the patients, comparable to the male predominance (around 55–60%) observed in the studies by Pandey et al. [8]and Ramya et al. [9], although some Western cohorts have shown a more equal sex distribution.

With respect to disease chronicity, **52% of patients had a disease duration of 1–5 years**, which is consistent with the chronic, relapsing course noted in previous Indian studies, including Dhar et al.[5] and Kumar MK et al.[7], who reported prolonged disease duration in nearly half of their cohorts. A positive **family history of atopy was present in 41%** of our subjects, which is comparable to the 35–50% range reported in studies by Čelakovská & Bukač et al.[11] and Kumar MK et al.[7], reinforcing the strong familial and genetic predisposition associated with the atopic diathesis.

Regarding associated atopic conditions, **asthma (19%)** and **allergic rhinitis (28%)** were common in our population, while **11% had both**, amounting to a total of 58% with some form of atopy. These findings closely mirror the prevalence of associated atopic manifestations reported by Pandey et al.[8], who documented eosinophilia and coexisting allergic diseases in a significant proportion of patients. The overall frequency of comorbid atopy in our study also aligns with published literature, which frequently reports that 40–70% of patients with atopic dermatitis exhibit at least one additional atopic condition. Identifying objective, easily measurable biomarkers that correlate with clinical severity is especially valuable in routine practice and in resource-limited settings where structured indices such as SCORAD may not always be applied consistently. In this context, the present study demonstrates a moderate–to–strong positive correlation between SCORAD score and Absolute Eosinophil Count (AEC) (r = 0.57), indicating that peripheral eosinophilia increases in parallel with disease severity. This finding is biologically plausible given the central role of Th2-driven inflammation in AD, with increased IL-4, IL-5, and IL-13 promoting IgE synthesis and eosinophil proliferation, activation, and survival. Several studies have shown that both circulating eosinophil counts and eosinophil granule proteins tend to be higher in AD and are often associated with more severe disease. [6]The clear upward trend seen in our scatter plot, with higher SCORAD categories associated with progressively higher AEC values, visually reinforces this pathophysiological link.

Comparison with Konangi Ramya et al**.**The study by Konangi Ramya et al.[9] evaluated 50 patients with AD and reported that 74% had elevated AEC and 82% had raised serum IgE, with both parameters correlating with disease severity. Their work established that hematological (AEC) and immunological (IgE) markers rise with increasing clinical severity, but did not quantify the correlation using a coefficient (r) or explore diagnostic performance metrics such as ROC curves.

Our study extends these observations in several ways:

We provide a quantitative estimate of the strength of association between SCORAD and AEC (r = 0.57), which falls in the moderate–strong range.

We support this numerically with graphical methods.

We additionally perform ROC analysis, showing an AUC of 1.00 for AEC in discriminating mild from moderate/severe disease in our dataset, suggesting excellent discriminatory capacity.

Thus, while both studies agree that AEC increases with severity, the present study adds more granular and methodologically rigorous evidence regarding the *strength* and *diagnostic utility* of AEC.

Comparison with Other Indian and Regional Studies

Our findings are consistent with several earlier studies from the Indian subcontinent and neighboring regions. Dhar et al.[5] studied Indian children with AD and reported that both

AEC and serum IgE were significantly higher in patients than in controls, and each parameter showed significant positive correlation with disease severity. Although specific correlation coefficients were not provided in the abstract, their conclusion that AEC tracks with severity aligns closely with our results.

In a Nepalese pediatric cohort, Pandey et al.[8] found a "reasonable positive correlation" between AEC and disease severity measured by SCORAD, with r = 0.514 ($p < 0.001$) in 53 patients. They also reported that children with higher AEC had significantly more severe disease and more frequent respiratory atopy. Our correlation estimate (r = 0.57) is slightly higher but broadly comparable to theirs, placing our result at the upper end of the correlation range reported in regional pediatric AD populations.

Similarly, Kumar et al.[7] studied 132 children with AD in Eastern India and found that mean AEC increased stepwise from mild to moderate to severe AD, and that patients with high AEC had significantly higher SCORAD scores than those with normal AEC within the same severity strata. This pattern closely mirrors the trend seen in our dataset, where AEC values rose progressively across SCORAD-defined severity categories.

A multicenter pediatric study by Batmaz et al.[10] reported a statistically significant but relatively *weaker* positive correlation between SCORAD and eosinophil count (rho ≈ 0.16, p = 0.002), and found that higher eosinophil counts were associated with greater improvement in SCORAD over time, particularly in extrinsic AD. The weaker correlation in that study, compared with ours (r = 0.57), highlights the heterogeneity seen across different populations, age groups, and phenotypes (intrinsic vs extrinsic AD).

Comparison with International Literature

Outside South Asia, several studies have similarly demonstrated a positive relationship between eosinophilia and AD severity. **Čelakovská** et al.[11] showed that peripheral eosinophil counts and eosinophil cationic protein were elevated in most adolescent and adult AD patients and tended to increase with more severe disease. A Taiwanese cohort cited by Pandey et al.[8] reported positive correlations between SCORAD and total eosinophil count (r ≈ 0.49, $p < 0.001$) and between SCORAD and serum IgE (r ≈ 0.32, p = 0.028), further supporting the association between these biomarkers and clinical severity. [12]

More recent immunological work has emphasized that combinations of biomarkers (e.g., serum IgE plus blood eosinophils) may better capture the inflammatory burden and phenotypic heterogeneity of AD than single markers alone. [12] However, in many routine clinical settings, particularly in low- and middle-income countries, AEC remains far more accessible and affordable than extended immunologic panels. Therefore, the demonstration of a robust correlation between AEC and SCORAD in our study has direct practical relevance.

Strength of Association, Clinical Implications, and Limitations

Across published literature, correlations between AEC and AD severity generally range from weak-to-moderate to moderate-to-strong, depending on study design and population characteristics. [13] The correlation coefficient observed in the present study (r = 0.57) falls toward the stronger end of this spectrum, suggesting that eosinophilia reasonably reflects clinical disease burden in our cohort.

However, these findings should be interpreted with appropriate clinical caution. Absolute eosinophil count should be regarded as an **adjunctive biomarker**, rather than a substitute for standardized clinical severity scoring systems such as SCORAD. While AEC may be particularly useful in busy outpatient settings, peripheral healthcare centers lacking dermatology specialists, and large epidemiological studies where structured clinical scoring is logistically challenging, several limitations of the present study must be acknowledged. These include its single-center design, relatively small sample size, cross-sectional nature, absence of multivariate adjustment for potential confounding variables, lack of routine parasitological screening, and the absence of longitudinal follow-up to assess changes in AEC with disease activity or treatment response.

Despite these limitations, the study provides clinically relevant evidence supporting the role of AEC as a simple, accessible biomarker that correlates with disease severity in atopic dermatitis. Nevertheless, future multicentric and longitudinal studies incorporating multivariate analyses are warranted to validate these findings and to further clarify the utility of AEC in disease monitoring and prognostication.

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5. Conclusion

This study demonstrates a statistically significant moderate–to–strong positive correlation between SCORAD score and absolute eosinophil count in patients with atopic dermatitis. The findings support the utility of AEC as a simple, objective, and widely available adjunctive biomarker for assessing disease severity, particularly in resource-limited clinical settings. While SCORAD remains the gold standard for comprehensive severity assessment, AEC may serve as a useful supportive tool for early identification of severe disease and guiding clinical decision-making. Further longitudinal and multicentric studies are recommended to validate these findings and explore the role of AEC in disease monitoring.

References

1. Kolb L, Ferrer-Bruker SJ. Atopic Dermatitis [Internet]. StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK448071/

2. Li Z, Gu J, Tang T, Huang X, Wu Y, Li Y, et al. The Global Burden of Atopic Dermatitis in Elderly Populations: Trends, Disparities, and Future Projections. Healthcare. 2025 Apr 1;13(7):788.

3. Yue C, Zhou H, Wang X, Yu J, Hu Y, Zhou P, et al. Atopic dermatitis: pathogenesis and therapeutic intervention. MedComm (Beijing). 2024 Dec 8;5(12).

4. Pala V, Rosset F, Mastorino L, Sciamarrelli N, Boskovic S, Borriello S, et al. The Central Role of Th2 Immune Response in Inflammatory Dermatoses: From Pathogenesis to Targeted Therapies. Int J Mol Sci. 2025 Nov 4;26(21):10720.

5. Dhar S, Malakar R, Chattopadhyay S, Dhar S, Banerjee R, Ghosh A. Correlation of the severity of atopic dermatitis with absolute eosinophil counts in peripheral blood and serum IgE levels. Indian J Dermatol Venereol Leprol. 2005;71(4).

6. Severity Scoring of Atopic Dermatitis: The SCORAD Index. Dermatology. 1993;186(1):23–31.

7. Kumar M, Singh P, Patel P. Clinico-immunological profile and their correlation with severity of atopic dermatitis in Eastern Indian children. J Nat Sci Biol Med. 2014;5(1).

8. Pandey S, Jha S, Pokharel NR, Kadel A. Atopic Dermatitis and its Severity Correlation With Absolute Eosinophil Counts. Journal of Nepalgunj Medical College. 2018 Jul 31;16(1):18–23.

9. Sree K, Ramya K, Shree M, Penchalaiah K. CORRELATION OF THE SEVERITY OF ATOPIC DERMATITIS WITH ABSOLUTE EOSINOPHIL COUNT IN PERIPHERAL BLOOD AND SERUM IgE LEVELS. Int J Curr Adv Res [Internet]. 2023; Available from: https://journalijcar.org/sites/default/files/issue-files/14136.pdf

10. Batmaz SB. Laboratory and severity evaluation of pediatric atopic dermatitis and moisturizer response in different phenotypes. Allergol Immunopathol (Madr). 2018 Nov;46(6):571–7.

11. Čelakovská J, Bukač J. Eosinophils in patients suffering from atopic dermatitis and the relation to the occurrence of food allergy and other atopic diseases. Food Agric Immunol. 2016 Sep 2;27(5):700–10.

12. Eguiluz-Gracia I, Tay TR, Hew M, Escribese MM, Barber D, O'Hehir RE, et al. Recent developments and highlights in biomarkers in allergic diseases and asthma. Allergy. 2018 Dec 30;73(12):2290–305.

13. Jacobson ME, Morimoto RY, Leshem YA, Howells L, Williams HC, Grinich E, et al. The Eczema Area and Severity Index: An update of progress and challenges in its measurement of atopic dermatitis after 20 years of use. Journal of the European Academy of Dermatology and Venereology. 2025 Jan 19;39(1):70–85.

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Non Surgical Prosthodontic Rehabilitation of a Prominent Anterior Maxillary Ridge with Labial Undercuts Using a Flangeless Complete Denture: A Case Report

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ABSTRACT:

Prominent anterior maxillary ridges associated with pronounced labial undercuts pose a significant prosthodontic challenge during complete denture fabrication, frequently resulting in over-contoured labial flanges that adversely affect facial aesthetics, lip support, phonetics, and patient comfort. This case report describes the prosthodontic management of such anatomical limitations using a conventional flangeless maxillary complete denture as a conservative, non-surgical alternative. Elimination of the anterior labial flange permitted improved adaptation of the denture base to the underlying ridge anatomy while minimizing soft-tissue impingement and excessive labial fullness. The clinical workflow, design rationale, and processing modifications are presented to highlight the functional and aesthetic advantages of this approach. This treatment approach demonstrated improved comfort, satisfactory retention, and enhanced facial aesthetics during post-insertion follow-up, supporting the clinical applicability of flangeless denture designs in cases with unfavorable ridge morphology.

KEYWORDS:

Flangeless denture; Labial undercut; Maxillary ridge; Denture aesthetics; Complete denture

1. Introduction

Residual alveolar ridge morphology is a primary determinant of complete denture success, as it directly influences retention, stability, and support, while also affecting phonetics, aesthetics, and overall patient comfort. Following tooth loss, the edentulous ridge undergoes continuous and often unpredictable remodeling, leading to marked anatomical variability ranging from broad, well-formed ridges to severely resorbed or anatomically compromised configurations. [1]. These morphological differences significantly modify the denture-bearing area, border seal effectiveness, stress distribution, and resistance to functional and parafunctional loads. Consequently, ridge form plays a decisive role in denture prognosis, necessitating thorough clinical assessment and individualized prosthodontic planning to achieve predictable functional outcomes and long-term patient satisfaction [1]. Among the various ridge configurations, a prominent anterior maxillary ridge with associated labial undercuts is a commonly encountered clinical condition that complicates maxillary complete denture construction [2]. The labial flange of a maxillary denture is critical for providing lip support, maintaining facial harmony, and facilitating proper phonetics. However, in the presence of a prominent ridge, conventional flange extension often results in over-contouring of the labial surface, leading to excessive lip fullness, altered facial profile, compromised speech, and patient dissatisfaction. Achieving an optimal balance between functional requirements and aesthetic demands in such cases remains a significant prosthodontic challenge [3].

Pre-prosthetic surgical procedures, such as alveoloplasty and ridge recontouring, have traditionally been recommended to correct unfavorable ridge anatomy and facilitate

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conventional denture fabrication. Despite their effectiveness, these surgical interventions may be contraindicated due to systemic health considerations, advanced patient age, or patient reluctance to undergo invasive treatment [4]. As a result, there is an increasing need for conservative, non-surgical prosthodontic alternatives that can accommodate existing ridge morphology while preserving function and aesthetics.

The flangeless complete denture represents a conservative prosthodontic approach for managing prominent maxillary ridges with labial undercuts. By eliminating or reducing the anterior labial flange, this design allows improved adaptation of the denture base to the underlying ridge anatomy while minimizing excessive acrylic bulk and soft tissue impingement. This modification contributes to enhanced facial aesthetics while maintaining clinically acceptable retention and stability [5]. The present case report highlights the clinical application of a conventional flangeless maxillary denture as an effective alternative to surgical intervention in patients with challenging ridge morphology.

