June 2025
Volume 5
Issue 1

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898

http://dx.doi.org/10.21622/AMPDR.2025.05.1.1060

Piper guineense (Swhan and Thon) Inhibits Lanosterol-14α-demethylase in Multi-Drug Resistant Non-*albicans Candida Species*: In vitro and In silico Studies

[1*]Olumide Oluyele, [2]Damilola Alex Omoboyowa, [3]Ayomide Esther Aderogba and [4]Koffi Adusa Osei

[1,3,4]Department of Microbiology, Adekunle Ajasin University Akungba-Akoko, Nigeria.

[2]Phyto-medicine and Computational Biology Lab., Department of Biochemistry, Adekunle Ajasin University Akungba-Akoko, Nigeria.

Emails: olumideoluyele@gmail.com, damilola.omoboyowa@aaua.edu.ng, aderogbaa989@gmail.com, oooomoowumi@gmail.com

Received on, 17 October 2024 - Accepted on, 15 January 2025 - Published on, 15 March 2025

ABSTRACT:

Background:
Candida species are globally recognized for invasive infections with poor prognosis. Burgeoning quest for the discovery of novel therapeutics has increased the scientific scrutiny of several medicinal plants. This study assessed the efficacy of Piper guineense crude extract and fractions against selected multi-drug resistant non- albicans Candida species.

Results
Both the crude and fractionated extracts of P. guineense elicited marked anti-candidal activity. Overall, the crude extract showed better efficacy over the fractions with the highest inhibition zone (25.0mm) recorded against Candida tropicalis; amongst the fractions, n-hexane fraction (F2) produced the highest inhibition zone (23mm) against Candida glabrata. The MIC ranged from 25mg/ml to 50mg/ml, while the MBC ranged between 100 mg/ml to 200 mg/ml. HPLC analysis revealed the presence of 14 compounds in the extract, with prominent members being quercetin, ellagic acid, persin, catechin, p-coumaric acid, and lutein. The binding affinity and free binding energy results reveal that most of these bioactive compounds were better than the standard drug (Fluconazole).

Conclusion:
Conclusively, P. guineense extracts demonstrated impressive anti-candidal properties against the tested multi-drug resistant non-albicans Candida species and could have potential as new drug lead for the treatment of infections resulting from these pathogens.

KEYWORDS:

P. guineense, non-albicans Candida, Lanosterol-14α-demethylase, Antimicrobial resistance

1. Introduction

Each year, more than 6.5 million people contract life-threatening fungal infections; about 1.5 million of these are attributable to invasive candidiasis, with a 63.6% mortality rate [1]. The clinical spectrum of candidiasis, which is caused by yeasts of the genus *Candida,* extends from superficial diseases such as cutaneous, nail, digestive, and genital candidiasis to systemic diseases such as candidemia [2]. *Candida* spp are generally commensal germs that develop in the skin, inside the body, in the mouth, throat, intestines, and vagina, without causing infection [3]. They express their pathogenic power only in the presence of factors favoring the origin of the translation of endogenous commensal to the disease-causing parasite. These factors can be intrinsic or extrinsic to the host, including overweight and prolonged use of broad-spectrum antibiotic therapy and corticosteroids, among others [4]. Immunosuppression remains one of the most prevalent risk factors [5]. The resurgence of diseases weakening the immune system, such as AIDS, and immunosuppressive

treatments, such as heavy chemotherapy, has led to a drastic increase in *Candida* infections, which have become a major cause of morbidity, mortality, and increased treatment expenses in hospitals. The disseminated forms of candidiasis can be life-threatening, with high mortality rates among immunocompromised cancer patients and those exposed to multiple treatments, such as broad-spectrum antibiotics, chemotherapy, immunosuppressive therapy, and antiretroviral therapy [2,6]. On the other hand, inappropriate medical practices such as misdiagnosis and inadequate medication are responsible for the exacerbation, spread, and persistence of the infection. The pathogenicity of *Candida spp.* Emanates from a diversity of factors, including its heightened ability to adapt to stressful conditions [7]. Moreover, virulence attributes such as the expression of surface molecules as adhesins, the ability to change its morphology, biofilm forming capacity, and the secretion of hydrolytic enzymes are essential for establishing infection [4].

Natural products obtained from plants have a vast repertoire of biologically active compounds that represent rich prospects for drug development [8]. *Piper guineense* (African black pepper), belonging to the order Piperales and the Piperaceae family, is one of the most commonly used spices. It is considered as "the king of spices" due to its trade in the international market [9,10]. African black pepper is cultivated in many tropical regions like Brazil, Nigeria, India, Ghana, Indonesia, and Senegal [2]. *P. guineense* adapts well to a broad range of environmental conditions, including vast altitudinal regions [10]. Interestingly, *P. guineense* has been used for several purposes as a natural medicinal agent for the treatment and alleviation of digestive and respiratory disorders [11]. It has also been used in human dietaries and perfumery as a preservative and biocontrol agent [12,13].

Despite therapeutic advances, the incidence of candidiasis continues to aggravate with increasing mortality. Moreover, non-*albicans Candida* species (NACs) are more frequently implicated in the epidemiology of the disease nowadays [6,14,15]. The poor prognosis of candidal infections is partly attributable to the limited antifungal armamentarium, toxicity of antifungal drugs, and the current challenge of antifungal resistance [16,17]. Against this background, it becomes pertinent to develop alternative therapies for treating *Candida* infections. This study, therefore, evaluated the efficacy of *Piper guineense* crude and fractionated leaf extracts against multi-drug resistant NACs.

