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Identification of Phenolic Compounds in Conocarpus lancifolius and Hedera helix by High-Performance Liquid Chromatography and Evaluation of Antifungal Activity Against Pathogenic Fungal Isolates

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ABSTRACT: Because clinical isolates show variability in susceptibility and most commercial synthetic antifungals are associated with resistance, toxicity and recrudescence, management of opportunistic fungal infections due to *Candida* spp. and similar yeast pathogens will become increasingly challenging. Therefore, this research evaluated and compared the composition of phenolic components in *Conocarpus lancifolius* and *Hedera helix* leaf extracts by High Performance Liquid Chromatography (HPLC) and determined the antifungal potential of ethanol and butanol extracts of both plants against clinically significant fungal isolates. Phenols were extracted from dry leaves using an Ultrasonic-Assisted Extraction method and each compound was measured individually using reversed phase-HPLC at 280 nm. Clinical isolates were cultured from one hundred patient samples collected from urine, sputum, and skin and subsequently isolated on Sabouraud dextrose agar. Each isolate was identified through morphological characteristics, microscopy, and chromogenic *Candida* agar. Isolate susceptibility to each plant extract (concentration of 250, 500, 750, and 1000 mg/ml) was examined. Responses of gallic acid and quercetin in relation to minimum inhibitory concentrations against *C. albicans* and *C. tropicalis* were demonstrated by plotting Principal Component Analysis (PCA) plots. In total, rutin, gallic acid, ferulic acid, sinapic acid, p-coumaric acid, caffeic acid were found in higher levels in the ethanol extract of *C. lancifolius* while the ethanol extract of *H. helix* showed gallic acid, kaempferol, quercetin, apigenin, caffeic acid, rutin and ferulic acid depending upon the extracting solvents. A maximum zone of inhibition was observed in *C. albicans* with *C. lancifolius* ethanol extract and in *C. glabrata* with *H. helix* ethanol extract, each reaching a zone of 12mm; however, all *Talaromyces* isolates were consistently resistant. Based on these results it appears that the ethanol extracts containing phenolics from both plants could represent potential antifungal candidates and therefore require further purification, determination of the minimum inhibitory concentrations and evaluation of cytotoxicity.

INTRODUCTION

Fungal diseases that exploit compromised hosts' defenses and disrupt normal microbial balance are now being recognized as emerging public health issues worldwide. Fungi within the *Candida* genus have been specifically targeted for attention due to the severity of disease they cause in humans and the growing prevalence of resistant strains of *Candida* (Dantas et al., 2025; WHO, 2022). Typically, *Candida* species colonize the epithelial surfaces of healthy individuals but can develop into superficial

or invasive lesions if there is a disruption to the host's immune system or to the host's normal flora. Due to the widespread nature of this problem, we elected to focus primarily on the *Candida* genus during our research even though several other opportunistic yeast species were also studied concurrently.

Plant-derived secondary metabolite phenolic compounds have been identified as potentially effective natural products capable of disrupting fungal cell membrane function, interfering with enzyme function, reducing biofilm production and otherwise impeding growth (Shariati et al., 2022; Zhang et al., 2022). Previous studies have indicated that ethanol/hydro-alcoholic extracts typically contain a greater diversity of phenolic compounds active against *Candida* species than either water soluble or nonpolar extracts alone (Maželienė et al., 2025; Fakhim et al., 2025); however, the effectiveness of such extracts remains highly dependent on both the type of plant used as well as on the specific fungal isolate(s) studied (Alharbi et al., 2026; Tan et al., 2023).

Gallic acid has previously been demonstrated to promote apoptotic death in *C. albicans* cells via induction of mitochondrial dysfunction (Valderrama et al., 2024; Alharbi et al., 2026), while quercetin has been reported to inhibit adhesion/invasion/biofilm development in various strains of *Candida* (Tan et al., 2023; Rahbari et al., 2025) which have varying response profiles based on preparation method/strain identity.

Standard techniques employed for the identification/quantification/separation of single phenolic compounds include Reversed Phase High Performance Liquid Chromatography (RP-HPLC), where ultrasonically assisted extraction methods have also proven effective for the isolation of phenols/flavones from plant material (Khursheed et al., 2024; Yusuff et al., 2022).

Recent investigations have revealed that both *Hedera helix* and *Conocarpus* species contain biologically active compounds; nevertheless the majority of the antifungal data published on these plants are fragmentary in nature and depend upon plant part/species investigated (Salim et al., 2025; Qabaha et al., 2025).

Therefore the purpose of this project was to provide a head-to-head comparative assessment of the phenolic content and antifungal activity of extracts prepared from both *C. lancifolius* and *H. helix* in two different solvents (ethanol/butanol) against locally derived clinically relevant fungal pathogens. We proposed that phenolic constituents would be recovered in extracts from both plants and that activity against fungal pathogens would be exhibited in a manner dependent upon both plant species and concentration.