2. Case Report

A 70-year-old male patient presented to the Department of Prosthodontics with complaints of difficulty in mastication, impaired speech, and compromised facial aesthetics due to complete edentulism of both arches for the past 12 years. Clinical examination revealed completely edentulous maxillary and mandibular arches, with a prominent anterior maxillary ridge exhibiting a pronounced labial undercut from canine to canine (Figure 1a-c). In contrast, the mandibular ridge was U-shaped with adequate height and width (Figure 1d). The ridge anatomy was considered unfavorable for conventional maxillary denture fabrication because it predisposed to excessive labial flange bulk, thereby adversely affecting aesthetic outcomes. After discussing treatment options, including pre-prosthetic surgery and implant-supported prostheses, the patient declined surgical intervention, and a conservative treatment plan involving a conventional flangeless maxillary complete denture opposing a conventional mandibular complete denture was planned. Written informed consent was obtained from the patient for treatment, following a thorough explanation of the procedure, alternative treatment options, and consent for the use of clinical data and photographs for publication.

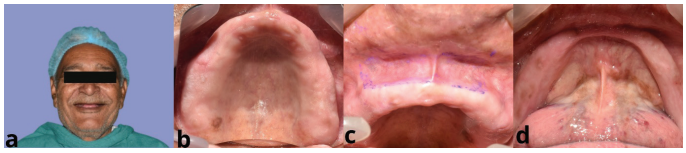

Figure 1: 1a. Extraoral view 1b. Maxillary arch 1c. Maxillary labial undercut 1d. Mandibular arch

3. Clinical procedure

Primary impressions of both the maxillary and mandibular arches were made using an irreversible hydrocolloid impression material (Orikam Neoalign Alginate; Orikam Healthcare, India) (Figure 2a, b), and the primary casts were poured using Type II dental plaster (Kalabhai Karson Pvt. Ltd., Kalrock, India) (Figure 2c). Wax spacers were adapted to the primary casts, and custom impression trays were fabricated for both arches using autopolymerizing acrylic resin. Border molding was performed using a low-fusing green stick impression compound (DPI Pinnacle Tracing Sticks; Dental Products of India Ltd., Mumbai, India) to achieve functional border extension. A definitive wash impression was recorded using a light-body elastomeric impression material (Aquasil Ultra LV; Dentsply Sirona, Konstanz, Germany) to accurately capture fine anatomical details and soft-tissue morphology (Figure 2d). The posterior palatal seal was recorded functionally at the vibrating line during border molding and was incorporated directly into the definitive impression. The definitive master casts were poured using Type III dental stone (Kalabhai Karson Pvt. Ltd., Kalrock, India) (Figure 2e). Subsequently, record bases and occlusal rims were fabricated, and the maxillomandibular relationship was recorded following conventional clinical procedure (Figure 2f). The vertical dimension of occlusion (VDO) was established using assessment of facial proportions, phonetics, and physiologic rest position, with verification of an adequate 2–4 mm freeway space. Trial evaluation confirmed balanced lower facial height and absence of muscular strain. Despite the proclined anterior ridge, sufficient interarch space was available, and no alteration of VDO was required.

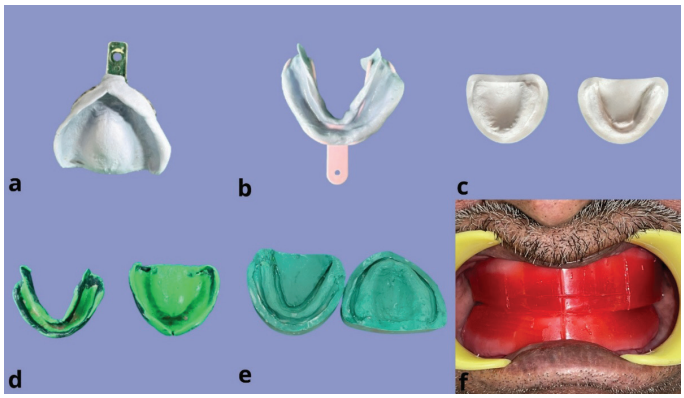

Figure 2: 2a, b. Maxillary and Mandibular Primary impression 2c. Primary casts 2d. Definitive Impressions 2e. Definitive cast 2f. Maxillomandibular relation record

The definitive casts were mounted on a mean-value articulator, and the arrangement of artificial teeth was performed. A bilateral balanced occlusal scheme was established to counteract potential rotational forces arising from the elimination of the anterior labial flange. Incisal guidance was deliberately reduced by increasing horizontal overlap and maintaining minimal vertical overlap, thereby decreasing anterior tipping forces during protrusive movements. Eccentric movements were adjusted to achieve simultaneous bilateral posterior contacts, verified intraorally using articulating paper, to maintain stability and prevent rotation around a potential fulcrum line in the premolar region. Protrusive movements were refined to ensure light anterior contact accompanied by posterior balancing contacts, thereby preserving the posterior palatal seal during functional excursions. Cuspal inclinations were selectively adjusted to minimize horizontal destabilizing forces. A clinical evaluation of the trial denture was subsequently performed to evaluate aesthetic parameters, phonetic accuracy, and occlusal relationships, ensuring harmonious maxillomandibular articulation before final processing (Figure 3a). Following the clinical evaluation, a labial window was created in the maxillary trial denture extending from canine to canine, thereby eliminating the anterior labial flange (Figure 3b). This modification permitted direct contact of the upper lip with the underlying residual ridge, reducing labial fullness and enhancing facial aesthetics. The remaining denture borders were maintained with adequate thickness to preserve strength and durability. A 19-gauge (≈1.0 mm) stainless steel orthodontic wire was temporarily adapted during the trial stage to evaluate the required anterior acrylic bulk and spatial clearance following flange reduction. The wire served solely as a dimensional reference during wax modification and was not incorporated into the definitive prosthesis (Figure 3c)

Following insertion of the trial denture, a clinical evaluation was performed to assess denture retention and stability following labial flange modification (Figure 3d). During processing, the addition silicone putty spacer was isolated from the acrylic resin using a separating medium to prevent chemical interaction and polymerization inhibition. The intaglio surface of the flangeless anterior region was carefully finished and highly polished to achieve a smooth, non-porous tissue-contacting surface, thereby minimizing plaque accumulation and mucosal irritation. Dewaxing was subsequently performed using conventional laboratory procedures.

Figure 3: 3a. Clinical evaluation of trial denture with conventional labial flange 3b. Labial flange removed and adaptation of support wire 3c. Modified wax-up of maxillary trial denture post flange reduction 3d. Clinical evaluation of trial denture with modified labial flange 3e. Flashing of the flangeless denture 3f. Putty indexing for denture processing.

The denture was processed using a conventional heat-polymerized polymethyl methacrylate (DPI Heat Cure; Dental Products of India Ltd., Mumbai, India) by the conventional compression molding technique. The definitive flangeless maxillary denture was subsequently finished, polished, and inserted for clinical evaluation (Figure 4a). Chairside occlusal adjustments were carried out to achieve optimal occlusal harmony, functional efficiency, and patient comfort (Figure 4b, c). The patient was instructed to avoid incising hard food items with the anterior teeth to reduce functional tipping stresses.

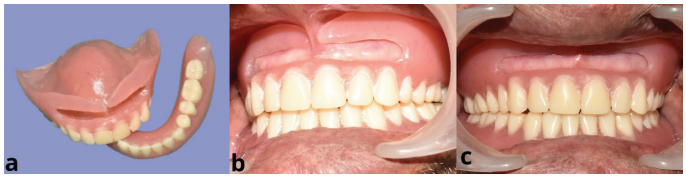

Figure 4: 4a. Definitive prosthesis 4b. Intraoral lateral view of the final prosthesis 4c. Intraoral frontal view of the final prosthesis.

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Post-insertion follow-up visits were scheduled at 24 hours, one week, and one month. At each review appointment, the patient reported satisfactory comfort, improved facial esthetics, and adequate functional performance of the prosthesis, with no evidence of soft-tissue irritation or postoperative complications (Figure 5a, 5b).

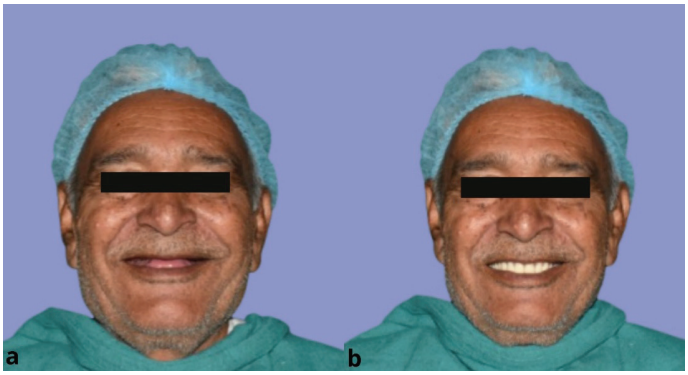

Figure 5: 5a. Pre-Operative Extra-Oral View, 5b. Postoperative Extra-Oral View

4. Discussion

The management of edentulous patients with unfavorable residual ridge morphology continues to present a biomechanical and aesthetic challenge in complete denture therapy [6]. Prominent or proclined anterior maxillary ridges with associated labial undercuts significantly alter the neutral zone and complicate denture base extension, often predisposing the prosthesis to compromised retention, instability, phonetic distortion, and compromised facial aesthetics. Conventional denture designs in such scenarios frequently rely on excessive labial flange extension to engage the undercut area, which may result in over-contoured denture bases, altered lip posture, soft-tissue impingement, and reduced patient acceptance.

Various treatment modalities have been advocated to manage pronounced labial undercuts. Pre-prosthetic surgical interventions such as alveoloplasty, ridge recontouring, and undercut reduction can improve the denture-bearing foundation; however, these procedures are invasive and associated with surgical morbidity, postoperative discomfort, and irreversible loss of alveolar bone. Their use is further limited in medically compromised or elderly patients, as well as in individuals unwilling to undergo surgical treatment [7]. Orthodontic correction and implant-supported prostheses have also been proposed, but these options are often constrained by cost, treatment duration, systemic health considerations, and anatomical limitations.

Several prosthodontic modifications have been described as conservative alternatives, including sectional dentures, flexible denture base materials, hollow labial flanges, window dentures, and modified flange contouring techniques. While flexible denture bases may aid insertion through undercuts, they frequently lack long-term dimensional stability and adequate support. Sectional dentures increase technical complexity and patient handling difficulty. Window dentures, although aesthetically acceptable, may compromise border seal and retention if not carefully executed [8].

In the present case, a flangeless maxillary complete denture design was selected as a conservative, non-surgical solution to accommodate the prominent anterior labial undercut while preserving facial aesthetics and functional efficiency [9]. Retention is primarily derived from a functionally recorded posterior palatal seal, maximal palatal coverage, interfacial surface tension, and controlled passive engagement of the favorable hard-tissue undercut. This design represents a conservative, non-surgical alternative when surgical ridge recontouring is contraindicated or declined. [10] A critical limitation of flangeless denture designs is the potential reduction in structural rigidity of the anterior denture base. A 19-gauge (≈1.0 mm) stainless steel orthodontic wire was temporarily adapted during the trial stage to simulate the anticipated anterior acrylic thickness and to assess spatial clearance following labial flange reduction. The wire functioned exclusively as a dimensional and contour guide during wax modification to prevent over- or under-contouring of the anterior region. It was removed before processing and was not incorporated into the definitive prosthesis. Additionally, the use of putty-consistency addition silicone over the labial undercut area before dewaxing served as an effective spacer, preventing acrylic resin overextension and ensuring accurate reproduction of ridge anatomy. This technique minimized polymerization shrinkage–related distortion, preserved soft-tissue contours, and contributed to improved comfort and phonetic performance. Compared with conventional wax relief or manual trimming methods, this approach offers greater precision and reproducibility during processing.

The combination of a flangeless design and

controlled acrylic contouring represents a biologically sound and mechanically stable approach tailored to the patient's anatomical constraints and treatment preferences. [11]. This integrated technique provides a distinct advantage over alternative conservative methods by simultaneously addressing aesthetics, strength, comfort, and functional performance without increasing clinical or laboratory complexity.

Overall, the favorable outcome observed in this case reinforces the clinical relevance of flangeless maxillary complete dentures as a predictable and patient-centered solution for managing prominent anterior maxillary ridges with labial undercuts. When appropriately indicated and reinforced, this technique offers an effective alternative to surgical intervention, aligning with contemporary principles of minimally invasive prosthodontic rehabilitation.

5. Conclusion

A flangeless maxillary complete denture represents a conservative and clinically effective treatment option for patients presenting with prominent anterior maxillary ridges and pronounced labial undercuts, particularly when surgical intervention is contraindicated or declined. Elimination of the anterior labial flange contributes to reduced lip prominence, enhanced facial aesthetics, and improved patient comfort while maintaining satisfactory denture retention and stability. This case report highlights the importance of individualized prosthodontic treatment planning and demonstrates the clinical versatility of conventional denture techniques in successfully managing complex ridge morphology to achieve favorable functional and aesthetic outcomes.

References

1. Bidra AS, Manzotti A, Wu R. Differences in Lip Support with and without Labial Flanges in a Maxillary Edentulous Population. Part 2: Blinded Subjective Analysis. Journal of Prosthodontics. 2017 May 31;27(1):17–21.

2. Stalin M, Jain P, Rathee M, Diwan K, Tomar SS, Balavignesh S. Prosthetic rehabilitation of edentulous atrophied Mandibular ridge using piezographic technique: a case report. Advances in Medical, Pharmaceutical and Dental Research. 2024 Apr 18;4(1):36.

3. Pathania N, Sailo JL, Noor SU, Nagpal A, Mahajan M. Prong maxillary complete denture for better facial aesthetic: A case report. Journal of Dental Specialities [Internet]. 2021 Oct 10;9(1):22–5. Available from: https://jdsits.in/archive/volume/9/issue/1/article/9594

4. Rathee M, Diwan K, Tomar S, Stalin M, Balavignesh S, Moungkhom N. Management of Unstable Dentures on Atrophied Mandibular Ridges Using Different Impression Materials for Recording the Neutral Zone: A Case Series. Acta Medica Bulgarica. 2025 May 1;52(s1):104–9.

5. Sikri A, Sikri J. Window denture: An unconventional approach for the bulky edentulous ridges. IP Annals of Prosthodontics and Restorative Dentistry. 2021 Sep 15;7(3):169–73.

6. Singh V, Rathi R. Flangeless complete denture and obliterated labial vestibule space. International Journal of Medical Reviews and Case Reports . 2020 Jul;4(7).

7. Manjrekar NS, Dange SP, Khalikar SA, Khalikar AN. Flangeless Denture:A Simple Yet Novel Approach For Management of Severe Labial Undercuts In Edentulous... IOSR Journal of Dental and Medical Sciences. 2017 Jan 30;16(1).