2. METHODS

Test Organisms and Preparation of Inoculum

The test organisms used in this study were multidrug resistant strains of *Candida krusei, Candida tropicalis,* and *Candida glabrata* obtained from the stock culture of previously identified isolates at the Microbiology Laboratory, AAUA. The test organisms were maintained on Sabauraud dextrose agar (SDA) agar slant and stored in the refrigerator at 4^0C for further studies. McFarland standard (0.5) was prepared by combining 0.05ml of 1% barium chloride Dihydrate ($BaCl_2.2H_2O$) with 9.95ml of 1% Sulfuric acid (H_2SO_4) to yield 1.0%w/v barium sulphate suspension. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer at 625 nm. The McFarland standard was vigorously agitated on a vortex mixer before use. The Inoculum of each test organism was prepared by picking five distinct colonies of approximately 1 mm in diameter from a 24-hour-old culture. Colonies were suspended in 5 mL of sterile 0.85% saline. The resulting suspension was vortexed for 15 seconds, and its turbidity was adjusted to 0.5 McFarland standards. This procedure yielded a yeast stock suspension of 1-5 x 10^6 cells per mL [6].

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2025.05.1.1060

Extraction and Fractionation of Plant Material

Fresh leaves of *Piper guineense* (Swhan and Thon) were sourced from AAUA medicinal garden, Akungba-Akoko (Latitude 7.4740 °N and Longitude 5.7379 °E), Ondo state, Nigeria. The plant was authenticated at the Department of Plant Science and Biotechnology Herbarium of our institution. Voucher specimen number PSBH 254 was deposited for the plant.

The maceration method, as described by Oluyele *et al.* [18], was used for the crude-extraction process. Briefly, the leaves of *Piper guineense* were air-dried at room temperature and pulverized. Thereafter, 850g of the powdered leaves were soaked in 2550 ml of 70% ethanol for 7 days with occasional shaking to allow the full extraction of the active ingredients. The mixture was sieved using a muslin cloth and then filtered using Whatman No.1 filter paper. The filtrates were concentrated using a rotary evaporator. The extract obtained was stored in the refrigerator at 4°C for further studies. Fractionation of *P. guineense* crude extract was performed using liquid-liquid extraction according to the method of Pham *et al.* [19]. Briefly, the crude extract was dissolved in distilled water (1:10, w/v), transferred into a separating funnel, and successively fractionated with n-hexane and ethyl acetate to yield different fractions, respectively designated hexane fractions (F1 and F2) and ethyl acetate fraction (F3). The residue was generated, namely aqueous fraction (F4). The resulting fractions (F1, F2, F3, F4) were then concentrated using a rotary evaporator. All fractions were then freeze-dried and stored at 4°C until further experiments.

Antifungal Assay of Piper guineense leaf extract

The agar well diffusion technique, as described by Oluyele *et al.* [18], was used to determine the antifungal activity of the crude extract and fractions. One (1ml) aliquot of each test organism suspension (standardized) was transferred onto the well dried sterile Sabauraud dextrose agar (SDA) plates and was spread evenly using sterile swab sticks. The plates were allowed to dry; a standard sterile cork borer of 6mm diameter was used to cut uniform wells on the SDA plates. Each well was appropriately labeled on the reverse side of the plates. Then, 50 µL of 100 mg/mL of the extract and fractions prepared in 5% dimethyl-sulfoxide (DMSO, Sigma Aldrich, Germany) were filled into the corresponding wells. Fluconazole was used as a control in one of the wells. The plates were allowed to stand for 15 minutes at room temperature to allow proper diffusion of the extract to occur. All the plates were incubated at 35°C for 48 hours, after which the zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MBC)

The MIC and MFC of the extract were determined using tube-dilution and plating methods respectively [18]. For MIC, different concentrations of the extract were prepared at 200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml. This was followed by the addition of 0.1 ml of the standardized test inoculum into each test tube. A set of test tubes containing only sterile Sabauraud dextrose broth (SDB) was used as a negative control, and another set of test tubes containing SDB plus test organisms was used as a positive control. All the test tubes were then incubated at 35°C for 48 hours. Growth in each tube was checked by visible observation and by using a spectrophotometer (Beckman model 35). The concentration that produced no visible turbidity was taken as the MIC. The MFC was determined using the plating method by subculturing from the MIC tube and other tubes that showed no turbidity onto freshly prepared Sabauraud dextrose agar and incubated at 35°C for 48 hours. The concentration that showed no visible growth after incubation was taken as the MFC.

High Performance Liquid Chromatography (HPLC) Analysis of Extract

About 2g of sample was measured into an

amber bottle, followed by the addition of 20mls of (Acetonitrile/methanol) and vigorous agitation for 30 minutes. Thereafter, the aqueous end was run off while the organic solvent end was collected into a 25ml standard flask, made up to the mark, and ready for analysis. The sample was run using gradient elution according to the following chromatographic conditions: reversed phase chromatography (Agilent Technologies 1200 HPLC), mobile phase composition: 0.1% formic acid + acetonitrile, stationary phase: Hypersil BDS C18 (Agilent), column dimension: 250mm x 4.0 mm, injection volume: 20µL, flow rate 0.6 ml/min, detector wavelength: 280 nm. The standard form of analyte profile was first injected into the HPLC, and this generated a chromatograph with a given peak area and peak profile. These were used to create a window in the HPLC in preparation for the test sample analysis. An Aliquot of the extracted test sample was also injected into the HPLC to obtain a corresponding peak area and peak profile in a chromatograph. By keeping track of retention time and analyzing UV spectra, the peaks were identified by comparing them with reference standards [20].