MATERIALS AND METHODS

Study Design and Plant Material

The study employed an in vitro design. Leaves of *Conocarpus lancifolius* and *Hedera helix* were taken, washed, dried, and ethanolic and butanic extracts made for use in assessing their antifungal activities at 4 different concentration levels: 250 mg/mL, 500 mg/mL, 750 mg/mL, and 1000 mg/mL. Additionally, ethyl acetate fractions were generated for HPLC analyses to identify phenolic compounds.

Extraction of Phenolic Compounds

To remove fat from homogenized plant material, 100 ml of chloroform were added to ten grams of ground sample. The mixture was shaken for three hours. Following removal of the chloroform layer, the sample was placed into a drying oven at 50°C and heated to dry all remaining solvent. Ten grams of defatted plant material were then subjected to extraction of either ethanol or butanol by placing them in an ultrasonic bath at room temperature for one hour according to the method described in Yusoff et al. (2022) and El Baakili et al. (2023). The resultant extracts were filtered through Whatman No. 41 filter paper, evaporated using a rotary evaporator at low pressure, dried to constant weight in a drying oven at 40°C, and stored at 4°C in glass containers prior to analysis.

HPLC Analysis

Phenolic compounds were isolated from individual extracts via reversed-phase HPLC. An SYKAM chromatographic system equipped with a UV detector and a C18-ODS column (25cm x 4.6mm) was used along with a pump. The column temperature was maintained at 30°C. A mobile phase composed of methanol (A) and 1% formic acid (B) was utilized in conjunction with the following elution profile: 40% B over 6 minutes, followed by a gradual increase to 50% B over 9

minutes. Flow rates were 1mL/minute. Injection volumes were 100µl. Detection was conducted at 280nm using previously established reversed-phase methods for extracting phenolics from plants (Chaharmiri-Dokhaharani et al., 2024; Susanti et al., 2024).

Clinical Fungal Isolates

Samples of clinical origin consisted of skin swab (n = 16), urine (n = 40), and sputum (n = 44) samples from patients admitted to teaching hospitals located in Nasiriyah City (Nasiriyah Teaching Hospital, Al-Hussein Teaching Hospital, and Chest Diseases Hospital). Samples were collected from these sources and transported to the laboratory where they were cultured onto Sabouraud dextrose agar. Each sample was subsequently preserved under refrigeration in a culture medium that had been supplemented with glycerol. The isolation and preservation of fungi was completed consistent with accepted practices in mycology (Abdulla & Ismael, 2023).

Identification and Assessment of Antifungal Activity of Pathogens

Species of pathogenic fungi were identified by evaluating colony morphology, microscopic evaluation, and chromogenic Candida agar. For the purpose of assessing antifungal activity, extracts were evaluated against several pathogens including *Candida tropicalis*, *Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, *Trichosporon*, *Talaromyces*, *Pichia kudriavzevii*, and *Nakaseomyces glabratus*. Zone diameter of inhibition was measured in millimeters. Each extract-concentration combination was tested in duplicate.

Data Statistical Analysis

Means ($\pm$ SD) and maxima for zone diameter of inhibition for each extract-isolate combination were determined. Means were grouped utilizing Tukey's multiple comparisons test and results are presented as letters indicating grouping. Concentration of individual phenols was determined based on peak area relative to that of authentic reference compound(s). Concentration is expressed as parts per million. Patterns of response to gallic acid and quercetin exhibited by *C. albicans* and *C. tropicalis* were interpreted via principal components analysis (PCA). All data handling, peak integration using HPLC software, determination of groupings using Tukey's tests and plotting using PCA, were accomplished using Microsoft Excel software version 2019 and IBM SPSS Statistics (Version 26).

RESULTS

Results for this project were developed with respect to the three research objectives: (i) identifying phenolic compounds, (ii) evaluating the antifungal properties of plant extracts and (iii) interpreting the MIC response patterns of selected phenolics using Principal Components Analysis (PCA).

Identification of Phenolic Compounds

C. lancifolius's phenolic compound content varied according to extraction solvent. All six phenols identified were found in the ethanol extract. Rutin was the largest amount detected (187.4 ppm), while gallic acid was second largest (166.0 ppm). Ferulic acid, sinapic acid, p-coumaric acid and caffeic acid were all found in smaller amounts. Compared to ethanol extracts, the butanol extracts had lower overall levels of phenolics as well as missing ferulic and sinapic acids. Ethyl acetate extracts did not contain p-coumaric acid (see Table 1).

Hedera helix also produced a solvent dependent phenolic composition. High levels of gallic acid were found in all three solvents. However, quercetin and apigenin were not found in the butanol extracts (see Table 2). In general, ethanol recovery yielded the most complete complement of phenolic compounds for each of these two species.

Table 1. Phenolic compounds in *Conocarpus lancifolius* extracts (ppm).

Compound	Ethanol	Butanol	Ethyl acetate
Rutin	187.4	98.9	68.7
Ferulic acid	141.0	—	95.0
Caffeic acid	85.9	77.5	90.6
Gallic acid	166.0	80.3	74.8

Sinapic acid	130.3	—	55.6
p-coumaric acid	124.1	62.3	—

The highest rutin and gallic acid concentrations, together with the widest phenolic range, were obtained with ethanol from *C. lancifolius*, indicating its greater expected antifungal potential.