8. Kaur M, Kaura SM, Kumar S, Mahajan A. A winged denture for the management of flabby prominent premaxilla in class III ridge relation: a case report. Journal of Advanced Medical and Dental Sciences Research. 2020;8(7):67–71.

9. Ismail HA, El-Danasory MB, Salma Abolgheit, Soliman IS. Evaluation of open-face maxillary complete denture for patients with prominent premaxilla: a crossover study. BMC Oral Health [Internet]. 2024 Apr 19;24(1). Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11031895/

10. Sunny G, Shetty G, Bukkapatnam S. Gum fit denture: Dentures befitting labial undercut to improve esthetics in anterior maxilla. International Journal of Applied Dental Sciences. 2021 Apr 1;7(2):285–7.

11. Srivastava S, Misra SK. Flangeless denture: an esthetic innovation. International Dental Journal of Student Research. 2018;6(1):24–5.

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Serum Zinc, Copper, and Copper–Zinc Ratio in Psoriasis: Association with Disease Severity

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ABSTRACT:

Background: *Psoriasis is a chronic immune-mediated inflammatory skin disorder in which oxidative stress and immune dysregulation play key pathogenic roles. Trace elements such as zinc and copper are essential for antioxidant defense and immune function, and their imbalance—often expressed as the copper–zinc (Cu/Zn) ratio—has been implicated in various inflammatory conditions. However, evidence regarding their association with psoriasis severity remains inconclusive.*

Aim: *To evaluate serum zinc, copper, and Cu/Zn ratio in patients with psoriasis and to assess their association with disease severity as measured by the Psoriasis Area and Severity Index (PASI).*

Materials and Methods: *This hospital-based cross-sectional observational study included 50 adult patients with clinically diagnosed psoriasis attending a tertiary care teaching hospital in South Gujarat, India. Disease severity was assessed using PASI scoring. Fasting serum zinc and copper levels were measured using atomic absorption spectrophotometry, and the Cu/Zn ratio was calculated. Data normality was assessed using the Shapiro–Wilk test, and non-parametric statistical methods were applied. Associations between PASI score and biochemical parameters were analyzed using Spearman's rank correlation coefficient.*

Results: *The mean PASI score was 8.38 ± 6.63, indicating predominantly mild to moderate disease severity. Serum zinc, copper, and Cu/Zn ratio showed considerable inter-individual variability. Serum copper levels differed significantly across PASI-defined severity groups ($p = 0.030$), whereas serum zinc levels and Cu/Zn ratio did not show significant variation. Correlation analysis demonstrated no association between PASI score and serum zinc ($r_s = +0.03$), while weak negative correlations were observed between PASI score and serum copper ($r_s = -0.31$) and Cu/Zn ratio ($r_s = -0.21$).*

Conclusion: *The study highlights altered trace-element homeostasis in psoriasis, with serum copper levels showing significant variation across disease severity categories. However, serum zinc levels and Cu/Zn ratio did not demonstrate a significant correlation with PASI score, suggesting limited utility as markers of disease severity. Further large-scale longitudinal studies incorporating dietary assessment and inflammatory biomarkers are needed to better elucidate the role of trace elements in psoriasis.*

KEYWORDS:

Psoriasis; Zinc; Copper; Copper–zinc ratio; Oxidative stress; Psoriasis Area and Severity Index (PASI)

1. Introduction

Psoriasis is a chronic, immune-mediated inflammatory skin disorder affecting approximately 2–3% of the global population. It is characterized by well-demarcated erythematous plaques covered with silvery scales, with disease severity ranging from limited cutaneous involvement to extensive body surface area involvement [1,2]. The pathogenesis of psoriasis involves a complex interplay between genetic predisposition, environmental triggers, and immune dysregulation, particularly mediated through pro-inflammatory cytokines

such as tumor necrosis factor-α (TNF-α) and interleukins, including IL-11, IL-23, and IL-22.[3–6].

Increasing evidence suggests that oxidative stress plays a significant role in the initiation and perpetuation of psoriatic inflammation. Enhanced production of reactive oxygen species, coupled with an impaired antioxidant defense system, contributes to keratinocyte hyperproliferation and sustained inflammatory responses in psoriasis [7,8]. Micronutrients with antioxidant properties, especially trace elements such as zinc and copper, are therefore of particular interest in understanding disease pathophysiology and potential prognostic relevance [9].

Zinc and copper are essential trace elements that function as cofactors for numerous enzymes involved in immune regulation, antioxidant defense, and cellular proliferation. Zinc is crucial for DNA synthesis, wound healing, and immune modulation, while copper plays an important role in connective tissue formation, melanin synthesis, and redox reactions [10–14]. However, excess copper can participate in free radical generation via redox cycling, leading to oxidative tissue damage and amplification of inflammatory pathways [12].

Zinc and copper exhibit a well-recognized antagonistic relationship, and their balance is often better reflected by the copper–zinc (Cu/Zn) ratio rather than absolute serum concentrations alone. The Cu/Zn ratio has been proposed as a sensitive marker of systemic oxidative stress and inflammation and has been shown to correlate with disease severity in various inflammatory and malignant conditions [15–18].

Several studies have reported altered serum zinc and copper levels in patients with psoriasis, though findings remain inconsistent, and their relationship with disease severity is not clearly established [15–17]. Variations in study design, population characteristics, nutritional status, and analytical methods may partly explain these discrepancies. Given the chronic inflammatory nature of psoriasis and the biological relevance of these trace elements, evaluating their levels in relation to disease severity may provide further insight into their role in disease progression and potential therapeutic implications.

In this context, the present study was undertaken to assess serum zinc, copper, and Cu/Zn ratio in patients with psoriasis and to evaluate their association with disease severity as measured by the Psoriasis Area and Severity Index (PASI).

2. Aim and Objectives

Aim

To assess the association between serum zinc, copper, copper–zinc ratio, and disease severity in patients with psoriasis.

Objectives

1. To measure serum zinc and copper levels and calculate the Cu/Zn ratio in psoriasis patients.
2. To evaluate the relationship between these trace elements and disease severity using the PASI score.

3. MATERIALS AND METHODS

This hospital-based cross-sectional observational study was carried out in the Department of Biochemistry in collaboration with the Department of Dermatology at a tertiary care teaching hospital in South Gujarat, India, after obtaining approval from the Institutional Ethics Committee. The study was conducted during the approved postgraduate study period. Clinically diagnosed cases of psoriasis attending the dermatology outpatient department were enrolled after obtaining informed written consent.

A total of 50 patients aged 18 years and above were included in the study. Diagnosis of psoriasis was made by a qualified dermatologist based on characteristic clinical features. Patients with other systemic inflammatory or autoimmune disorders, hepatic or renal diseases, metabolic illnesses, pregnant or lactating women, and those receiving mineral or antioxidant supplementation within the preceding three months were excluded to avoid confounding effects on trace element levels.

A detailed clinical evaluation was performed for each patient. Assessment of disease severity was carried out using the Psoriasis Area and Severity Index (PASI), which was evaluated by a dermatologist trained in PASI scoring to minimize inter-observer variability. PASI scoring was based on the assessment of erythema, induration, and desquamation over four body regions—head, upper limbs, trunk, and lower limbs—with

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appropriate weighting according to the extent of body surface area involvement.

After an overnight fast, 5 mL of venous blood was collected from each participant under strict aseptic precautions. The blood samples were allowed to clot and centrifuged to obtain serum, which was stored at -20°C until biochemical analysis. Serum zinc and copper concentrations were estimated using Atomic Absorption Spectrophotometry (AAS) with an iCE 3500 Atomic Absorption Spectrophotometer employing flame atomization, following standard operating procedures and manufacturer's guidelines. Calibration was performed using standard reference solutions, and internal quality control measures were maintained throughout the analysis to ensure accuracy and reproducibility of results.

The copper–zinc (Cu/Zn) ratio was calculated by dividing the serum copper concentration by the serum zinc concentration for each subject. Collected data were entered into Microsoft Excel and analyzed using appropriate statistical software. The normality of continuous variables was assessed using the Shapiro–Wilk test. Since most variables showed non-normal distribution, non-parametric statistical tests were applied. Continuous variables were expressed as mean ± standard deviation for descriptive purposes and median with interquartile range. Comparisons across different severity groups were performed using appropriate non-parametric tests, and correlations between PASI score and biochemical parameters were assessed using Spearman's rank correlation coefficient. A p-value less than 0.05 was considered statistically significant.

4. RESULTS

Table 1. Baseline Characteristics of Psoriasis Patients (n = 50)

Variable	Mean ± SD	Median (IQR)	Range
Age (years)	41.4 ± 10.1	40.5 (33–47)	25–70
PASI score	8.38 ± 6.63	4.0 (3.0–14.7)	1.1–21.2
Zinc (µg/dl)	211.1 ± 65.1	193.4 (170.4–242.1)	109.3–398.3
Copper (µg/dl)	124.2 ± 45.9	116.5 (92.0–145.1)	54.9–294.4
Cu/Zn ratio	0.65 ± 0.35	0.56 (0.43–0.78)	0.24–2.10

Table 1 summarizes the baseline demographic, clinical, and biochemical characteristics of the study population. The patients exhibited a wide age range with predominantly mild to moderate disease severity, as reflected by the median PASI score. Serum zinc and copper levels showed considerable inter-individual variability, as indicated by broad ranges and interquartile distributions. The Cu/Zn ratio likewise demonstrated substantial variation among patients, suggesting heterogeneity in trace-element balance within the study population.

Table 2. Normality Testing of Study Variables (Shapiro–Wilk Test)

Variable	p value	Distribution
PASI score	<0.001	Non-normal
Zinc (µg/dl)	0.001	Non-normal
Copper (µg/dl)	0.002	Non-normal
Cu/Zn ratio	<0.001	Non-normal

As shown in Table 2, the Shapiro–Wilk test demonstrated that PASI score, serum zinc, serum copper, and the Cu/Zn ratio all significantly deviated from a normal distribution ($p < 0.05$). This indicates that the assumptions of normality required for parametric statistical tests were not met for these variables. Consequently, non-parametric statistical methods were applied for group comparisons and correlation analyses in the present study to ensure the validity and robustness of the statistical inferences.

Table 3. Comparison of Biochemical Parameters Across the Severity of Psoriasis

Parameter	Test	H value	p value
PASI score	Kruskal–Wallis	37.21	<0.001
Zinc (µg/dl)	Kruskal–Wallis	2.51	0.285
Copper (µg/dl)	Kruskal–Wallis	7.00	0.030
Cu/Zn ratio	Kruskal–Wallis	3.38	0.185

Interpretation:

PASI scores increased significantly with increasing severity of psoriasis. Serum copper levels also

differed significantly across severity groups, whereas zinc levels and Cu/Zn ratio showed no statistically significant variation across PASI-defined severity categories.

Figure 1. Scatter Plot with Regression Line Showing the Correlation Between PASI Score and Serum Zinc (µg/dl)

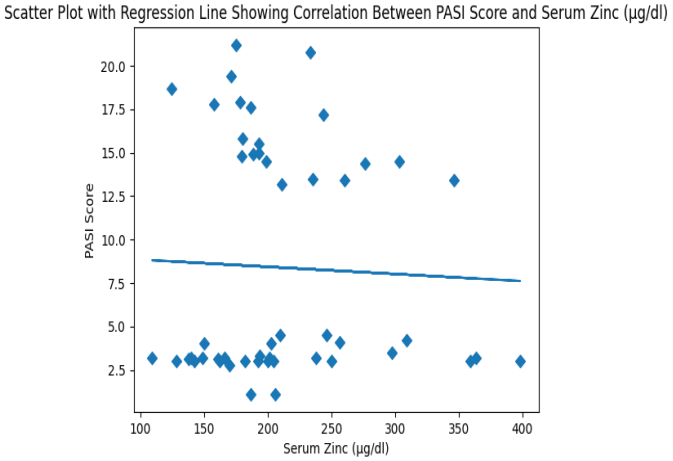

Figure 1 shows the regression line is nearly flat with a very slight negative slope, confirming no meaningful association between PASI score and serum zinc levels.

Figure 2: Scatter Plot with Regression Line Showing the Correlation Between PASI Score and Serum Copper (µg/dl)

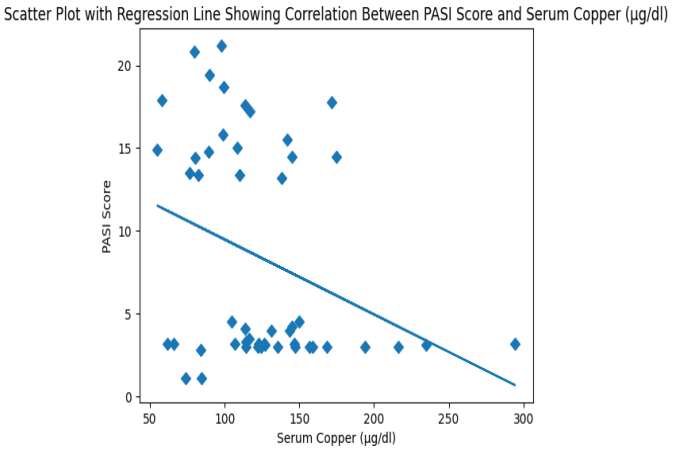

Figure 2: The regression line demonstrates a downward slope, indicating a weak negative relationship between PASI score and serum copper levels.

Figure 3: Scatter Plot with Regression Line Showing the Correlation Between PASI Score and Cu/Zn Ratio

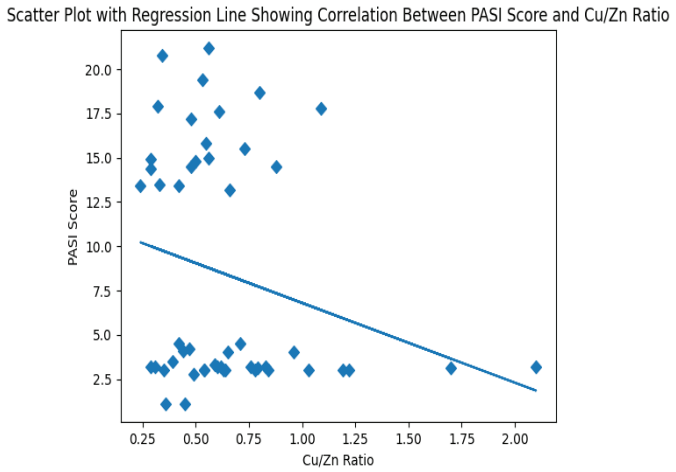

Figure 3 shows a negative slope is observed, suggesting a weak inverse relationship between PASI score and Cu/Zn ratio.