Generation and Preparation of Compound Library

The compounds that were identified from *P. guineense* by HPLC analysis were downloaded from the PubChem (https:// pubchem.ncbi.nlm.nih.gov) repository alongside with the protein standard drug in structure data file (sdf) format. These molecules were exported onto Schrodinger workspace (Schrodinger, 2021v2) and prepared using Ligprep tool for the *in silico* study.

Protein Preparation

The research collaborator for the structural bioinformatics protein databank (RCSB PDB) [www.rcsb.org] website provided the x-ray crystallographic structure of the lanosterol-14α-demethylase complex with ketoconazole having PDB ID of 3LD6. The missing residues and loop in the protein and other side chain anomalies were resolved, followed by energetic optimization with force field OPLS3 using the protein preparation wizard of Schrodinger suit 2021. The receptor grid generator was used to generate a glide grid on the co-ligand (digoxin) attached site with glide coordinates of x = 42.47, y = 4.96, and z = 2.02. The prepared protein crystallographic structure and Ramachandran residues' distribution are shown in Figure 1.

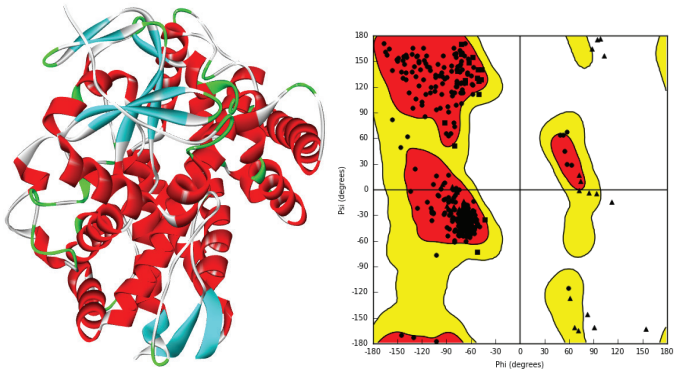


Figure 1: Crystal structure of Lanosterol-14α-demethylase and Ramachandran plot of residues distribution

Structure-based virtual screening

The prepared compounds from *P. guineense* and the standard ligand were screened against lanosterol-14α-demethylase using the extra precision (XP) GLIDE docking filtering procedure in the Maestro Schrodinger suite (v 2021). This scoring function is known for its robustness and discriminating ability, but it requires more time to run [21].

Prime/MM-GBSA calculations

The lanosterol-14α-demethylase-ligand complexes were minimized by using the local optimization feature in Prime; the binding energy (Δ^{bind}) for the complexes was determined using the OPLS3 force field. Molecular mechanics generalized the Born surface area (MM/GBSA) calculation, which was carried out on the docking complexes. The following equation was used to calculate the binding free energy:

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2025.05.1.1060

3. RESULTS

Anti-candidal Activity of Piper guineense Extracts

As shown in Tables 1 and 2, the extracts of *P. guineense* showed marked anti-candidal activity in this study. The highest activity was observed against *Candida tropicalis* 25.0mm for the crude extract of *P. guineense*, and against *Candida glabrata* (N-hexane fraction 2) amongst the fractions. The MIC ranged from 25mg/ml to 50mg/ml; while the MBC ranged between 100 mg/ml to 200 mg/ml.

Table 1: Antifungal Potency of Piper guineense Extracts

Organism	CE	F 1	F 2	F 3	F 4	FLC
Candida glabrata	23.0^{b}	21.5^{c}	23.0^{c}	17.5 ± 0.29^{c}	20.5^{b}	26.0^{a}
Candida tropicalis	25.0 ± 0.29^{c}	19.5^{b}	5.5^{a}	4.5^{a}	18.5 ± 0.29^{a}	26.0^{a}
Candida krusei	22.0 ± 0.29^{a}	17.0 ± 0.29^{a}	11.5 ± 0.29^{b}	16.5^{b}	21.5 ± 0.33^{c}	28.0^{a}

Legend: CE- Crude extract, F1- N-hexane fraction 1, F2- N-hexane fraction 2, F3- Ethyl acetate fraction, F4- Aqueous fraction, FLC - Fluconazole. Values with the same superscript across the column are not significantly different.

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal concentration (MFC) of Piper guineense Crude-Extract against Test Organisms

Organism	MIC	MBC
Candida glabrata	50mg/ml	100mg/ml
Candida tropicalis	25mg/ml	100mg/ml
Candida krusei	50mg/ml	200mg/ml

HPLC Identified Compounds of Piper guineense Crude-Extract

As presented in Figure 2 and Table 3, HPLC analysis revealed fourteen compounds in the crude leaf extract of *P. guineense*. Some of the notable constituents include quercetin, ellagic acid, persin, catechin, p-coumaric acid, and lutein.