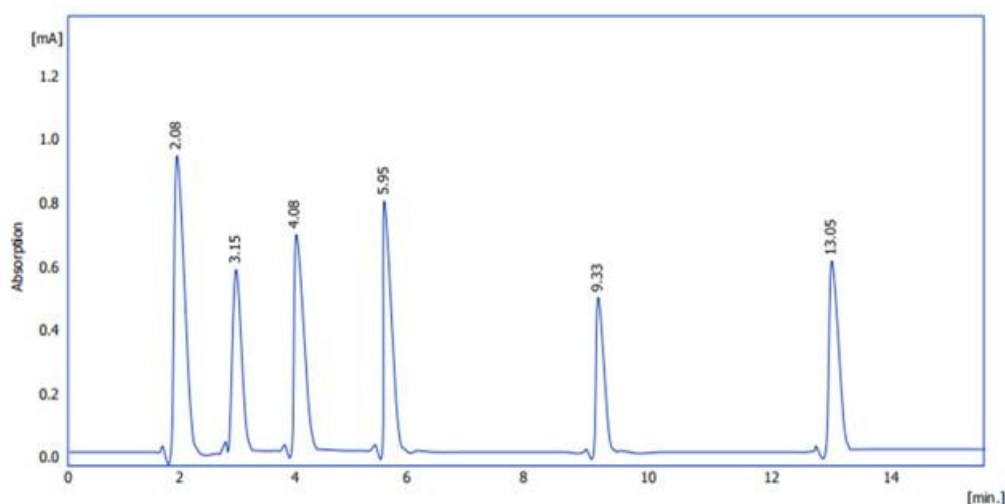


Figure 1. Chromatogram from an HPLC analysis of *Conocarpus lancifolius* ethanol extract which was run on a SYKAM system utilizing a 25 cm X 4.6 mm C18-ODS Column at 30° C with a Methanol / 1 % Formic Acid gradient at 1 ml/min, detected by U.V. at 280nm. Phenolics were present as discrete peaks each having their own unique retention time within the chromatogram. This confirmed that the sample is comprised entirely of six major components each differing in polarity and relative amounts. As shown in figure 1, the peak with the largest concentration/absorbance (Peak #2 @ 2.08) represents the largest amount of the component(s) of interest; whereas the smallest absorbance (peak #13 @ 13.05) represents the least amount of this compound(s).

Table 2. Phenolic compounds in *Hedera helix* extracts (ppm).

Compound	Ethanol	Butanol	Ethyl acetate
Rutin	—	66.3	114.2
Ferulic acid	90.2	—	—
Caffeic acid	69.8	92.5	66.0
Gallic acid	157.8	124.6	132.5
Quercetin	118.7	—	74.1
Apigenin	81.4	—	92.5
Kaempferol	133.2	74.9	50.6

The extraction solvents used yielded gallic acid in all fractions from *H. helix*. Quercetin and apigenin were recovered mostly in the ethanol and ethyl acetate fractions, respectively.

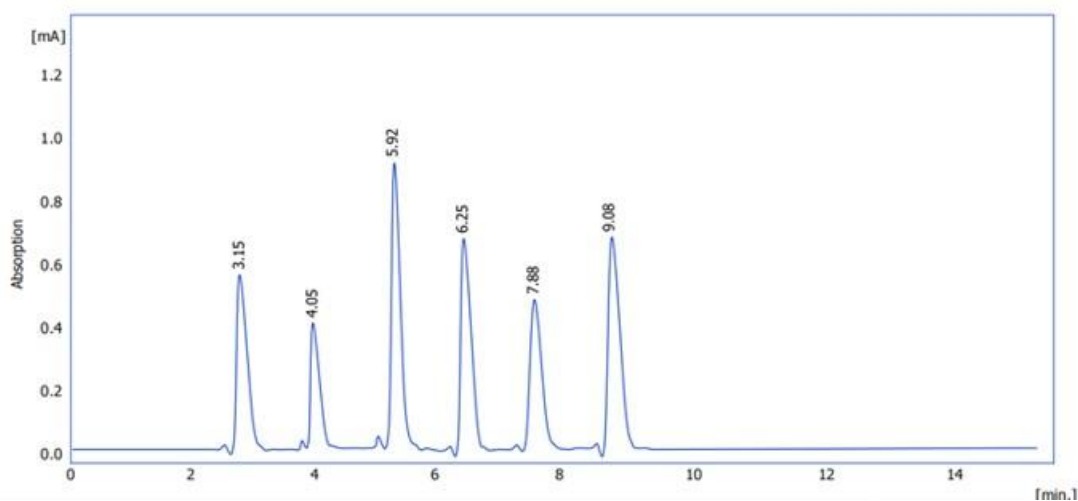


Figure 2. HPLC chromatogram of the ethanol extract of *Hedera helix*, using the same instrument conditions as those described in figure 1. A multi-component profile of phenols is evident in the form of several distinct chromatographic peaks whose areas and retention times are dependent on the solvent being used