Table 4. Correlation of PASI Score with Biochemical Parameters (Spearman's Rank)

Variables	r_s	Strength
PASI vs Zinc	+0.03	No correlation
PASI vs Copper	-0.31	Weak negative
PASI vs Cu/Zn ratio	-0.21	Weak negative

5. Discussion

Psoriasis is a chronic immune-mediated inflammatory disorder characterized by keratinocyte hyperproliferation and systemic immune activation, processes that may influence trace-element homeostasis. Copper and zinc play essential roles in antioxidant defense, immune modulation, and epidermal differentiation, and disturbances in their metabolism have been increasingly explored in psoriasis. The biological relevance of zinc and copper in psoriasis can be better understood in the context of the psoriatic inflammatory cycle. Psoriasis is characterized by activation of dendritic cells and T helper (Th1 and Th17) lymphocytes, leading to increased production of pro-inflammatory cytokines such as **TNF-α, IL-17, IL-22, and IL-23**, which stimulate keratinocyte proliferation and sustain cutaneous inflammation. This inflammatory cascade generates increased levels of **reactive oxygen species (ROS)**, contributing to oxidative stress within the epidermis.[17]

Trace elements play critical modulatory roles in this process. **Zinc** functions as a cofactor for numerous antioxidant enzymes, including **superoxide dismutase**, and contributes to

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stabilization of cell membranes, regulation of keratinocyte proliferation, and modulation of immune responses. Zinc deficiency may therefore impair antioxidant defense mechanisms and promote inflammatory signaling pathways. **Copper**, on the other hand, participates in redox reactions and serves as a cofactor for enzymes involved in oxidative metabolism and connective tissue formation. While physiologic copper supports antioxidant activity through copper-dependent enzymes, excess copper may also contribute to **free radical generation via redox cycling**, thereby amplifying oxidative damage in inflammatory conditions.[18-22]

The balance between these two trace elements is reflected in the **copper–zinc ratio**, which has been proposed as an indicator of systemic inflammation and oxidative stress. Alterations in this ratio may therefore influence the inflammatory microenvironment in psoriasis by modifying the balance between oxidative injury and antioxidant defense mechanisms.[23]

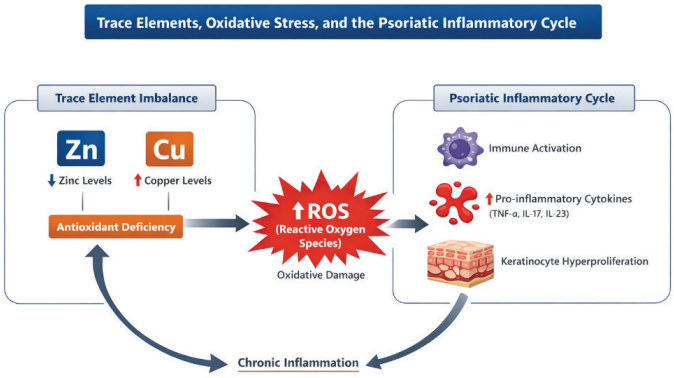

Figure 4: a simple schematic figure showing the "Trace Elements–Oxidative Stress–Psoriasis Inflammatory Cycle

In the present study, serum copper levels demonstrated a statistically significant difference across psoriasis severity groups, whereas serum zinc levels and the Cu/Zn ratio did not show significant variation with disease severity. Correlation analysis revealed no meaningful association between PASI score and serum zinc levels, while serum copper and Cu/Zn ratio showed weak negative correlations with PASI score. These weak correlations did not reach clinical or statistical significance, suggesting limited utility as severity markers.

Several Indian studies have reported significantly decreased serum zinc levels and elevated serum copper levels in psoriasis patients compared with healthy controls. Sheikh et al.[24] observed markedly reduced zinc and increased copper levels, along with altered serum protein fractions, suggesting chronic inflammation and enhanced epidermal turnover as contributory mechanisms. Basavaraj et al.[25] also reported elevated copper levels in both mild and severe psoriasis, supporting the concept of copper behaving as an acute-phase reactant through increased ceruloplasmin synthesis during inflammation. In contrast, Rao et al.[26] reported significantly reduced zinc, copper, and selenium levels, indicating a possible generalized micronutrient deficiency state influenced by dietary or regional factors.

International studies have similarly demonstrated heterogeneous findings. Ala et al.[27] reported elevated copper levels without significant zinc deficiency in Iranian patients, while Tasaki et al. [28] highlighted increased serum copper and the diagnostic relevance of the Cu/Zn ratio in inflammatory skin diseases, including psoriasis. Compared with these studies, the present findings show that although serum copper levels differ across severity categories, neither zinc levels nor the Cu/Zn ratio reliably correlates with PASI score. The weak inverse relationship observed between PASI score and serum copper contrasts with earlier reports of rising copper levels with increasing severity and may reflect differences in disease chronicity, treatment exposure, inflammatory burden, or adaptive metabolic responses. The weak inverse relationship observed between PASI score and serum copper levels in the present study contrasts with several earlier reports in which copper levels increased with greater disease activity. Copper is widely recognized as an acute-phase reactant, largely due to increased hepatic synthesis of ceruloplasmin during systemic inflammation. Elevated copper levels in psoriasis have therefore often been interpreted as reflecting enhanced inflammatory activity and oxidative stress. However, several factors may explain the inverse trend observed in the present study.

First, the majority of patients in our cohort had **mild to moderate disease severity**, with relatively low PASI scores. In such patients, the systemic inflammatory burden may be insufficient to produce a consistent rise in copper levels. Second, copper metabolism may undergo **adaptive metabolic regulation during chronic inflammation**, where prolonged inflammatory exposure leads to redistribution of trace elements between plasma, liver, and tissues. In chronic inflammatory states, copper may be

increasingly utilized by antioxidant enzymes such as **superoxide dismutase and ceruloplasmin**, potentially resulting in lower circulating levels despite ongoing oxidative stress.[20,21,23]

Third, **nutritional status and dietary micronutrient intake** can significantly influence serum copper concentrations. Regional variations in diet, particularly in populations with variable micronutrient intake, may partly explain discrepancies between studies conducted in different geographic settings. Fourth, **treatment exposure and disease chronicity** may modify trace-element dynamics. Patients receiving topical therapies or having long-standing disease may exhibit metabolic adjustments that alter circulating copper levels independently of current disease severity.[21,23]

Another possible explanation is that serum copper levels alone may not accurately reflect **functional copper activity within inflammatory pathways**, as copper is largely protein-bound in circulation and participates in multiple enzymatic processes within tissues rather than remaining freely available in serum. Consequently, the **Cu/Zn ratio or intracellular trace-element balance** may better represent oxidative stress status than absolute serum copper levels.[29]

The absence of a significant association between zinc levels and disease severity suggests that zinc imbalance, when present, may represent a background metabolic alteration rather than a dynamic marker of psoriasis activity. Moreover, serum zinc levels may not accurately reflect intracellular zinc distribution or tissue utilization, which may be altered during chronic inflammatory states.[18]

Overall, the findings of the present study support the presence of trace-element imbalance in psoriasis while demonstrating that serum zinc levels and the Cu/Zn ratio may have limited utility as markers of disease severity. The variability of findings across studies underscores the influence of nutritional status, inflammatory burden, methodological differences, and population characteristics. Future large-scale longitudinal studies incorporating dietary assessment, inflammatory biomarkers, and treatment variables are warranted to further clarify the role of trace elements in the pathophysiology and clinical monitoring of psoriasis.

Strengths of the Study

- The study employed **standardized severity assessment using PASI scoring**, allowing objective stratification of disease severity.
- Appropriate **non-parametric statistical methods** were used in view of the non-normal distribution of biochemical variables.
- The evaluation of **both individual trace elements and the Cu/Zn ratio** provides a more comprehensive understanding of trace-element balance in psoriasis.
- The findings contribute **region-specific data**, adding to the limited Indian literature on trace elements and psoriasis severity.

Limitations of the Study

- The **cross-sectional design** precludes causal inference between trace-element alterations and disease severity.
- The **sample size**, though adequate for exploratory analysis, may limit the detection of subtle associations.
- **Dietary intake, supplementation status, and inflammatory markers** were not assessed, which may influence serum trace-element levels.
- Serum measurements may not accurately reflect **intracellular or tissue trace-element status**, potentially underestimating their biological relevance.
- Treatment history and disease duration were not fully accounted for, which could have influenced biochemical parameters.

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References

1. Kremers HM, McEvoy MT, Dann FJ, Gabriel SE. Heart disease in psoriasis. Journal of the American Academy of Dermatology. 2007;57(2).

2. Tsoi LC, Spain SL, Knight J, Ellinghaus E, Stuart PE, Capon F, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. Nature Genetics. 2012 Dec;44(12):1341–8.

3. Huerta C, Rivero E, García LA. Incidence and risk factors for psoriasis in the general population. Archives of Dermatology. 2007;143(12).

4. Ricketts JR, Rothe MJ, Grant-Kels JM. Nutrition and psoriasis. Clinics in Dermatology. 2010 Nov;28(6):615–26.

5. Nazlroğlu, Mustafa , Yildlz K, Burcu Tamtürk, Ijlal Erturan, Flores-Arce M. Selenium and psoriasis. Biological Trace Element Research. 2012;150(1-3).

6. Popov I, Lewin G. A deficient function of the antioxidative system of the organism as an aetiopathogenetic factor in psoriasis. Medical Hypotheses. 1991;35(3).

7. Zadorozhny BA, Adolf EV, Shevchenko VP. Free radicals in the skin of patients with psoriasis and their relationship to oxidative and reductive processes (Russian). Vestnik Dermatologii i Venerologii. 1973;47(5).

8. Osredkar, Josko . Copper and zinc, biological role and significance of Copper/Zinc imbalance. Journal of Clinical Toxicology. 2011;s3(01).

9. Koyanagi A, D. Kuffó, L. Gresely, A. Shenkin, Cuevas LE. Relationships between serum concentrations of C-reactive protein and micronutrients, in patients with tuberculosis. Annals of Tropical Medicine and Parasitology. 2004;98(4).

10. Kiouri DP, Evi Tsoupra, Massimiliano Peana, Perlepes SP, Stefanidou ME, Chasapis CT. MULTIFUNCTIONAL ROLE OF ZINC IN HUMAN HEALTH: AN UPDATE. EXCLI Journal. 2023;22.

11. Prasad R, Kaur G, Walia S. A critical evaluation of copper metabolism in indian wilson's disease children with special reference to their phenotypes and relatives. Biological Trace Element Research. 1998 Nov;65(2):153–65.

12. Dubey P, Thakur V, Chattopadhyay M. Role of minerals and trace elements in diabetes and insulin resistance. Nutrients. 2020 Jun;12(6):1864.

13. Chan S, Gerson B, Subramaniam S. The role of copper, molybdenum, selenium, and zinc in nutrition and health. Clinics in Laboratory Medicine. 1998;18(4).

14. Mohamad NS. Trace elements homeostatic imbalance in mild and severe psoriasis: a new insight in biomarker diagnostic value for psoriasis. Our Dermatology Online. 2013;4(4).

15. Dadras MS, Namazi N, S. Khalilazar, Shima Younespour. Trace elements status in psoriasis and their relationship with the severity of the disease. Iranian Journal of Dermatology. 2012;15(60):38–41.

16. Mezzetti A, Pierdomenico SD, Costantini F, Romano F, Cesare DD, Cuccurullo F, et al. Copper/zinc ratio and systemic oxidant load: effect of aging and aging-related degenerative diseases. Free Radical Biology and Medicine. 1998 Oct;25(6):676–81.

17. Pleńkowska J, Gabig-Cimińska M, Paweł Mozolewski. Oxidative stress as an important contributor to the pathogenesis of psoriasis. International Journal of Molecular Sciences. 2020;21(17).

18. Prasad AS. Zinc in human health: Effect of zinc on immune cells. Molecular Medicine. 2008;14(5-6).

19. Oteiza PI. Zinc and the modulation of redox homeostasis. Free Radical Biology and Medicine. 2012;53(9).

20. Gaetke LM, Chow CK. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology. 2003;189(1-2).

21. Uriu-Adams JY, Keen CL. Copper, oxidative stress, and human health. Molecular Aspects of Medicine. 2005;26(4-5 SPEC. ISS.).

22. Ogawa Y, Kinoshita M, Shimada S, Kawamura T. Zinc and skin disorders. Nutrients. 2018;10(2).

23. Malavolta M, Piacenza F, Basso A, Robertina Giacconi, Costarelli L, Mocchegiani E. Serum copper to zinc ratio: Relationship with aging and health status. Mechanisms of Ageing and Development. 2015;151.

24. Sheikh G, Masood Q, Majeed S, Hassan I. Comparison of levels of serum copper, zinc, albumin, globulin and alkaline phosphatase in psoriatic patients and controls: A hospital based casecontrol study. Indian Dermatology Online Journal. 2015;6(2).

25. Basavaraj KH, Darshan MS, P. Shanmugavelu, R. Rashmi, A. Yuti Mhatre, Dhanabal SP, et al. Study on the levels of trace elements in mild and severe psoriasis. Clinica Chimica Acta. 2009;405(1-2).

26. Rao VM, M. Deepthi, Ramalingam K, Naidu MP, Shaik MV. Study on serum copper, zinc and selenium trace element levels in Psoriasis. IP Indian Journal of Clinical and Experimental Dermatology. 2019;5(3).

27. Ala S, Shokrzadeh M, Masoud Golpour, Ebrahim Salehifar, Alami M, Ahmadi A. Zinc and copper levels in Iranian patients with psoriasis: A case control study. Biological Trace Element Research. 2013;153(1-3).

28. Tasaki M, Hanada K, Hashimoto I. Analyses of serum copper and zinc levels and copper/ zinc ratios in skin diseases. Journal of Dermatology. 1993;20(1).

29. Linder MC. Ceruloplasmin and other copper binding components of blood plasma and their functions: An update. Metallomics. 2016;8(9).