Table 3: Compounds identified in the Piper guineense Leaf Extract

Compounds	Retention	Area	Height
Ellagic acid	3.70	1781.7085	48.923
Zeaxanthin	5.883	308.7870	8.077
P-Coumaric Acid	7.966	609.2790	13.901
Camphene	9.116	64.5150	3.573
Catechin	10.500	128.7600	3.217
Epicatechin	11.300	108.8825	5.707
Obovaten	12.000	59.3410	4.334
Obovatinal	13.833	75.0890	4.515
Persin	15.500	863.8795	14.749
Quercetin	17.233	2761.1350	37.005
Persenone A	19.166	105.4600	7.437
Lutein	19.950	84.0050	5.832
Scorpoletein	20.500	62.7400	5.332
Afzelin	21.416	66.7880	2.356

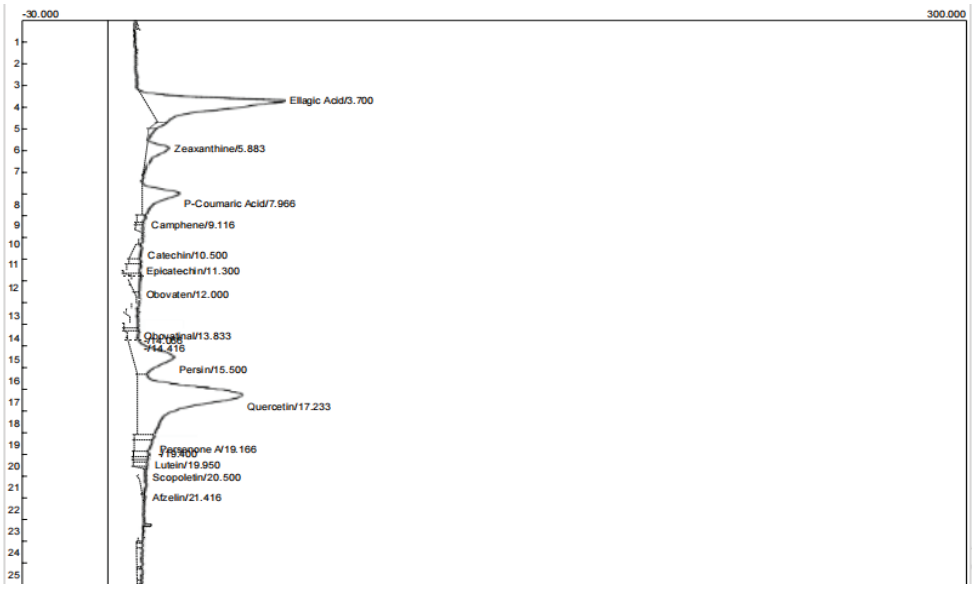


Figure 2: HPLC Chromatograph of Piper guineense

Molecular Docking and MM/GBSA of the bioactive compounds against the target

As shown in Figure 3, the active compounds had varying binding affinities against lanosterol-14α-demethylase ranging from -5.752 to -10.227 kcal/mol, which is comparable with the standard drug (fluconazole = -8.454 kcal/mol). Quercetin, persin, persenone A, afzelin, epicatechin, catechin, and ellagic acid were observed to have better binding affinities (-10.227, -10 027, -9.555, -9.948, -9.081, -9.310 and −9.286 kcal/mol respectively) against lanosterol-14α-demethylase compared with the standard drug. The binding affinities obtained might be attributed to the formation of various interactions between the functional groups of the bioactive compounds and the amino acid residues at the binding site of lanosterol-14α-demethylase. From Figure 4, the active compounds from the plant interacted with various amino acids present at the pocket of the protein through various molecular interactions like van der Waals, alkyl bond hydrogen bonds, and pi-alkyl bonds. Catechin and ellagic acid formed four (4) hydrogen bonds with other hydrophobic interactions while afzelin and obovatin formed three (3) hydrogen bonds with other hydrophobic bonds which is equivalent to the number of hydrogen bonds formed by fluconazole (standard drug) with TYR 131, ILE 397 and MET 378.

The binding free energies of the complex were determined by calculating the molecular mechanics generalized born surface area (MM/GBSA). From Figure 2, all the compounds except p-coumaric acid and camphene has binding free energy more than -40 kcal/mol. Obovaten, ellagic acid, catechin, persin, persenone A and afzelin have better binding free energies than the standard drug (fluconazole = -49.21 kcal/mol).

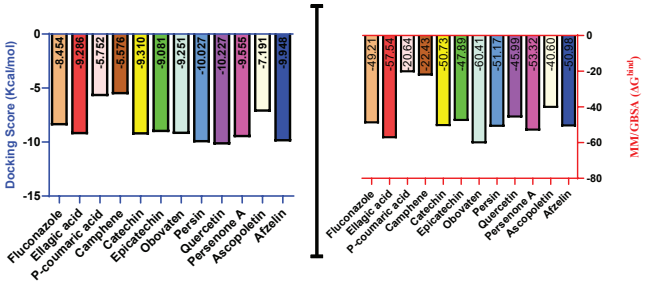
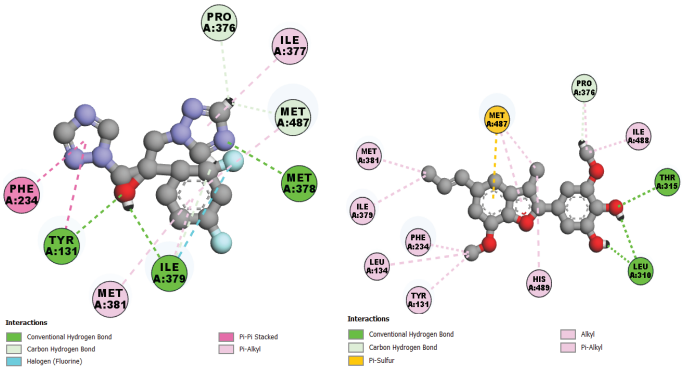