Six major compounds were identified through this chromatography; the compound eluting at 5.92 min represented the majority component of the mixture (the compound with the greatest concentration), while the compound eluting at 3.15 min represented the most polar component and the compound eluting at 9.08 min represented the least polar component. Antifungal Activity of Plant Extracts Species-specific, concentration-specific inhibition patterns were seen among all species examined in our study. The highest total response was from the ethanol extract of *C. lancifolius* (9.47 ± 1.95 mm against *C. albicans* and 8.50 ± 2.02 mm against *P. kudriavzevii*) which had an upper limit of 12 mm inhibition against *C. albicans* when tested at 1000 mg/mL.

The *C. lancifolius* butanol extract exhibited lower overall activity than did its ethanol counterpart, however it still demonstrated a 10 mm diameter zone against *P. kudriavzevii* when tested at 750 mg/mL. The ethanol extract of *H. helix* demonstrated significant activity against three different species of fungi, including the largest single measurement of 12 mm inhibition against *C. glabrata* when tested at 500 mg/mL. The butanol extract of *H. helix* showed a narrow range of antifungal activity primarily targeting *C. albicans*, *C. parapsilosis*, and *N. glabratus*. None of the extracts examined inhibited *Talaromyces*.

Table 3. Summary data demonstrating antifungal activity of plant extracts against clinical isolates of fungi, as measured by inhibitory zones (mean $\pm$ SD values, max values of two measurements per each sample per four concentrations: 250 mg/mL, 500 mg/mL, 750 mg/mL, and 1000 mg/mL).

Extract	Fungal isolate	Mean $\pm$ SD (mm)	Maximum (mm)
<i>C. lancifolius</i> ethanol	<i>C. tropicalis</i>	3.75 ± 4.10	9.00
<i>C. lancifolius</i> ethanol	<i>C. albicans</i>	9.47 ± 1.95	12.00
<i>C. lancifolius</i> ethanol	<i>C. parapsilosis</i>	6.62 ± 0.99	7.50
<i>C. lancifolius</i> ethanol	<i>C. glabrata</i>	7.06 ± 1.47	8.50
<i>C. lancifolius</i> ethanol	<i>Trichosporon</i>	5.50 ± 1.13	6.50
<i>C. lancifolius</i> ethanol	<i>Talaromyces</i>	0.00 ± 0.00	0.00
<i>C. lancifolius</i> ethanol	<i>P. kudriavzevii</i>	8.50 ± 2.02	11.00
<i>C. lancifolius</i> ethanol	<i>N. glabratus</i>	6.12 ± 0.95	7.00

<i>C. lancifolius</i> butanol	<i>C. tropicalis</i>	0.00 ± 0.00	0.00
<i>C. lancifolius</i> butanol	<i>C. albicans</i>	7.28 ± 0.66	8.50
<i>C. lancifolius</i> butanol	<i>C. parapsilosis</i>	4.38 ± 0.92	6.00
<i>C. lancifolius</i> butanol	<i>C. glabrata</i>	6.94 ± 0.62	7.50
<i>C. lancifolius</i> butanol	<i>Trichosporon</i>	0.00 ± 0.00	0.00
<i>C. lancifolius</i> butanol	<i>Talaromyces</i>	0.00 ± 0.00	0.00
<i>C. lancifolius</i> butanol	<i>P. kudriavzevii</i>	5.31 ± 4.50	10.00
<i>C. lancifolius</i> butanol	<i>N. glabratus</i>	6.56 ± 0.32	7.00
<i>H. helix</i> ethanol	<i>C. tropicalis</i>	1.56 ± 2.90	6.50
<i>H. helix</i> ethanol	<i>C. albicans</i>	8.25 ± 0.85	9.50
<i>H. helix</i> ethanol	<i>C. parapsilosis</i>	8.25 ± 0.53	9.00
<i>H. helix</i> ethanol	<i>C. glabrata</i>	3.62 ± 5.07	12.00
<i>H. helix</i> ethanol	<i>Trichosporon</i>	6.75 ± 0.89	8.00
<i>H. helix</i> ethanol	<i>Talaromyces</i>	0.00 ± 0.00	0.00
<i>H. helix</i> ethanol	<i>P. kudriavzevii</i>	5.56 ± 3.52	8.50
<i>H. helix</i> ethanol	<i>N. glabratus</i>	6.38 ± 0.79	7.00
<i>H. helix</i> butanol	<i>C. tropicalis</i>	0.00 ± 0.00	0.00
<i>H. helix</i> butanol	<i>C. albicans</i>	7.75 ± 0.67	8.75
<i>H. helix</i> butanol	<i>C. parapsilosis</i>	7.25 ± 0.65	8.00
<i>H. helix</i> butanol	<i>C. glabrata</i>	1.38 ± 1.60	4.00
<i>H. helix</i> butanol	<i>Trichosporon</i>	0.00 ± 0.00	0.00
<i>H. helix</i> butanol	<i>Talaromyces</i>	0.00 ± 0.00	0.00
<i>H. helix</i> butanol	<i>P. kudriavzevii</i>	0.00 ± 0.00	0.00
<i>H. helix</i> butanol	<i>N. glabratus</i>	6.69 ± 0.80	8.00

Table 4. The best results from all the different extract-isolate combinations with the highest concentration (in mg/mL) that produced the largest antifungal Inhibition Zone (IZ) in relation to each individual isolate shown in Figure 1.