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The cardiovascular patient in dental practice: current clinical considerations

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ABSTRACT:

Background: *Cardiovascular diseases are increasingly prevalent due to lifestyle factors and population aging. It is essential for dentists to be familiar with the most common cardiovascular conditions, the medications used to treat them, the risks associated with dental treatment, and the strategies to prevent medical emergencies.*

Material and methods: *A bibliographic search was performed in PubMed/MEDLINE and the Cochrane Library, including articles published in English on cardiovascular patients in dentistry.*

Results and discussion: *Cardiovascular diseases encompass a wide range of conditions, from arterial hypertension to ischemic heart disease. Patients with these conditions should be managed on an individualized basis in the dental clinic in order to prevent complications and medical emergencies during dental treatment.*

Conclusions: *A comprehensive medical history is essential in cardiovascular patients, including a detailed review of their pharmacological treatment and potential drug interactions. Proper risk assessment, individualized treatment planning, and effective communication with the patient's physician when necessary are key to preventing complications and medical emergencies during dental care. Continuous updating of cardiovascular knowledge is therefore fundamental in daily dental practice.*

KEYWORDS:

cardiovascular diseases, dental treatment, hypertension, ischemic heart disease, oral surgery, valvular heart diseases.

1. Introduction

Cardiovascular diseases are increasingly prevalent, and dentists must be familiar with these conditions in order to provide appropriate dental care without increasing patient risk.

They comprise a broad group of conditions ranging from arterial hypertension to arrhythmias or valvular heart diseases. Hypertension is the most common cardiovascular condition and may cause serious cardiac damage. Elevated blood pressure can lead to angina pectoris, myocardial infarction, heart failure, and cardiac arrhythmias, which in severe cases may result in sudden cardiac death. Hypertension may also cause obstruction or rupture of the arteries supplying blood and oxygen to the brain, leading to stroke, as well as renal damage resulting in renal failure.

In this literature review, we analyze the main cardiovascular diseases and the precautions that should be taken during dental treatment of these patients.

2. Methodology of the literature review

A narrative literature review was conducted using electronic databases, including PubMed, Scopus, and Web of Science. The search strategy included combinations of keywords such as "cardiovascular diseases", "dental treatment", "hypertension", "ischemic heart disease", "oral surgery", and "valvular heart diseases".

Inclusion criteria: Articles published in English, clinical studies, systematic reviews, guidelines, and studies focusing on the dental management of cardiovascular patients.

Exclusion criteria: Case reports with limited generalizability, non-peer-reviewed articles

The selection process involved screening titles and abstracts, followed by full-text review of relevant articles.

3. Analytical Discussion

Vascular diseases

Arterial hypertension is defined as blood pressure ≥140/90 mmHg according to the European Society of Cardiology, or ≥130/80 mmHg according to the American College of Cardiology. (1)

The basic principles of dental treatment in hypertensive patients are:

Pain should be avoided; therefore, the use of vasoconstrictors is recommended. However, adrenaline can increase blood pressure and may interact with non-selective beta-blockers, causing hypertension and reflex bradycardia. It may also interact with diuretics, potentially worsening hypocalcemia. In hypertensive patients, the use of up to two cartridges of lidocaine 1:100.000 (0.018 mg of adrenaline) is recommended, or alternatively up to four cartridges of articaine 1:200.000, which contains 0.009 mg of adrenaline per cartridge. (2-4)

Short appointments, preferably in the morning; diazepam (5-10 mg) may be prescribed when indicated. To prevent orthostatic hypotension, the patient should be raised gradually. In addition, nonsteroidal anti-inflammatory drugs (NSAIDs) are not recommended, as they may increase blood pressure. Avoid extensive surgical procedures to reduce the risk of bleeding. (2)

The patient's medication must be known and carefully recorded in the medical history. There are several groups of antihypertensive drugs:

Diuretics (hydrochlorothiazide, furosemide, indapamide) promote renal excretion of sodium and water, reducing plasma volume and consequently blood pressure. They are usually the first-line drugs prescribed for hypertensive patients. (5)

Beta-blockers (atenolol, carvedilol, propranolol, metoprolol) are commonly prescribed and lower blood pressure by reducing heart rate and myocardial contractility. (6)

Angiotensin-converting enzyme inhibitors (ACE inhibitors) (enalapril, captopril, lisinopril) block the conversion of angiotensin I to angiotensin II, thereby reducing vasoconstriction and sodium retention. (7)

Calcium channel blockers (amlodipine, nifedipine, diltiazem, verapamil) reduce blood pressure by limiting calcium entry into cardiac muscle and vascular smooth muscle. This results in decreased total peripheral resistance, and some agents also reduce heart rate and myocardial contractility. (8)

Alpha-adrenergic antagonists (doxazosin, prazosin, terazosin) block α_1-adrenergic receptors in vascular smooth muscle, leading to reduced arteriolar and venous tone. They are less commonly used, usually in combination with other agents, and may increase the risk of falls in elderly patients. (9)

Direct vasodilators (hydralazine, minoxidil) act directly on vascular smooth muscle, producing arteriolar vasodilation and thereby reducing peripheral vascular resistance. They are mainly used in severe or resistant hypertension, usually in combination with other drugs such as diuretics and beta-blockers. (10)

Angiotensin II receptor blockers (ARBs) (losartan, valsartan, irbesartan) selectively block type 1 angiotensin II receptors, reducing vasoconstriction and aldosterone secretion, thereby lowering blood pressure and providing cardiovascular and renal protective effects. (11)

Dentists must be aware of the oral manifestations and effects associated with these medications: Gingival hyperplasia, xerostomia, lichenoid reaction, dysgeusia, ageusia, oral ulceration, angioedema, facial flushing, increased risk of gingival bleeding and infection. (12-17)

Gingival enlargement associated with antihypertensive drugs, particularly calcium channel blockers such as amlodipine and nifedipine, requires a multidisciplinary approach. Initial management is based on non-surgical measures, including intensive plaque control (both professional and at-home), scaling and root planing, oral hygiene instruction, and the use of adjunctive antimicrobial agents such as chlorhexidine. In parallel, consultation with the patient's physician is recommended to evaluate

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the possibility of substituting the causative medication when clinically feasible. In cases where gingival overgrowth persists and results in functional or aesthetic impairment, surgical intervention, such as gingivectomy or periodontal flap surgery, may be indicated. (14,18)

Xerostomia is a common finding and is typically diagnosed based on a subjective complaint of dry mouth, reduced salivary flow (unstimulated <0.1 mL/min), and clinical signs such as dry mucosa, fissured tongue, and increased risk of dental caries. Management includes frequent hydration and salivary stimulation (e.g., sugar-free chewing gum), the use of saliva substitutes and oral moisturizers, and, in severe cases, sialogogues such as pilocarpine when not contraindicated. Topical fluoride should be recommended to prevent caries, and alcohol-containing mouthrinses should be avoided. (19)

Oral lichenoid reactions may present as unilateral or bilateral white striations, often accompanied by erythematous or erosive areas, and are frequently associated with medications such as ACE inhibitors or beta-blockers. Diagnosis is primarily clinical, although biopsy may be required to differentiate these lesions from oral lichen planus. Management involves identification and, when possible, substitution of the causative drug in consultation with the patient's physician, along with the use of topical corticosteroids as first-line therapy. Regular follow-up is recommended due to the low but relevant risk of malignant transformation. (17)

Dysgeusia or ageusia may also occur in patients taking cardiovascular medications. Management includes reviewing the patient's medication list to identify potential causative agents and implementing strategies to improve salivary function. These conditions can significantly affect nutrition and quality of life, particularly in elderly cardiovascular patients. (16)

It is also essential to consider the potential interactions between cardiovascular drugs and commonly prescribed medications in dental practice, as these may lead to significant adverse effects. (20) Macrolide antibiotics, such as erythromycin and clarithromycin, can increase the plasma levels of calcium channel blockers, thereby increasing the risk of hypotension and cardiac arrhythmias. (21)

Similarly, azole antifungals, including ketoconazole and fluconazole, may elevate the serum concentrations of several cardiovascular drugs, such as statins, calcium channel blockers, and certain anticoagulants, potentially leading to toxicity. Metronidazole is also of concern, as it can potentiate the anticoagulant effect of vitamin K antagonists, increasing the risk of bleeding complications. (22)

Opioid analgesics, such as codeine and tramadol, may interact with beta-blockers or other central nervous system depressants, resulting in enhanced sedation and an increased risk of adverse effects, particularly in elderly or medically compromised patients. In addition, nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce the efficacy of antihypertensive medications and contribute to an increased cardiovascular risk. (20)

Therefore, a thorough drug history is essential, and when necessary, consultation with the patient's physician should be undertaken to minimize the risk of drug-related complications and ensure safe dental care.

Patients with a history of **thrombosis or embolism** are at risk of stroke or myocardial infarction due to blood clots that obstruct or travel through the vascular system. These patients are commonly treated with anticoagulant or antiplatelet therapy. (23-25)

In oral surgery, the protocol for patients receiving antiplatelet or anticoagulant therapy is as follows, depending on the medication: Acetylsalicylic acid. For minor interventions, such as simple extractions (fewer than three teeth) or uncomplicated surgeries (e.g., implants without grafting), discontinuation of antiplatelet therapy is not necessary. Local hemostatic measures should be implemented. The risk of discontinuing antiplatelet therapy generally outweighs the risk of bleeding. (26)

Patients treated with acenocoumarol should not receive modifications or suspension of their treatment. However, the patient must be asked for an INR on the same day or the day before the procedure. If the value is under 3, surgery can be performed. (27)

If the value of INR is above 3 and an important bleeding is expected due to procedures such as multiple extractions, multiple implants, or a flap surgery combined with ostectomy, the dose must be adjusted, or the acenocoumarol should be changed to heparin by the treating physician

two days prior to surgery and resumed the same day as the extraction, about 12 hours after. Also, the patient must continue with heparin treatment until the anticoagulation levels are reached. However, it is preferable to postpone surgery until the INR is below 3, measured on the same day or within 24 hours prior to the procedure, and to avoid complex interventions by, for example, staging the treatment, limiting the number of extractions, or avoiding multiple implant placements. (27)

Patients treated with direct oral anticoagulants (DOACs):

Dabigatran, a thrombin inhibitor, is recommended at a dosage of 150 mg twice daily. (28)

Rivaroxaban, a direct Factor Xa inhibitor, is recommended at 15-20 mg once daily. (29)

Apixaban, another Factor Xa inhibitor, is typically prescribed at 5 mg twice daily. (30)

Edoxaban, also a Factor Xa inhibitor, has a recommended dosage of 60 mg once daily. (31)

The time to reach maximum plasma concentration ranges from 1 to 4 hours, and the anticoagulant effect usually diminishes within 12 to 24 hours after administration. (30)

For non-invasive oral surgeries, current recommendations advise against interrupting anticoagulant therapy to reduce the risk of thromboembolism. (32)

For more invasive surgical procedures, such as multiple extractions, implant placement with grafting, wide mucoperiosteal flaps, impacted third molar removal involving ostectomy, or long surgical duration (>45–60 minutes), it is essential to evaluate both bleeding and thrombotic risks. Treatment should be scheduled early in the day. For patients taking apixaban or dabigatran (administered twice daily), some clinicians recommend that the morning dose be omitted. Patients taking rivaroxaban or edoxaban (once daily) should delay the dose until at least four hours after the procedure. If the usual dosing time is in the afternoon, no adjustment is necessary. (33)

The procedure must be performed as late as possible after the last intake (at least 12 hours after in twice daily dose and 24 hours after in single daily dose. (33)

In periodontal therapy, the protocol varies depending on the procedure.

Non-surgical periodontal therapy (scaling and root planing) is considered a low-risk procedure; anticoagulant/antiplatelet therapy should not be interrupted, and local hemostatic measures (pressure, tranexamic acid) are usually sufficient.

Periodontal surgery (e.g., flap surgery): moderate bleeding risk, procedures should be limited in extent (quadrant-based approach recommended), careful flap design and atraumatic technique are essential, and use of local hemostatic agents and sutures is mandatory.

Regenerative procedures (bone grafts, membranes): higher bleeding risk, require individualized assessment, and in selected cases, temporary modification of anticoagulant therapy may be considered in consultation with the physician.

Renal function must be considered, especially for dabigatran, which is primarily renally excreted.

Unlike vitamin K antagonists, direct oral anticoagulants (DOACs) do not usually require bridging therapy with heparin due to their short half-life and rapid onset of action. Bridging is generally discouraged, as it may increase bleeding risk without reducing thromboembolic events. (34)

The use of hemostatic measures during or after the procedure can often eliminate the need to suspend antiplatelet or anticoagulant therapy. The most recommended techniques include alveolar curettage to remove granulation tissue, use of sutures (preferably resorbable), gauze compression, fibrin sponges, resorbable gelatin sponges, oxidized cellulose, and bone wax. Although bone wax can be effective for controlling bleeding from cancellous bone, its use is associated with potential complications, including foreign body reactions, chronic inflammation, infection, and impaired bone healing. Therefore, its use should be limited to cases where other hemostatic measures are insufficient.

Antifibrinolytic agents may also be employed, such as Tranexamic acid (500 mg injectable solution), which can be used to soak gauze pads for gentle rinsing during the first two days, at least one minute every 6 hours. Also, Epsilon-

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aminocaproic acid (4 g injectable solution) may be applied in the same manner as tranexamic acid. (35)

Tranexamic acid is the most widely studied agent. It acts by inhibiting plasminogen activation, thereby stabilizing the fibrin clot.

Epsilon-aminocaproic acid (EACA) has a similar mechanism of action but is less potent and less extensively studied in dental settings. Current evidence suggests that tranexamic acid provides more predictable hemostasis and is therefore preferred in clinical practice.

However, both agents are effective when used adjunctively with local measures such as sutures and gelatin sponges. (35)

Peripheral arterial disease (PAD) is characterized by obstruction of arteries in the extremities, most commonly in the lower limbs, and represents a manifestation of systemic atherosclerosis. It is associated with a high cardiovascular and cerebrovascular risk, including myocardial infarction and stroke. These patients are treated with antiplatelet therapy, with the same implications regarding bleeding risk during oral surgery.

Cerebrovascular diseases

Stroke. These patients are commonly treated with antiplatelet agents, anticoagulants, antihypertensive drugs, and other cardiovascular medications.