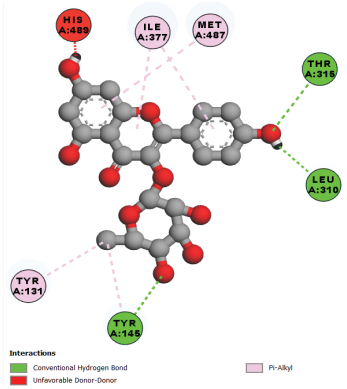
Figure 3: Graphical representation of the binding affinity and binding free energy calculation of bioactive compounds against lanosterol-14α-demethylase



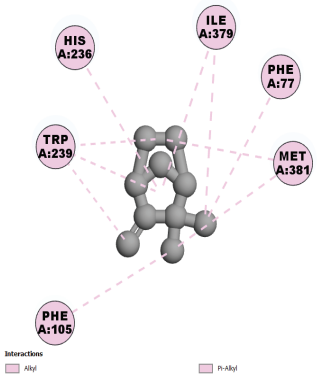
Fluconazole

Obovaten

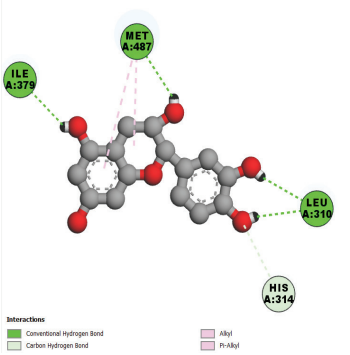
Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2025.05.1.1060



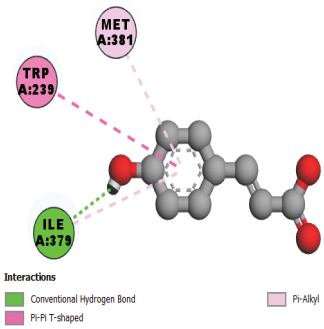
Afzelin



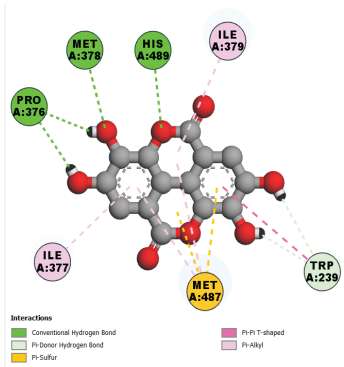
Camphene



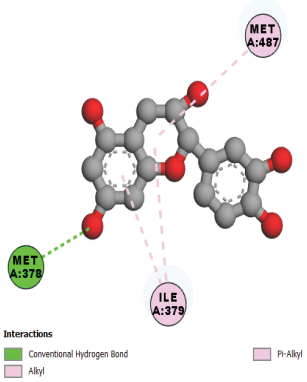
Catechin



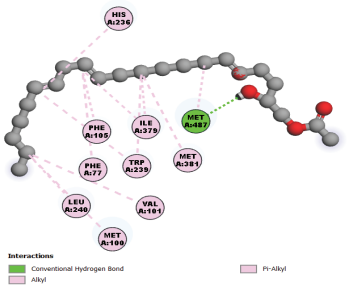
P-coumaric acid



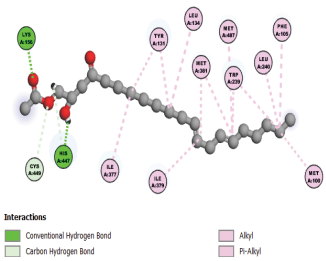
Ellagic acid



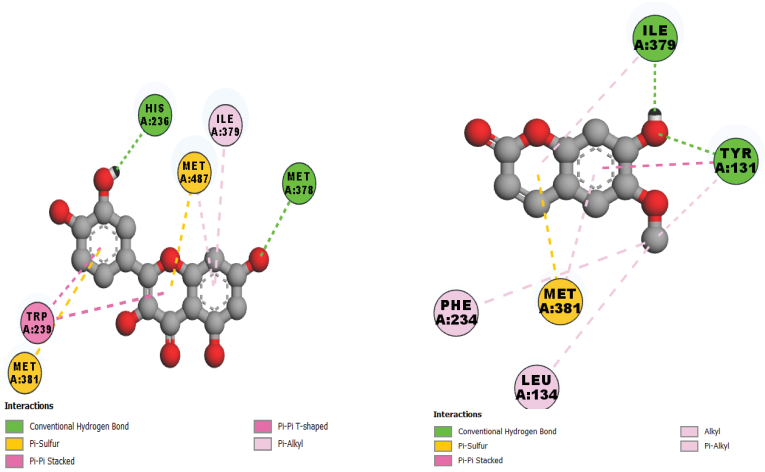
Epicatechin



Persenone A



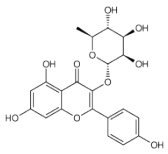
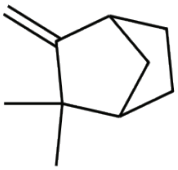
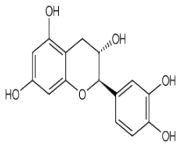
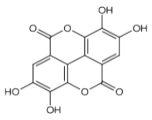
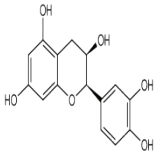
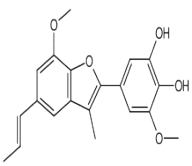
Persin



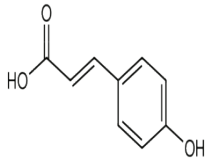
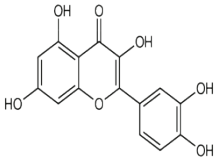
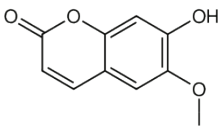
Quercetin ***Scopoletein***