Extract	Most inhibited isolate	Concentration (mg/mL)	Maximum zone (mm)
<i>C. lancifolius</i> ethanol	<i>C. albicans</i>	1000	12.00
<i>C. lancifolius</i> butanol	<i>P. kudriavzevii</i>	750	10.00
<i>H. helix</i> ethanol	<i>C. glabrata</i>	500	12.00
<i>H. helix</i> butanol	<i>C. albicans</i>	500	8.75

The highest inhibition zone (12 mm) against *Candida* isolates was obtained with the ethanol extracts of *C. lancifolius* and *H. helix*.

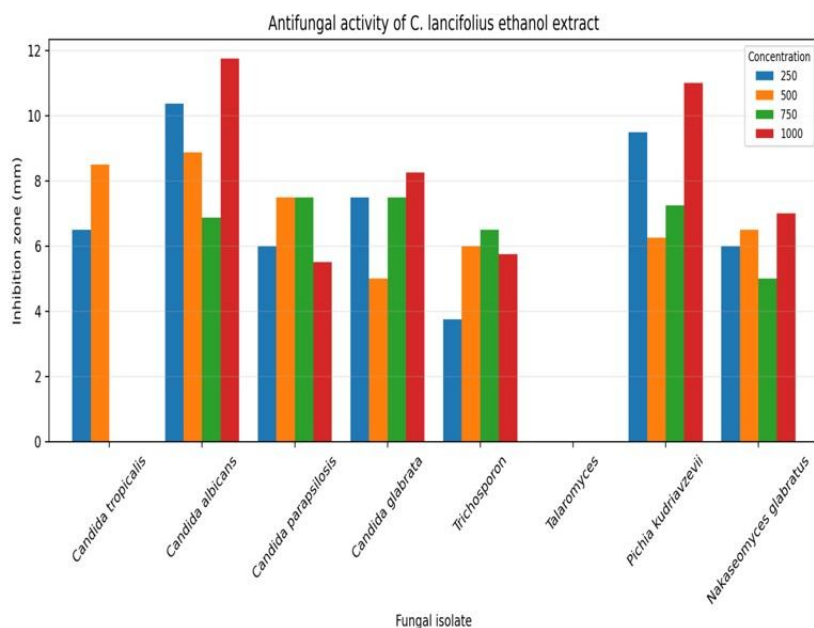


Figure 3. Antifungal inhibitory activities of an ethanol extract from *Conocarpus lancifolius* on the same eight fungi at concentrations of 250, 500, 750 and 1000 mg/mL.

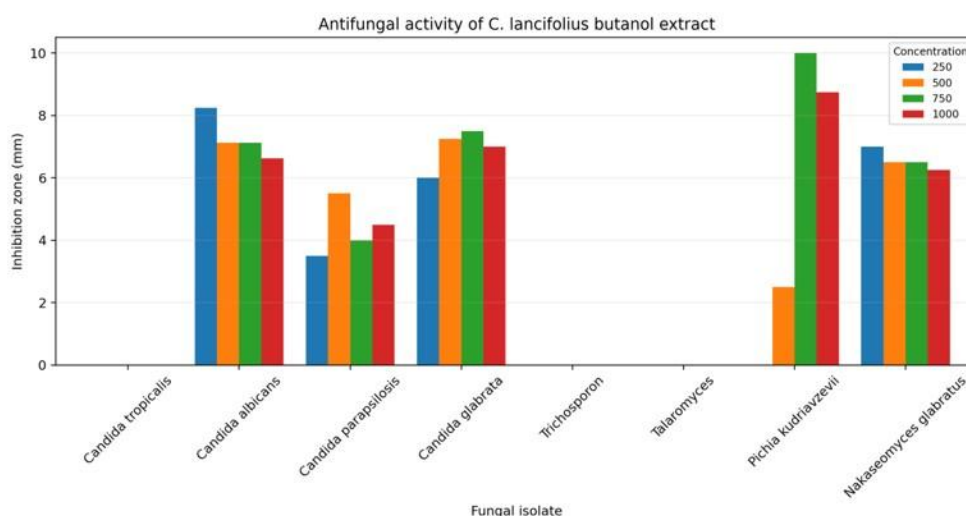


Figure 4. Spectrum of antifungal activities of the butanol extract of *Conocarpus lancifolius* (at concentrations 250; 500; 750; and 1000 mg/mL).