Traditionally, it has been recommended to wait between 6 and 12 months before providing dental treatment to these patients; however, maintaining good oral health and oral care is essential, and with appropriate medical supervision, dental treatment may be performed before the 6-month period. (36) In these patients, oral health is often neglected due to limited ability to maintain oral hygiene; therefore, early oral assessment is essential to prevent complications such as aspiration pneumonia and malnutrition. (37)

Aneurysms are abnormal dilatations of blood vessel walls, such as the aorta. These patients are usually polymedicated and receive antihypertensive drugs, antiplatelet therapy such as acetylsalicylic acid or clopidogrel, sometimes dual antiplatelet therapy, and, in some cases, anticoagulants when atrial fibrillation is present. They have an increased risk of bleeding during oral surgery, and medication should not be discontinued without a medical indication. Short appointments and adherence to antihemorrhagic protocols are recommended. (33)

Cardiac diseases

A thorough medical history should be obtained to identify the type and severity of the cardiac

condition, its duration, any previous complications, and the medication the patient is receiving.

It is important to emphasize that periodontal disease and cardiovascular diseases, particularly atherosclerosis and ischemic heart disease, exhibit a well-documented bidirectional relationship. Chronic periodontal inflammation contributes to systemic inflammatory burden through the release of pro-inflammatory mediators such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), which are known to play a role in endothelial dysfunction and atheroma formation.

Periodontal pathogens, including *Porphyromonas gingivalis*, have been identified in atherosclerotic plaques, suggesting a direct microbial contribution to vascular disease.

Conversely, cardiovascular patients often present with worsened periodontal status due to reduced immune response, polypharmacy, and limited ability to maintain oral hygiene.

Therefore, periodontal therapy should be considered part of the global management of cardiovascular patients, as it may contribute to reducing systemic inflammation and improving cardiovascular outcomes. (38)

Periodontal evaluation in cardiovascular patients should include probing depth, bleeding on probing, and radiographic bone loss, as these parameters are essential for assessing disease severity and guiding treatment planning.

Antimicrobial mouthrinses, particularly chlorhexidine (0.12-0.2%), are recommended in the perioperative management of cardiovascular patients undergoing periodontal or surgical procedures. Preoperative rinsing reduces bacterial load and the risk of bacteremia, while postoperative use may help prevent infection and support wound healing.

Ischemic heart disease (IHD) is characterized by insufficient blood supply to the heart, usually due to obstruction of the coronary arteries, and includes conditions such as angina pectoris and acute myocardial infarction.

The main symptom of ischemic heart disease is precordial pain, which occurs when the balance between myocardial oxygen supply and demand is altered. This pain may radiate to the left arm, neck, jaw, palate, or tongue, and can be triggered by meals, physical exertion, or stress.

Orofacial pain may represent, therefore, the initial manifestation of ischemic heart disease and should be carefully distinguished from odontogenic or temporomandibular disorders. Pain of cardiac origin is typically diffuse, poorly localized, and relieved following administration of nitroglycerin. In contrast, odontogenic pain is usually well localized and associated with thermal stimuli, while temporomandibular disorder-related pain is often linked to jaw movement and presents as muscular or joint discomfort. Given that cardiac-related orofacial pain may occur in the absence of evident dental pathology, clinicians must maintain a high index of suspicion; if such an origin is suspected, dental treatment should be discontinued immediately, and the patient referred for urgent medical evaluation. (39)

In stable angina, the pain lasts for a few minutes and resolves when the triggering stimulus ceases, whereas in myocardial infarction and unstable angina, it persists for a longer duration. The general treatment of ischemic heart disease is based on nitrates, beta-adrenergic blockers, calcium channel blockers, antiplatelet agents, and anticoagulants. It is recommended to wait at least 6 months before providing dental treatment to a patient who has suffered a myocardial infarction. In this case, the use of adrenaline is also recommended with caution. No more than two cartridges of lidocaine 1:100.000 with vasoconstrictor (equivalent to 0.036 mg of adrenaline) or four cartridges of articaine 1:200.000 containing the same total amount of adrenaline should be administered. (2)

In anxious patients, benzodiazepines such as diazepam (5-10 mg) may be administered; however, caution is required in elderly patients (risk of oversedation and falls), patients with respiratory disease or sleep apnea, and those taking opioids or consuming alcohol. Additionally, potential drug interactions must be considered, as benzodiazepines may potentiate the effects of opioids, increasing the risk of respiratory depression, and may also enhance sedation when combined with beta-blockers or other antihypertensive medications. (40)

Blood pressure and pulse oximetry should be monitored. Patients with unstable angina must be medically stabilized before dental treatment. In case of chest pain, sublingual nitroglycerin and oxygen therapy (3 L/min) should be provided. Postoperative bleeding and pain should be minimized. (41,42)

Heart failure is a condition in which the heart is unable to pump blood efficiently, leading to its accumulation in the lungs, lower limbs, or other organs. Patients may present with cough, fatigue, and dyspnea. It commonly coexists with hypertension, ischemic heart disease, valvular heart disease, and arrhythmias, and these patients are often polymedicated. For dental management, appointments should be short, and benzodiazepines may be administered to reduce stress. The patient should not be placed fully supine to avoid dyspnea and pulmonary congestion, and the dental chair should be raised gradually. Adrenaline use should be limited to a maximum of two cartridges of 1:100.000 or four cartridges of 1:200.000. In patients with

severe functional limitation, only short and urgent treatments should be performed after consultation with the cardiologist. Nonsteroidal anti-inflammatory drugs should be avoided, with paracetamol recommended instead. Due to increased bleeding risk, prolonged surgical procedures in a single session should be avoided. (2) In some cases, these patients may receive a ventricular assist device. Oral sanitation should be performed prior to device implantation to prevent infections, and patients should be instructed on proper oral hygiene. In addition, these patients are usually receiving anticoagulant therapy. (43)

Valvular heart diseases include rheumatic heart disease, mitral valve prolapse, aortic valve disease, prosthetic valves, prior infective endocarditis, and congenital valve disorders. Patients should maintain good oral hygiene and receive regular professional dental care. Strict infection control is required, and antibiotic prophylaxis is recommended before high-risk dental procedures, including extractions, oral and periodontal surgery, gingival or periapical manipulation, and dental implant placement. (2)

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High-risk patients include those with a history of infective endocarditis, those with prosthetic heart valves or any implanted material used for valve repair, patients with congenital heart disease associated with a risk of infective endocarditis, whether untreated or treated with prosthetic material, and patients with ventricular assist devices implanted for heart failure. (2,44)

Antibiotic prophylaxis for infective endocarditis consists of a single dose administered 30-60 minutes before the procedure. Amoxicillin is the first-line antibiotic (2 g orally in adults; 50 mg/kg in children). In penicillin-allergic patients, recommended alternatives include cephalexin (2 g orally in adults; 50 mg/kg in children), azithromycin (500 mg orally), clarithromycin (500 mg orally; 15 mg/kg in children), or doxycycline (100 mg orally; 2.2 mg/kg in patients <45 kg). Clindamycin is discouraged due to the increased risk of *Clostridioides difficile* infection. (44)

Arrhythmias include atrial fibrillation, ventricular tachycardia, and bradycardia. Patients with atrial fibrillation are usually anticoagulated and require bleeding management protocols. (45) Anxiety control and limited use of adrenaline are essential to prevent arrhythmias. (2) Some patients may have pacemakers, and dental clinics should be equipped with a semi-automatic external defibrillator. (46) Patients with cardiomyopathies, heart failure, or valvular disease have a higher arrhythmic risk and are commonly treated with beta-blockers. (2,47)

4. Conclusions

Cardiovascular diseases represent the leading cause of mortality worldwide and are highly prevalent among patients attending dental clinics. Specific guidelines must be followed to prevent potentially serious complications.

Epidemiological studies indicate that a significant proportion of adult dental patients present with at least one cardiovascular risk factor, such as hypertension, diabetes, or dyslipidemia.

Socioeconomic disparities significantly influence both cardiovascular and oral health outcomes. Patients with lower socioeconomic status often exhibit a higher prevalence of cardiovascular diseases and periodontal disease, compounded by reduced access to dental and medical care. Barriers include financial limitations, low health literacy, and limited access to preventive services. These factors contribute to delayed diagnosis, poor disease control, and increased risk of complications.

Community-based strategies should focus on improving access to affordable dental care, integrating oral health into primary healthcare systems, and promoting interdisciplinary collaboration between dentists and physicians. In addition, enhancing patient education on the relationship between oral and systemic health is essential to increase awareness and encourage preventive behaviors, particularly in populations at higher risk of both oral and cardiovascular diseases.

Continuous professional development is essential to ensure that dental practitioners are adequately prepared to manage patients with cardiovascular diseases. Training should include medical risk assessment, management of emergencies, and pharmacological knowledge, particularly regarding drug interactions and anticoagulation. In addition, public health initiatives should promote cardiovascular screening in dental settings and interdisciplinary collaboration. These strategies can improve patient safety and reinforce the role of dentistry within the broader healthcare system.

The dental team, including dentists and dental hygienists, plays a critical role in the early detection of undiagnosed cardiovascular disease through routine clinical assessment. A thorough evaluation should include inquiry about symptoms such as chest pain or discomfort, dyspnea on exertion, palpitations, or syncope, as well as a review of the patient's medical history for known hypertension, previous myocardial infarction or stroke, and current medication use, particularly anticoagulants and antihypertensive agents. In addition, measurement of blood pressure during the dental visit is an essential screening tool. Patients presenting with suspicious findings should be referred for medical evaluation prior to undergoing elective dental treatment, thereby contributing to improved patient safety and overall healthcare outcomes.

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References

1. Bergler-Klein J. What's new in the ESC 2018 guidelines for arterial hypertension. Wiener Klinische Wochenschrift. 2019;131(7):180–5.

2. Gupta K, Kumar S, Anand Kukkamalla M, Taneja V, Syed GA, Pullishery F, et al. Dental Management Considerations for Patients with Cardiovascular Disease—A Narrative Review. Reviews in Cardiovascular Medicine. 2022 Jul 20;23(8):261.

3. Anna G, Tomasz S, Marcin J, Katarzyna ST. Clinical assessment of the safe use local anaesthesia with vasoconstrictor agents in cardiovascular compromised patients: A systematic review. Medical Science Monitor. 2014;20:393–8.

4. Abu-Mostafa N, Aldawssary A, Assari A, Alnujaidy S, Almutlaq A. A prospective randomized clinical trial compared the effect of various types of local anesthetics cartridges on hypertensive patients during dental extraction. Journal of Clinical and Experimental Dentistry. 2015 Feb 1;7(1):e84-88.

5. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA. 2014 Feb 5;311(5):507–20.

6. Thomopoulos C, Bazoukis G, Tsioufis C, Mancia G. Beta-blockers in hypertension: overview and meta-analysis of randomized outcome trials. Journal of Hypertension. 2020 Jul 7;38(9):1669–81.

7. Cutrell S, Alhomoud IS, Mehta A, Talasaz AH, Van BW, Dixon DL. ACE-Inhibitors in Hypertension: A Historical Perspective and Current Insights. springer link. 2023 Jun 7;25(9).

8. Zhu J, Chen N, Zhou M, Guo J, Zhu C, Zhou J, et al. Calcium channel blockers versus other classes of drugs for hypertension. Cochrane Database of Systematic Reviews. 2021 Oct 17;2021(10).

9. Mansbart F, Kienberger G, Sönnichsen A, Mann E. Efficacy and safety of adrenergic alpha-1 receptor antagonists in older adults: a systematic review and meta-analysis supporting the development of recommendations to reduce potentially inappropriate prescribing. BMC Geriatrics. 2022 Sep 28;22(1).

10. Sica DA, Gehr TWB. Direct Vasodilators and their Role in Hypertension Management: Minoxidil. The Journal of Clinical Hypertension. 2001 Mar;3(2):110–4.

11. Munger MA. Use of Angiotensin Receptor Blockers In Cardiovascular Protection: Current Evidence and Future Directions. Pharmacy and Therapeutics. 2011;36(1):22.

12. Villa A, Wolff A, Aframian D, Vissink A, Ekström J, Proctor G, et al. World Workshop on Oral Medicine VI: a systematic review of medication-induced salivary gland dysfunction: prevalence, diagnosis, and treatment. Clinical Oral Investigations. 2015 May 22;19(7):1563–80.

13. Kataoka M, Kido J, Shinohara Y, Nagata T. Drug-Induced Gingival Overgrowth—a Review. Biological and Pharmaceutical Bulletin. 2005;28(10):1817–21.

14. Mainas G, Santamaria P, Zoheir N, Alamri MM, Hughes F, Lu EMC, et al. Association between calcium-channel blockers and gingival enlargement: A case-control study. Journal of dentistry. 2024 Oct;149:105315.

15. Gopal S, Joseph R, Santhosh V, Kumar VH, Joseph S, Shete A. Prevalence of gingival overgrowth induced by antihypertensive drugs: A hospital-based study. Journal of Indian Society of Periodontology. 2015;19(3):308.

16. Balakumar P, Kavitha M, Nanditha S. Cardiovascular drugs-induced oral toxicities: A murky area to be revisited and illuminated. Pharmacological Research. 2015 Dec 1;102:81–9.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.2025

17. Kamath V, Setlur K, Yerlagudda K. Oral lichenoid lesions - A review and update. Indian Journal of Dermatology. 2015;60(1):102.

18. Dongari-Bagtzoglou A, Research, Science and Therapy Committee, American Academy of Periodontology. Drug-associated gingival enlargement. Journal of Periodontology. 2004 Oct;75(10):1424–31.

19. Villa A, Wolff A, Narayana N, Dawes C, Aframian D, Lynge Pedersen A, et al. World Workshop on Oral Medicine VI: a systematic review of medication-induced salivary gland dysfunction. Oral Diseases. 2016 Jan 20;22(5):365–82.

20. Becker DE. Cardiovascular Drugs: Implications for Dental Practice Part 1 — Cardiotonics, Diuretics, and Vasodilators. Anesthesia Progress. 2007 Dec;54(4):178–86.

21. Wright AJ, Gomes T, Mamdani MM, Horn JR, Juurlink DN. The risk of hypotension following co-prescription of macrolide antibiotics and calcium-channel blockers. Canadian Medical Association Journal. 2011 Jan 17;183(3):303–7.

22. Neuvonen P, Niemi M, Backman J. Drug interactions with lipid-lowering drugs: Mechanisms and clinical relevance. Clinical Pharmacology & Therapeutics. 2006 Dec;80(6):565–81.

23. Little JW, Miller CS, Henry RG, McIntosh BA. Antithrombotic agents: Implications in dentistry. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2002 Jun 25;93(5):544–51.