Figure 4: 2D interaction between the compounds-lanosterol-14α-demethylase complexes

Table 4: Post-docking analysis of Ligand-protein complexes

Name	Structure	Binding Affinity (kcal/mol)	No of H-bonds	Amino Acids
Afzelin		-9.948	3	THR 315; LEU 310; TYR 145
Camphene		-5.576	Nil	Nil
Catechin		-9.310	4	ILE 379; MET 487; LEU 310
Ellagic acid		-9.286	4	PRO 376; MET 378; HIS 489
Epicatechin		-9.061	1	MET 378
Persin		-10.027	2	LYS 156; HIS 467
Obovaten		-9.251	3	THR 315; LEU 318

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2025.05.1.1060

P-coumaric acid		-5.752	1	ILE 378
Quercetin		-10.227	2	HIS 236; MET 378
Ascopoletin		-7.191	2	ILE 379; TYR 131
Persenone A		-9.555	1	MET 487

4. DISCUSSION

Candida spp are globally recognized for invasive infections with poor prognosis [6]. The burgeoning quest for the discovery of novel therapeutics has increased the scientific scrutiny of several medicinal plants. In this study, we evaluated the efficacy of *Piper guineense* crude extract and fractions against some pathogenic multi-drug resistant non-*albicans Candida* species. Both the crude extract and the fractions of *P. guineense* elicited impressive activities against the test pathogens in this study. Overall, the crude extract showed higher efficacy compared to the fractions. This higher activity observed could be attributed to the crude extract containing a larger proportion of the bioactive constituents of the plant, owing to the ability of the extraction solvent to retrieve these constituents. The results obtained showed variations in the inhibition zones (IZs) of the extracts on the test yeast strains. The highest inhibition zones were observed against *Candida tropicalis* for the crude extract, against *Candida glabrata* for F1, F2, and F3, and against *Candida krusei* for F4. Notably, F2 and F3 had minimal effect on *Candida tropicalis,* and the inhibition zones observed were the least overall. The variation seen in the inhibition zones is presumed to be due to different active compounds present in each extract and the susceptibility profile of the *Candida* strains studied.

In tandem with this report, the hexane and ethanolic fruit extracts of *P. guineense* were chronicled to be effective against the growth of *C. albicans* and *C. glabrata* [22]; the hexane leaf extract elicited appreciable activity against *Sarcina* sp., *Staphylococcus aureus,* and *Enterobacter aerogenes*. However, the water extracts were not active against the bacterial strains tested [23]. Moreover, another study revealed that the seed extracts of *P. guineense* showed antimicrobial potency against *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae, Aspergillus flavus, Aspergillus niger* and *Candida albicans* [24].

The MIC and MFC of the crude extract were further determined since the IZs were the most prominent of all the extracts. The MIC was 50mg/ml for *Candida glabrata* and *Candida krusei* and 25 mg/ml for *Candida tropicalis,* while the MFC was 100mg/ml for *Candida glabrata* and *Candida tropicalis* and 200mg/ml for *Candida krusei.* The activities of the extracts (especially the crude) compared favorably with the standard drug (Fluconazole) employed in this study, although Fluconazole produced higher inhibition zones. The better activity of the standard drug over the extracts could be connected to the fact that organic extracts are in crude form compared to synthetic antibiotics, which have a high degree of purity; hence, the secondary active metabolites could be present in low concentrations or masked in the extracts [18].

Considering that the crude extract portrayed the overall higher activity in this study, it was subjected to HPLC profiling. Distinctive among the compounds identified include quercetin, ellagic acid, persin, catechin, p-coumaric

acid, and lutein. Notably, these compounds are different from those reported by a previous study [23]. The compounds identified from our analysis are most likely responsible for the observed efficacy of *P. guineense* in our experiment. For instance, quercetin a flavonoid with antioxidant properties and many beneficial effects on health [25], has been reported to show a broad inhibitory effect on bacteria, and its combination with amphotericin-B and fluconazole produced promising synergistic antifungal-activity [26,27]. Quercetin enhanced fluconazole-resistant *Candida albicans*-induced apoptosis by regulating quorum sensing [28]. The presence of ellagic acid in *P. guinesse* studied is of great importance, as it is a bioactive polyphenolic compound naturally occurring as a secondary metabolite in many plant taxa [25]. Varying concentrations of ellagic acid were reported to inhibit the growth of *Candida albicans* [29]. Persin is a fungicidal compound present in avocados [30]. Catechin has been proven to exhibit antimicrobial activity against clinical isolates of methicillin resistant *Staphylococcus aureus* [31]. Lutein from extract of *Helianthus annuus* was found to be active against *Bacillus subtilis, Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* [32].