The butanol extract showed a reduced spectrum of activity as compared to the ethanol extracts. Good levels of inhibition were shown in the case of *C. albicans*, *C. glabrata*, *N. glabratus*, and *P. kudriavzevii*.

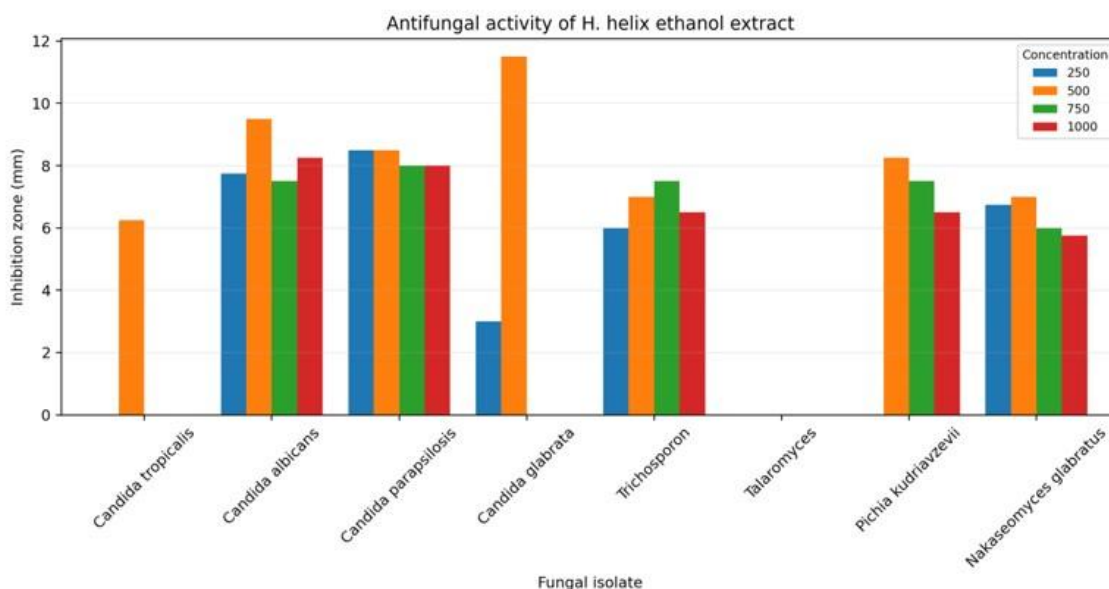


Fig. 5. Spectrum of antifungal activities of the ethanol extract of *Hedera helix* (at concentrations 250; 500; 750; and 1000 mg/mL).

Hedera helix (the ethanol extract) inhibited good numbers of strains of fungi including *C. albicans*, *C. parapsilosis*, and *C. glabrata*, however no inhibitory effects were recorded for *Talaromyces*.

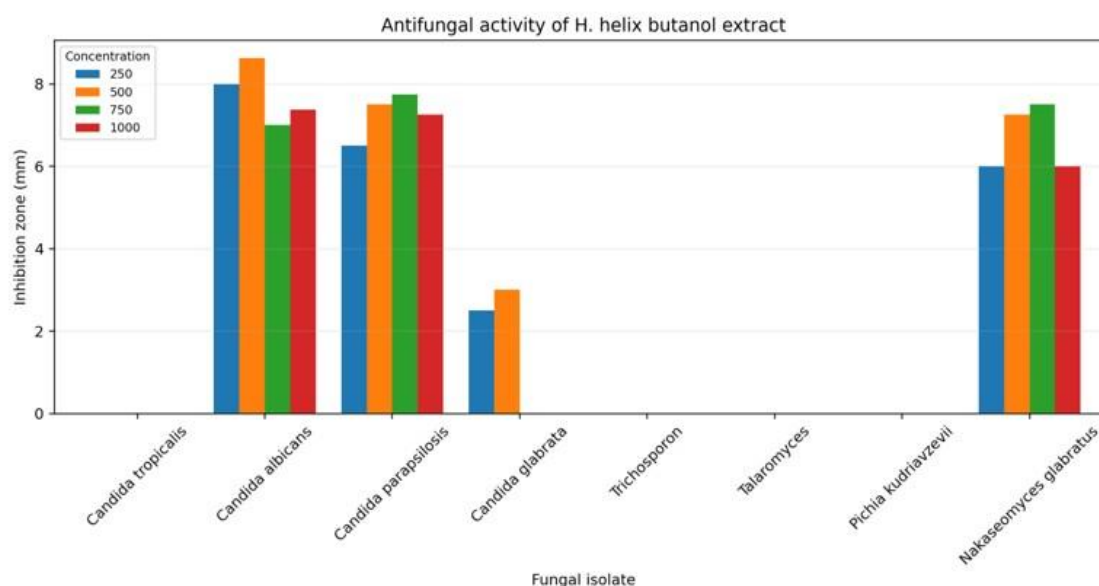


Fig. 6. Antifungal activity of the butanol extract from *Hedera helix* (at 250, 500, 750, 1000 mg/mL).