24. Curtis J, Henderson DP, Zarghami M, Rashedi S, Behnood Bikdeli. Management of Antithrombotic Therapy in Patients Undergoing Dental Procedures. Journal of Thrombosis and Haemostasis. 2024 Oct 1;23(1):47–72.

25. Inchingolo F, Inchingolo AM, Piras F, Ferrante L, Mancini A, Palermo A, et al. Management of Patients Receiving Anticoagulation Therapy in Dental Practice: A Systematic Review. Healthcare. 2024 Aug 2;12(15):1537–7.

26. Napeñas JJ, Hong CHL, Brennan MT, Furney SL, Fox PC, Lockhart PB. The Frequency of Bleeding Complications After Invasive Dental Treatment in Patients Receiving Single and Dual Antiplatelet Therapy. The Journal of the American Dental Association. 2009 Jun;140(6):690–5.

27. Kämmerer PW, Frerich B, Liese J, Schiegnitz E, Al-Nawas B. Oral surgery during therapy with anticoagulants—a systematic review. Clinical Oral Investigations. 2014 Nov 22;19(2):171–80.

28. Breik O, Cheng A, Sambrook P, Goss A. Protocol in managing oral surgical patients taking dabigatran. Australian Dental Journal. 2014 Jul 30;59(3):296–301.

29. Hanken H, Gröbe A, Heiland M, Smeets R, Kluwe L, Wikner J, et al. Postoperative bleeding risk for oral surgery under continued rivaroxaban anticoagulant therapy. Clinical Oral Investigations. 2015 Oct 26;20(6):1279–82.

30. Lopez-Galindo M, Bagan J. Apixaban and oral implications. Journal of Clinical and Experimental Dentistry. 2015;7(4):e528–34.

31. Curto A, Curto D, Sanchez J. Managing patients taking edoxaban in dentistry. Journal of Clinical and Experimental Dentistry. 2017;9(2):e308-e311.

32. Izzetti R, Cinquini C, Nisi M, Mattiozzi M, Marotta M, Barone A. Direct Oral Anticoagulants and Bleeding Management Following Tooth Extractions—A Prospective Cohort Study. Dentistry Journal. 2024 Aug 30;12(9):279–9.

33. Lupi SM, Rodriguez y Baena A. Patients Taking Direct Oral Anticoagulants (DOAC) Undergoing Oral Surgery: A Review of the Literature and a Proposal of a Peri-Operative Management Protocol. Healthcare. 2020 Aug 20;8(3):281.

34. Boccatonda A, Frisone A, Lorusso F, Bugea C, Carmine MD, Schiavone C, et al. Perioperative Management of Antithrombotic Therapy in Patients Who Undergo Dental Procedures: A Systematic Review of the Literature and Network Meta-Analysis. International Journal of Environmental Research and Public Health. 2023 Mar 28;20(7):5293–3.

35. Engelen ET, Schutgens RE, Mauser-Bunschoten EP, van Es RJ, van Galen KP. Antifibrinolytic therapy for preventing oral bleeding in people on anticoagulants undergoing oral or dental procedures. Cochrane Database of Systematic Reviews. 2016 Aug 2;7(7).

36. Elad S, Zadik Y, Kaufman E, Leker R, Finfter O, Findler M. A new management approach for dental treatment after a cerebrovascular event: a comparative retrospective study. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics. 2010 Aug 1;110(2):145–50.

37. Lyons M, Smith C, Boaden E, Brady MC, Brocklehurst P, Dickinson H, et al. Oral care after stroke: Where are we now? European Stroke Journal. 2018 May 8;3(4):347–54.

38. Sanz M, Marco del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, et al. Periodontitis and cardiovascular diseases: Consensus report. Journal of Clinical Periodontology. 2020 Feb 3;47(3):268–88.

39. Kreiner M, Okeson JP, Michelis V, Lujambio M, Isberg A. Craniofacial pain as the sole symptom of cardiac ischemia. The Journal of the American Dental Association. 2007 Jan;138(1):74–9.

40. Boon M, van Dorp E, Broens S, Overdyk F. Combining opioids and benzodiazepines: effects on mortality and severe adverse respiratory events. Annals of Palliative Medicine. 2020 Mar;9(2):542–57.

41. Silvestre FJ, Miralles-Jorda L, Tamarit C, Gascon R. Dental management of the patient with ischemic heart disease: an update. Medicina Oral: Organo Oficial De La Sociedad Espanola De Medicina Oral Y De La Academia Iberoamericana De Patologia Y Medicina Bucal. 2002 May 1;7(3):222–30.

42. Silvestre-Donat FJ. Dental management of ischaemic heart disease. Medicina oral : organo oficial de la Sociedad Espanola de Medicina Oral y de la Academia Iberoamericana de Patologia y Medicina Bucal. 2003;8(3):230.

43. Javed F, Khan J, Youssef M, Divakar DD, Michelogiannakis D. Dental management of patients with congestive heart failure before and after implantation of ventricular assist devices: linking the missing protocol. Scandinavian Cardiovascular Journal. 2020 Mar 18;54(4):206–11.

44. Imazio M. The 2023 new European guidelines on infective endocarditis: main novelties and implications for clinical practice. Journal of Cardiovascular Medicine. 2024 Jun 25;25(10):718–26.

45. Tsolka P. Dental Procedures in Patients with Atrial Fibrillation and New Oral Anticoagulants. Arrhythmia & Electrophysiology Review. 2014;3(2):85.

46. Rhodus NL, Little JW. Dental management of the patient with cardiac arrhythmias: an update. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2003 Dec;96(6):659–68.

47. Chaudhry S, Jaiswal R, Sachdeva S. Dental considerations in cardiovascular patients: A practical perspective. Indian Heart Journal. 2016 Jul;68(4):572–5.

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Antimicrobial Activity of Volatile and Non-Volatile Extracts of Hyptis Suaveolens Leaves

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ABSTRACT:

Medicinal plants serve as valuable reservoirs of bioactive compounds with therapeutic potential, offering alternatives in the fight against drug-resistant infections. Hyptis suaveolens (L.) Poit., though widely used in traditional medicine, remains underexplored despite reports of antimicrobial, anti-inflammatory, and other pharmacological activities.

Given the global need for novel antimicrobials, this study investigated the volatile (essential oils) and non-volatile (methanolic) extracts of H. suaveolens leaves, aiming to assess their antimicrobial activity against selected bacterial and fungal pathogens. Fresh Hyptis suaveolens leaves were collected and authenticated at Adekunle Ajasin University, Nigeria. Essential oils were obtained via hydrodistillation, while methanolic extracts were prepared by maceration. Antimicrobial activity against clinical isolates (E. coli, P. aeruginosa, B. cereus, K. pneumoniae, and C. albicans) was assessed using agar well diffusion, with MIC and MBC determined through macro-broth dilution techniques. Hydrodistillation of fresh leaves yielded 2.7% essential oil, while methanol extraction of dried leaves produced 1.5% crude extract. Both extracts inhibited all six clinical isolates, with the methanolic extract showing greater efficacy. The highest inhibition zone was recorded against Candida albicans (32 mm), and the lowest against Staphylococcus aureus (27 mm).

Minimum inhibitory concentration (MIC) testing revealed consistent activity of the methanolic extract across all concentrations, whereas the essential oil displayed variable inhibition. Minimum bactericidal concentration (MBC) assays confirmed bactericidal activity of both extracts, with E. coli and Pseudomonas aeruginosa eradicated at 25–50 mg/mL, while Klebsiella pneumoniae required the highest concentration (12.5 mg/mL). Hyptis suaveolens extracts exhibited broad-spectrum antimicrobial and bactericidal activity, with the methanolic extract showing superior potency, highlighting its potential as a natural alternative antimicrobial source.

KEYWORDS:

Hyptis suaveolens; antimicrobial activity; essential oil; methanolic extract; minimum inhibitory concentration.

1. Introduction

Medicinal plants, formally recognized for containing bioactive substances useful in disease treatment, have been integral to healthcare for millennia [1,2]. Derivation of therapeutic agents from plants has attracted global attention due to their perceived safety, accessibility, and cost-effectiveness. Herbal medicines are typically prepared from leaves, roots, bark, seeds, or flowers and administered via oral, inhalation, or topical routes [3]. Beyond their cultural and historical relevance, medicinal plants remain a cornerstone for drug discovery and development, serving as reservoirs of bioactive compounds with proven pharmacological potential [4].

The therapeutic activities of medicinal plants are primarily attributed to their phytochemical

constituents such as alkaloids, flavonoids, tannins, terpenoids, saponins, phenolic compounds, and essential oils. These natural compounds not only contribute to traditional healing practices but also form the structural backbone of many modern pharmaceuticals [5]. Historically, the medicinal use of plants emerged through empirical observation, where beneficial and toxic species were distinguished through trial and error. Knowledge was preserved through generations, forming the basis of traditional medicine across diverse cultures. Despite the scientific progress of synthetic chemistry, there remains a strong interest in plant-based bioactive compounds, both for their direct therapeutic use and as lead molecules for novel drug design [6].

Hyptis suaveolens (L.) Poit., commonly referred to as "mosquito plant," represents one such species of significant ethnomedicinal value. Native to tropical and subtropical regions, H. suaveolens is often considered a pervasive weed but has been documented to possess diverse pharmacological properties. Its leaves are rich in pharmacologically active secondary metabolites with antispasmodic, anti-colic, anti-rheumatic, and anti-fertility effects [7]. Additional reports highlight sedative, diuretic, aromatic, anti-inflammatory, anti-pyretic, anti-catarrhal, anti-rheumatic, anti-soporific, and anti-cancer activities [8]. The essential oils of H. suaveolens have demonstrated antimicrobial and antifungal properties, while the roots contain ursolic acid, a triterpenoid with documented anti-retroviral potential through inhibition of retroviral integrases and proteases [9,10,11]. Phytochemical studies of the leaves have revealed alkaloids as the dominant metabolites, followed by tannins and saponins [12].

Although H. suaveolens is widely used in traditional medicine, its chemical diversity and antimicrobial potential remain underexplored compared to other medicinal plants of global relevance. Reports on its essential oil profile and non-volatile metabolites are still limited, particularly in relation to their activity against clinically important pathogens. This represents a significant knowledge gap, given the urgent global demand for alternative antimicrobial agents in the face of rising antimicrobial resistance.

Here, we investigate the volatile and non-volatile constituents of H. suaveolens leaves and evaluate their antimicrobial activities against selected pathogens. Specifically, essential oils were extracted by hydrodistillation using a Clevenger apparatus, while methanol was employed for solvent extraction of non-volatile compounds. The antimicrobial potential of both extracts was subsequently tested to establish their comparative efficacy.

2. Materials and Methods

Plant Material

Fresh leaves of Hyptis suaveolens were collected from the campus of Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. The plant was authenticated by Dr. Obembe, Department of Plant Science and Biotechnology, Adekunle Ajasin University, and a voucher specimen was deposited in the departmental herbarium.

Extraction of Plant Constituents

Essential Oil Extraction

Volatile oils were extracted from 250 g of fresh leaves by hydrodistillation using a Clevenger-type apparatus for 3 h, following standard protocols [9]. The oil was dried over anhydrous sodium sulfate and stored at 4 °C in airtight vials until use.

Methanol Extraction

Powdered air-dried leaves (50 g) were extracted with 200 mL of methanol by maceration for 72 h. The extract was filtered (Whatman No. 1) and concentrated under reduced pressure at 40 °C using a rotary evaporator (Büchi, Switzerland). The dried extract was stored at 4 °C in sterile containers.

Test Microorganisms

Clinical isolates of Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Klebsiella pneumoniae, and Candida albicans were obtained from the Microbiology Laboratory of Adekunle Ajasin University. The strains were subcultured on nutrient agar and maintained at 4 °C until use.

Standardization of Inoculum

The bacterial inocula were standardized to a 0.5 McFarland turbidity standard, corresponding to ~1.5 × 10^8 CFU/mL, by adjusting the turbidity of 24 h broth cultures with sterile saline [17].

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Preparation of Extract Solutions

Stock solutions (100 mg/mL) of the methanol extract were prepared in 30% dimethyl sulfoxide (DMSO) and sterile distilled water (1:3, v/v). Two-fold serial dilutions yielded working concentrations of 50, 25, 12.5, 6.25, and 3.125 mg/mL. Essential oil dilutions were prepared similarly.

Antimicrobial Susceptibility Testing

The antimicrobial activity of extracts was evaluated using the agar well diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. Mueller–Hinton agar was seeded with standardized inocula of test organisms, and wells (5 mm diameter) were bored aseptically. Each well received 100 µL of extract solution at different concentrations. Erythromycin (10 µg/mL) served as the positive control, while solvent served as the negative control. Plates were incubated at 37 °C for 24 h, and the zones of inhibition were measured in millimeters.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MICs were determined by macro-broth dilution following the method of Parvekar et al. (2020) with slight modifications. Serial dilutions of extracts (50–3.125 mg/mL) were prepared in Mueller–Hinton broth. MIC was defined as the lowest concentration with no visible growth after 24 h incubation at 37 °C. To determine MBC, aliquots from MIC tubes showing no visible growth were plated onto Mueller–Hinton agar. MBC was recorded as the lowest concentration, yielding no colony growth after incubation. For Candida albicans, Sabouraud dextrose broth and agar were used.

3. Results and Discussion

Chemical Composition of Hyptis suaveolens Extracts

The chemical composition of essential oils obtained from Hyptis suaveolens (L.) Poit. has been reported by several studies, showing marked variation across geographic regions and even within countries. The species is widely distributed in tropical and subtropical zones of Asia, the Americas, and Africa, and at least seven major chemotypes have been identified, including 1,8-cineole, sabinene, β-caryophyllene, eugenol, aromadendrene/alloaromadendrene, fenchone, and menthol types [13].

Comparisons among samples from Cameroon, India, and Nigeria illustrate both inter- and intra-country differences in oil profiles (Table 2). In many Indian and Nigerian collections, monoterpene hydrocarbons predominate, ranging from 32.5–64.1% and 22.9–55.5%, respectively [14]. In contrast, specific populations show divergent trends: leaves from Dehra Dun (India) contained higher oxygenated sesquiterpenes (≈22.3%), while Cameroonian samples typically yielded 31.2–38.2% oxygenated sesquiterpenes [13].