The virtual screening analysis of the active molecules from the HPLC analysis of the crude extract against the binding site lanosterol-14α-demethylase was carried out. The fungal lanosterol-14α-demethylase is a vital protein in the pathway that synthesizes ergosterol and cholesterol biosynthetic pathways in humans [33]. This cytochrome P450 enzyme remains the target for the azole antifungals used in the treatment of fungal infections in humans [34]. Inhibition of the binding domain of this protein results in the termination of ergosterol biosynthesis and the formation and accumulation of the intermediates that might lead to inhibition of microbial growth [35]. The virtual screening of active molecules through a molecular docking procedure is one of the vital methods of drug design. This method forecasts the interaction between small molecules and the amino acid residues at the binding site of the target [21]. Inhibition of lanosterol-14α-demethylase by bioactive compounds from *Piper guineense* was selected to study the antifungal mechanism of the compounds from the plant and complement the in vitro antifungal activity of the plant observed in this study. The result of the binding affinities obtained in this study, as shown in Fig. 3, suggests that eight out of the eleven active compounds from *Piper guineense* were potent inhibitors of lanosterol-14α-demethylase with better binding affinity compared with fluconazole (standard drug). This result agrees with the findings of Oladimeji *et al*. [33], who observed the inhibition of sterol-14α-demethylase by the active compounds from *E. coccinea* essential oil. Rosam *et al*. [35] have also reported that sterol-14α-demethylase-ligand binding pocket mediated acquired and intrinsic azole resistance in fungal pathogens. The ligand-protein interactions in molecular docking contributed to the binding affinity of small molecules against the target, which has been observed to be a key regulator of their action [36]. Figure 4 showed that afzelin interacted with THR 315, LEU 310, and TYR 145 with hydrogen bonds, catechin interacted with ILE 379, MET 487, and LEU 310 with four hydrogen bonds, ellagic acid interacted with PRO 376, MET 378, HIS 489 with four hydrogen bonds and obovaten interacted with THR 315 and LEU 310 with three hydrogen bonds. These active compounds have the highest hydrogen bond interactions among the compounds identified from *Piper guineense*, which is comparable with the standard drug (fluconazole) interacting with TYR 131, ILE 379, and MET 378 with three hydrogen bonds. Other hydrophobic interactions were also observed in the protein-ligand complex, which might contribute to the docking score. The observed inhibitory activity of these active compounds against fungi lanosterol-14α-demethylase presents the enzyme as the antifungal mechanism of the plant, as observed in the *in vitro* study.

5. Conclusion

Conclusively, *P. guineense* extracts demonstrated impressive anti-candidal efficacy against the test multi-drug resistant non-*albicans Candida* species and could have potential as a new therapeutic agent for the treatment of infections resulting from these pathogens. The efficacy of *P. guineense* recorded in our study could be attributed to the diversity of bioactive compounds identified with the inhibition of lanosterol-14α-demethylase proposed as the mechanism of anti-fungi action. Further, *in vivo* studies on the isolated bioactive compounds are necessary to validate the findings from the *in vitro* and *in silico* analyses in this study for safe therapeutic resolution of Candidal infections.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2025.05.1.1060

References

1. Denning DW. Global incidence and mortality of severe fungal disease. Lancet Infect Dis. 2024 Jul;24(7):e428–38.
2. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nat Rev Dis Primers. 2018 May 11;4(1):18026.
3. Netea MG, Joosten LAB, van der Meer JWM, Kullberg BJ, van de Veerdonk FL. Immune defence against Candida fungal infections. Nat Rev Immunol. 2015 Oct 21;15(10):630–42.
4. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence. 2013 Feb 15;4(2):119–28.
5. Pfaller MA. Antifungal drug resistance: Mechanisms, epidemiology, and consequences for treatment. American Journal of Medicine. 2012;125(1 SUPPL.).
6. Oluyele O, Oladunmoye MK, Ogundare AO, Onifade AK, Okunnuga NA. 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.
7. Dhanya K, Kizhakkayil J, Syamkumar S, Sasikumar B. Isolation and Amplification of Genomic DNA from Recalcitrant Dried Berries of Black Pepper (Piper nigrum L.)—A Medicinal Spice. Mol Biotechnol. 2007 Sep 13;37(2):165–8.
8. Oluyele O, Oladunmoye MK, Ogundare AO. Toxicity Studies on Essential Oil from Phoenix dactylifera (L.) Seed in Wistar Rats. Biologics. 2022;2(1).
9. Singh VK, Singh P, Patel AMA, Yadav KKM. Piperine: delightful surprise to the biological world, made by plant "pepper" and a great bioavailability enhancer for our drugs and supplements. World J Pharm Res. 2014;3(6).
10. Matsuda H, Ninomiya K, Morikawa T, Yasuda D, Yamaguchi I, Yoshikawa M. Protective effects of amide constituents from the fruit of Piper chaba on d-galactosamine/TNF-α-induced cell death in mouse hepatocytes. Bioorg Med Chem Lett. 2008 Mar;18(6):2038–42.
11. Damanhouri ZA. A Review on Therapeutic Potential of Piper nigrum L. (Black Pepper): The King of Spices. Med Aromat Plants (Los Angel). 2014;03(03).
12. Mathew PJ, Mathew PM, Kumar V. Graph clustering of Piper guineense L. (black pepper). Vol. 18. Euphytica; 2020. 257–264 p.
13. Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, Khan MA. Biological role of Piper nigrum L. (Black pepper): A review. Asian Pac J Trop Biomed. 2012 Jan;2(3):S1945–53.
14. Hernández-Pabón JC, Tabares B, Gil Ó, Lugo-Sánchez C, Santana A, Barón A, et al. Candida Non-albicans and Non-auris Causing Invasive Candidiasis in a Fourth-Level Hospital in Colombia: Epidemiology, Antifungal Susceptibility, and Genetic Diversity. Journal of Fungi. 2024 Apr 30;10(5):326.
15. Jannati B, Pourdad A, Izadjoo A, Zarrinfar H, Najafzadeh Mj, Fata A. The prevalence of Non-albicans Candida and Candida mixed-species in vulvovaginal candidiasis in Iran. Clin Exp Obstet Gynecol. 2024;51(3):77.
16. Galocha M, Pais P, Cavalheiro M, Pereira D, Viana R, Teixeira MC. Divergent approaches to virulence in C. Albicans and C. Glabrata: Two sides of the same coin. Int J Mol Sci. 2019;20(9).
17. Ceballos Garzon A, Amado D, Robert E, Parra Giraldo CM, Le Pape P. Impact of calmodulin inhibition by fluphenazine on susceptibility, biofilm formation and pathogenicity of caspofungin-resistant Candida glabrata. Journal of Antimicrobial Chemotherapy. 2020;75(5).
18. Oluyele O, Oladunmoye M. Susceptibility Patterns of Staphylococcus aureus Isolated from Wounds Swabs to Extracts of Vernonia amygdalina. J Adv Med Pharm Sci. 2017;13(4).
19. Pham DC, Truong DH, Tran QH, Ho QT, Nguyen TAD, Nguyen TNH, et al. Fractionation, identification of chemical constituents, and biological properties of cashew (Anacardium occidentale L.) leaf extracts. Food Sci Nutr. 2023;11(12).
20. Khan S, Mirza KJ, Anwar F, Abdin MZ. Development of RAPD markers for