The butanol extracts showed an even narrower spectrum of antifungal activities than the gallic acid or quercetin alone with main effects on *Candida albicans*, *Candida parapsilosis*, and *Neosartorya glabrata*.

Interpretation of MIC-response patterns through PCA

PCA of the gallic acid and quercetin data sets demonstrated variability among the tested isolates. Isolate clusters for *C. albicans* indicated some level of similar susceptibility/ resistance profiles among the majority of the isolates tested; however, isolates outside these clusters exhibited different sensitivities to the same compound as the concentration increased. In contrast, there were greater levels of isolate specificity observed when examining the distributions of *C. tropicalis* indicating

isolate specific responses to the compounds examined. The lengths of the concentration vectors illustrated which concentrations of each compound resulted in the greatest contribution to isolate separation in the PCA plots.

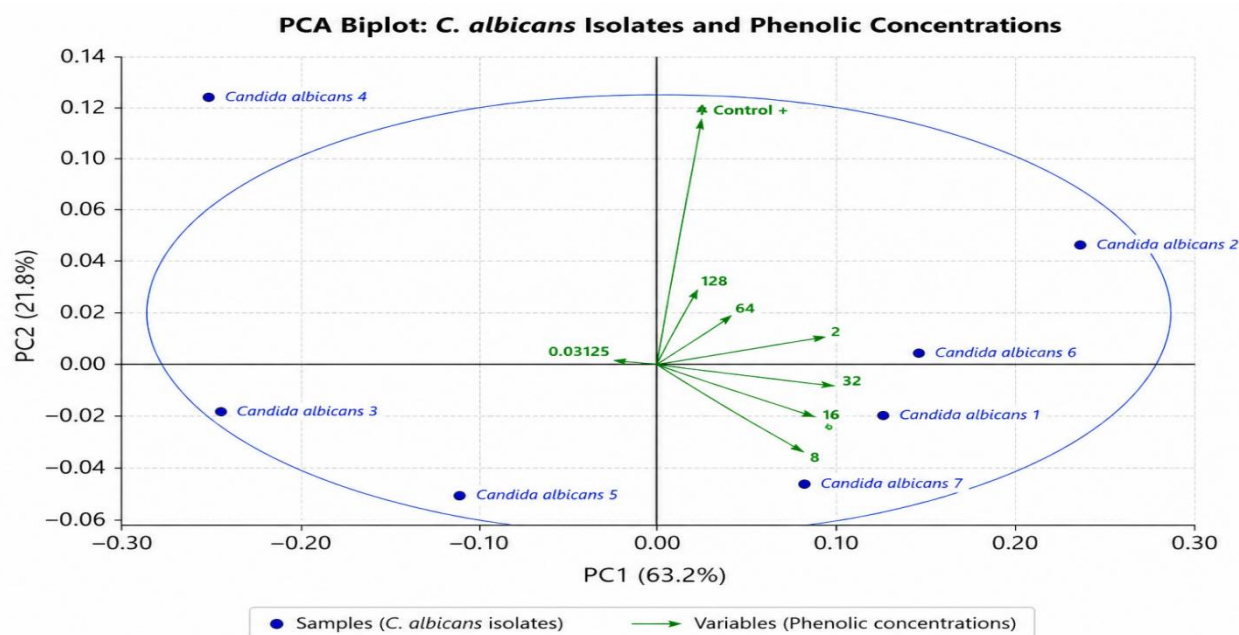


Figure 7: Representation of *Candida albicans* isolate responses to gallic acid (Gallic Acid/GA), through the use of Principal Component Analysis/Minimum Inhibitory Concentration (PCA/MIC) of gallic acid. Clustering of isolates indicate the major sensitivity profiles for *Candida albicans* isolates, while outlier isolates can reflect differences within sensitivity or resistance of *Candida albicans* to the action of gallic acid.