Within Nigeria, compositional diversity is evident. Oils from Ibadan and Lagos share only a limited set of common compounds (limonene, linalool, p-cymene, α-pinene, and α-phellandrene). The Lagos specimen, as described by Tonzibo et al. (2009), may represent a distinct chemotype, with unusually high α-pinene (13.6%) and p-cymene (11.7%) levels. Similarly, Chung et al. (2020) reported that juvenile leaves contained elevated concentrations of β-caryophyllene (22.3%), α-phellandrene (10.6%), and caryophyllene oxide (10.3%), suggesting that both leaf age and local environmental conditions strongly influence oil composition. These findings highlight the chemical plasticity of H. suaveolens, shaped by geography, genotype, phenology, and environmental factors such as climate and soil.

In the present study, hydrodistillation of fresh leaves yielded 2.7% (w/w) essential oil, characterized as a dark green semi-viscous liquid, while methanol extraction of air-dried leaves gave 1.5% (w/w) crude extract, appearing as a green liquid (Table 1). These yields are consistent with reported values but may vary according to extraction technique, plant maturity, and origin, underscoring the importance of standardized collection and processing methods when comparing results across studies.

Table 1: Physicochemical properties and yields of Hyptis suaveolens leaf extracts

Extraction method	Yield (%)	Colour	Appearance
Hydrodistillation (fresh leaves)	2.7	Dark green	Semi-viscous liquid
Methanol solvent extraction (air-dried leaves)	1.5	Green	Liquid

Table 2: Representative compounds identified in the essential oils of Hyptis suaveolens collected from Cameroon, India, and Nigeria

Compound	Cameroon (Nga)	Cameroon (Yao)	India (Kum)	India (Luc)	Nigeria (Iba)	Nigeria (Lag)
ar-Abietadiene	–	–	–	–	–	0.3
ar-Abietatriene	9.6	–	4.4	–	–	–
ar-Abietatrienol	–	–	0.4	–	–	–
neo-Abietol	–	–	–	–	0.2	–
Acetophenone	–	–	–	–	–	0.2
allo-Aromadendrene	0.4	1.3	–	–	–	–
Aromadendrene	–	3.5	–	–	–	–
Atiserene	0.6	–	–	–	–	–
Bergamotol	–	6.2	–	–	–	–
trans-α-Bergamotene	–	–	–	–	–	5.2
trans-α-Bergamotene	–	–	–	–	–	19.8
trans-α-Bergamotol	–	–	–	0.3	–	1.9
(Z)-trans-α- Bergamotol	12.8	–	2.0	–	–	–

Nga. = Ngaoundere; Yao. = Yaoundé; Kum. = Kumaun; Luc. = Lucknow; Iba. = Ibadan; Lag. = Lagos

Antimicrobial Activity: Zone of Inhibition

Both the methanolic extract and essential oil of Hyptis suaveolens demonstrated inhibitory activity against all six tested clinical isolates. The methanolic extract consistently showed higher antimicrobial efficacy than the essential oil (Table 3). The strongest activity was observed against Candida albicans (32 mm zone of inhibition), while the weakest effect was noted against Staphylococcus aureus (27 mm). These results corroborate earlier findings by Pachkore et al. (2011), who also reported broad-spectrum activity of H. suaveolens extracts.

The superior performance of the methanolic extract relative to the essential oil likely reflects the higher solubility and wider range of bioactive phytochemicals recovered by methanol compared with volatile oil distillation. Among the tested organisms, Pseudomonas aeruginosa exhibited the lowest susceptibility, particularly to the essential oil, which is consistent with its known intrinsic resistance mechanisms such as biofilm formation, efflux activity, and encapsulation [21,22]. Overall, the results highlight that H. suaveolens contains compounds with notable antimicrobial activity, with methanolic extracts being especially potent against yeast and Gram-negative bacteria.

Table 3: Zone of inhibition (mm) of clinical isolates exposed to methanolic extract and essential oil of Hyptis suaveolens

Isolate	*E. coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Bacillus cereus*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
Essential oil	24	20	23	21	20	22
Methanolic extract	28	29	32	30	30	27
Control	30	28	32	50	30	32

Sensitive (S ≥ 25 mm); Intermediate (I = 20–24 mm); Resistant (R ≤ 19 mm)

Minimum Inhibitory Concentration (MIC)

The methanolic extract of Hyptis suaveolens inhibited all six test organisms at every concentration tested, including the lowest level (3.125 mg/mL), indicating strong and consistent antimicrobial potency (Table 4). In contrast, the essential oil displayed variable inhibition across the organisms (Table 5). E. coli and Pseudomonas aeruginosa were inhibited at higher concentrations (≥12.5 mg/mL) but not at the lowest levels. Candida albicans

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and Staphylococcus aureus were inhibited at 25–50 mg/mL, while Bacillus cereus and Klebsiella pneumoniae required relatively higher concentrations compared with the methanolic extract. Overall, the methanolic extract demonstrated broader and more consistent inhibitory activity than the essential oil. These findings align with earlier studies [22,23], which reported stronger antimicrobial performance of polar solvent extracts compared to essential oils. Variations in susceptibility across organisms may reflect structural differences in microbial cell walls and membranes.

Table 4: Minimum inhibitory concentration (MIC) of the methanolic extract of Hyptis suaveolens

Isolate	50	25	12.5	6.25	3.125
E. coli	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+
Candida albicans	+	+	+	+	+
Bacillus cereus	+	+	+	+	+
Klebsiella pneumoniae	+	+	+	+	+
Staphylococcus aureus	+	+	+	+	+

+ = Growth observed, – = No growth observed

Table 5: Minimum inhibitory concentration (MIC) of the essential oil of Hyptis suaveolens

Isolate	50	25	12.5	6.25	3.125
E. coli	+	+	+	–	–
Pseudomonas aeruginosa	+	+	+	–	–
Candida albicans	+	+	+	–	–
Bacillus cereus	+	+	–	–	–
Klebsiella pneumoniae	+	+	–	–	–
Staphylococcus aureus	+	+	+	–	–

+ = Growth observed, – = No growth observed

Minimum Bactericidal Concentration (MBC)

In this study, the methanolic extract of Hyptis suaveolens demonstrated bactericidal activity against the tested clinical isolates. Complete eradication was observed for E. coli and Pseudomonas aeruginosa at 50 and 25 mg/L. Candida albicans required 12.5 mg/L for bactericidal effect, while Bacillus cereus was eliminated at 50 and 25 mg/L. Klebsiella pneumoniae succumbed only at 12.5 mg/L, and Staphylococcus aureus was killed at 50 and 25 mg/L. Similarly, the essential oil showed bactericidal effects, with E. coli and P. aeruginosa eradicated at 50 and 25 mg/L. Candida albicans and Staphylococcus aureus required 12.5 mg/L, while B. Cereus was eliminated at 50 and 25 mg/L. K. pneumoniae was the least susceptible, responding only at the higher concentrations. These variations in MBC values highlight differences in microbial susceptibility, likely influenced by structural and physiological features such as cell wall composition and membrane permeability [24]. Overall, both the methanol extract and the essential oil of H. suaveolens exhibited promising bactericidal potential across bacterial and fungal pathogens, though activity was strain-dependent.

Table 6: Minimum bactericidal concentration (MBC) of the essential oil of Hyptis suaveolens

Isolate	50	25	12.5	6.25	3.125
E. coli	–	–	+	+	+
Pseudomonas aeruginosa	–	–	+	+	+
Candida albicans	–	–	–	+	+
Bacillus cereus	–	–	–	+	+
Klebsiella pneumoniae	–	–	+	+	+
Staphylococcus aureus	–	–	–	+	+

+ = Growth observed, – = No growth observed

Table 7: Minimum bactericidal concentration (MBC) of the methanolic extract of Hyptis suaveolens

Isolate	50	25	12.5	6.25	3.125
E. coli	–	–	+	+	+
Pseudomonas aeruginosa	–	–	+	+	+
Candida albicans	–	–	–	+	+
Bacillus cereus	–	–	–	+	+
Klebsiella pneumoniae	–	–	–	+	+
Staphylococcus aureus	–	–	–	+	+

+ = Growth observed, – = No growth observed

4. Conclusion

The present study demonstrates that Hyptis suaveolens contains diverse bioactive compounds with significant antimicrobial activity. Both methanolic and essential oil extracts inhibited and killed a range of bacterial and fungal pathogens, with the methanolic extract showing superior efficacy and a broader spectrum. These findings support the potential of H. suaveolens as a source of natural antimicrobial agents, though activity varied by strain and warrants further pharmacological evaluation.

References

1. Bashir DSF. Antibacterial Activity of Hyptis suaveolens Leaves Extract on some Selected Clinical Isolates. Antibacterial Activity of Hyptis suaveolens Leaves Extract on some Selected Clinical Isolates [Internet]. 2019 Jan 1;33(2):4652–9. Available from: https://www.academia.edu/51034008/Antibacterial_Activity_of_Hyptis_suaveolens_Leaves_Extract_on_some_Selected_Clinical_Isolates

2. Bezerra JWA, Costa AR, da Silva MAP, Rocha MI, Boligon AA, da Rocha JBT, et al. Chemical composition and toxicological evaluation of Hyptis suaveolens (L.) Poiteau (LAMIACEAE) in Drosophila melanogaster and Artemia salina. South African Journal of Botany. 2017 Nov;113:437–42.

3. Kumar S, Bhattacharya A, Tiwari P, Sahu P. A review of the phytochemical and pharmacological characteristics of Moringa oleifera. Journal of Pharmacy And Bioallied Sciences. 2018;10(4):181.

4. Bonilla-Landa I, Callejas-Linares JM, Marian E, Farías FR, Guevara-Valencia M, Monribot-Villanueva JL, et al. Actividad antibacteriana y perfil fenólico del extracto metanólico de las partes aéreas de Hyptis suaveolens (Lamiaceae). Acta Botanica Mexicana. 2022 Jan 18;(129).

5. Chung NT, Huong LT, Dai DN, Ogunwande IA. Chemical Compostions of Essential Oils and Antimicrobial Activity of Hyptis suaveolens (L.) Poit. (Lamiaceae) from Vietnam. European Journal of Medicinal Plants. 2020 May 30;31(8):114–23.

6. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st Edition. Journal of Clinical Microbiology. 2021 Sep 22;59(12).

7. Kaggwa B, Kyeyune H, Munanura EI, Anywar G, Lutoti S, Aber J, et al. Safety and Efficacy of Medicinal Plants Used to Manufacture Herbal Products with Regulatory Approval in Uganda: A Cross-Sectional Study. Bourhia M, editor. Evidence-Based Complementary and Alternative Medicine. 2022 Apr 13;2022:1–21.

8. Kaur R, Sharma P, Gupta GK, Ntie-Kang F, Kumar D. Structure-Activity-Relationship and Mechanistic Insights for Anti-HIV Natural Products. Molecules [Internet]. 2020 Jan 1;25(9):2070. Available from: https://www.mdpi.com/703680

9. Krishnapriya, Sasikumar P, Aswathy M, Prem PT, Radhakrishnan KV, Baby Chakrapani PS. Plant derived bioactive compounds and their potential to enhance adult neurogenesis. Phytomedicine Plus. 2022 Feb;2(1):100191.

10. Kujawska M, Pardo-de-Santayana M. Management of medicinally useful plants by European migrants in South America. Journal of Ethnopharmacology. 2015 Aug 22;172:347–55.

11. Kumar N, Khanna A, Kaur K, Kaur H, Sharma A, Bedi PMS. Quinoline derivatives volunteering against antimicrobial resistance: rational approaches, design strategies, structure activity relationship and mechanistic insights. Molecular Diversity. 2022 Oct 5;27(4).

12. Li R, Tang G, Liu X, Li J, Wang D, Ji S. An ethnopharmacological review of Hyptis suaveolens (L.) Poit. Tropical Journal of Pharmaceutical Research: TJPR. 2020 Nov 18;19(7):1541–50.

13. McNeil M, Facey P, Porter R. Essential Oils from the Hyptis genus- A Review (1909-2009). Natural Product Communications. 2011 Nov;6(11):1775–96.

14. Mirković S, Martinović M, Tadić VM, Nešić I, Jovanović AS, Žugić A. Antimicrobial and

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1952

Antioxidant Activity of Essential Oils from Selected Pinus Species from Bosnia and Herzegovina. Antibiotics. 2025 Jul 3;14(7):677–7.

15. Mishra P, Sohrab S, Mishra SK. A review on the phytochemical and pharmacological properties of Hyptis suaveolens (L.) Poit. Future Journal of Pharmaceutical Sciences. 2021 Mar 12;7(1).

16. Moreira ACP, Lima E de O, Wanderley PA, Carmo ES, Souza EL de. Chemical composition and antifungal activity of Hyptis suaveolens (L.) poit leaves essential oil against Aspergillus species. Brazilian Journal of Microbiology. 2010 Mar;41(1):28–33.

17. Pachkore GL, Dhale DA, Dharasurkar AN. Antimicrobial and phytochemical screening of Hyptis suaveolens (L.Poit) Lamiaceae. 2011 Jan 1;1(4):1-03. Available from: https://www.researchgate.net/publication/215728752_Antimicrobial_and_phytochemical_screening_of_Hyptis_suaveolens_LPoit_Lamiaceae

18. Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. Biomaterial Investigations in Dentistry. 2020;7(1):105–9.

19. Petrovska BB. Historical review of medicinal plants' usage. Pharmacognosy Reviews [Internet]. 2012;6(11):1. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3358962/

20. Samanta S. Potential Bioactive Components and Health Promotional Benefits of Tea (Camellia sinensis). Journal of the American College of Nutrition. 2020 Nov 20;41(1):1–29.

21. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. African Journal of Traditional, Complementary and Alternative Medicines. 2013 Aug 14;10(5):210–29.

22. Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: an Updated Review. Evidence-Based Complementary and Alternative Medicine [Internet]. 2016;2016(PMC5206475):1–21. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5206475/

23. 23.Félix TZ, Florence AB, Bedi G, Chalchat JC. Chemical composition of essential oil of Hyptis suaveolens from Côte d'Ivoire. European Journal of Scientific Research [Internet]. 2009;38(4):565–71. Available from: https://www.researchgate.net/publication/261955341_Chemical_composition_of_essential_oil_of_Hyptis_suaveolens_from_Cote_d

24. 24.Veeresham C. Natural products derived from plants as a source of drugs. Journal of Advanced Pharmaceutical Technology & Research. 2012;3(4):200–1.

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