authentication of Piper nigrum (L.) . Environmental International Journals of Science Technology . 2010;5:47–56.

21. Omoboyowa DA. Exploring molecular docking with E-pharmacophore and QSAR models to predict potent inhibitors of 14-α-demethylase protease from Moringa spp. Pharmacological Research - Modern Chinese Medicine. 2022;4.

22. Mgbeahuruike EE, Holm Y, Vuorela H, Amandikwa C, Fyhrquist P. An ethnobotanical survey and antifungal activity of Piper guineense used for the treatment of fungal infections in West-African traditional medicine. J Ethnopharmacol. 2019;229.

23. Mgbeahuruike EE, Fyhrquist P, Vuorela H, Julkunen-Tiitto R, Holm Y. Alkaloid-Rich Crude Extracts, Fractions and Piperamide Alkaloids of Piper guineense Possess Promising Antibacterial Effects. Antibiotics. 2018 Nov 9;7(4):98.

24. Ogunmefun OT, Akharaiyi FC, Adegunle SJ. Phytochemical and Antimicrobial Properties of Piper guineense (Shumach and Thonn) on Selected Human Pathogens. J Chem Pharm Res. 2020;9(11):180–6.

25. Chitlange SS, Payal BS, Sanjay D, Nipanikar S, Dheeraj N. Development and validation of RPHPLC method for quantification of piperine from single herb formulation containing Piper guineense extract. . International Journals of Pharmacological Science Revised. 2020;6(2):16–21.

26. Gao M, Wang H, Zhu L. Quercetin Assists Fluconazole to Inhibit Biofilm Formations of Fluconazole-Resistant Candida Albicans in In Vitro and in Vivo Antifungal Managements of Vulvovaginal Candidiasis. Cellular Physiology and Biochemistry. 2016;40(3–4).

27. Oliveira VM, Carraro E, Auler ME, Khalil NM. Quercetin and rutin as potential agents antifungal against Cryptococcus spp. Brazilian Journal of Biology. 2016 May 6;76(4):1029–34.

28. Singh BN, Upreti DK, Singh BR, Pandey G, Verma S, Roy S, et al. Quercetin Sensitizes Fluconazole-Resistant Candida albicans To Induce Apoptotic Cell Death by Modulating Quorum Sensing. Antimicrob Agents Chemother. 2015 Apr;59(4):2153–68.

29. Nejatbakhsh S, Ilkhanizadeh-Qomi M, Razzaghi-Abyaneh M, Jahanshiri Z. The Effects of Ellagic Acid on Growth and Biofilm Formation of Candida albicans. Journal of Medical Microbiology and Infectious Diseases. 2020 Jan 1;8(1):14–8.

30. G V, K MA, M KC, M RM, R S, H S, et al. REVIEW OF PLANTS USED AS KSHAR OF FAMILY PIPERACEAE. International Journal of Ayurvedic Medicine. 2010 Oct 9;1(2).

31. Sinsinwar S, Vadivel V. Catechin isolated from cashew nut shell exhibits antibacterial activity against clinical isolates of MRSA through ROS-mediated oxidative stress. Appl Microbiol Biotechnol. 2020;104(19).

32. Kusmiati, Ningsih EB, Ramadhani I, Amir M. Antibacterial and antioxidant activity test of crude lutein extracted from sunflower (Helianthus annuus L.). In: AIP Conference Proceedings. 2021.

33. Oladimeji AO, Karigidi KO, Yeye EO, Omoboyowa DA. Phytochemical composition, antioxidant and antimicrobial activities of essential oils extracted from Emilia coccinea (Sims) G. Don using in-vitro and in-silico approaches. Vegetos. 2024 Aug 28;

34. Monk BC, Sagatova AA, Hosseini P, Ruma YN, Wilson RK, Keniya M V. Fungal Lanosterol 14α-demethylase: A target for next-generation antifungal design. Vol. 1868, Biochimica et Biophysica Acta - Proteins and Proteomics. 2020.

35. Rosam K, Monk BC, Lackner M. Sterol 14α-Demethylase Ligand-Binding Pocket-Mediated Acquired and Intrinsic Azole Resistance in Fungal Pathogens. Journal of Fungi. 2020 Dec 22;7(1):1.

36. Rampogu S, Baek A, Zeb A, Lee KW. Exploration for novel inhibitors showing back-to-front approach against VEGFR-2 kinase domain (4AG8) employing molecular docking mechanism and molecular dynamics simulations. BMC Cancer. 2018 Dec 7;18(1):264.