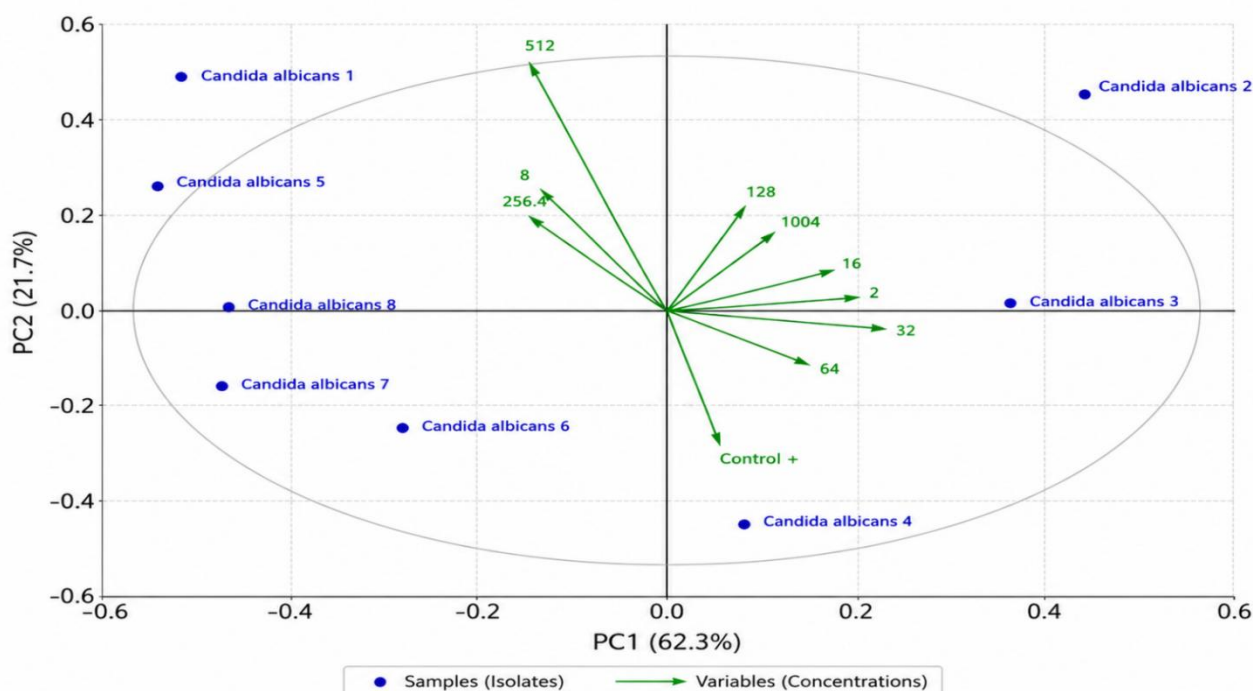


Figure 8. Response of *Candida albicans* isolates to quercetin. Variation in response to quercetin was observed among isolates as well as with regard to the concentration of quercetin tested. This observation indicates a possible existence of genetic/functional variations (phenotypic variability) among the *Candida albicans* isolates. Isolates (1, 5, 6, 7, 8) were sensitive

at lower levels than isolates (2, 3, 4). It is possible that there are differences in the resistance/susceptibility profiles for these compounds among the *Candida albicans* isolates. Isolate #4 was most like the Control + condition; isolate #3 showed higher sensitivity/susceptibility to the lower to moderate level quercetin concentrations.

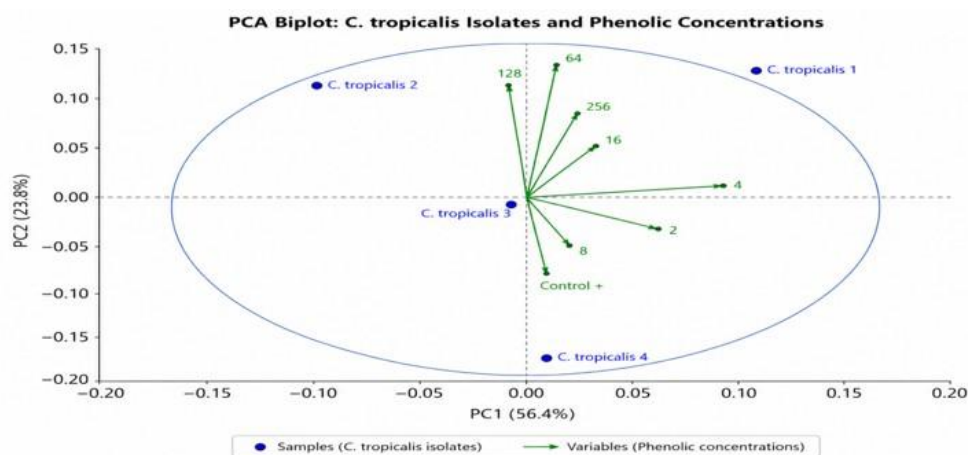


Figure 9: PCA/MIC represents how *Candida tropicalis* yeast isolates respond to gallic acid. The diagram shows the individual patterns of each isolate's response to the phenolics. It was observed that there is no uniform sensitivity of all *C. tropicalis* isolates to the same concentration of gallic acid; instead, the isolates were separated into different quadrants on the PCA/MIC diagrams, indicating variations in their responses to gallic acid. Effect of high concentrations: In addition, it can be inferred from the data presented in figure 9 that two isolates (isolates 1 & 2) show distinct behaviors towards medium to high phenolic concentrations (i.e. 64, 128, 256); possibly indicative of either differing modes of resistance or metabolism to cope with high levels of phenolic stress. Similarity to the control: Similarly, the behavior of *C. tropicalis* 4 (the isolated strain) was found to relate closely to that of the positive control (Control +) and low concentrations of gallic acid, suggesting that the growth or activity of this strain are comparable to those of the control group under normal conditions and that high concentrations of gallic acid have a severe impact on both strains' growth/activity. Furthermore, it appears that the higher concentrations (particularly 128, 64, and 256) exhibit greater effectiveness in producing an identifiable separation between the isolates as measured by the lengths of their respective arrows; therefore, they represent the most significant and effective concentrations used in order to study the biological or inhibitory effects of these phenolic compounds.

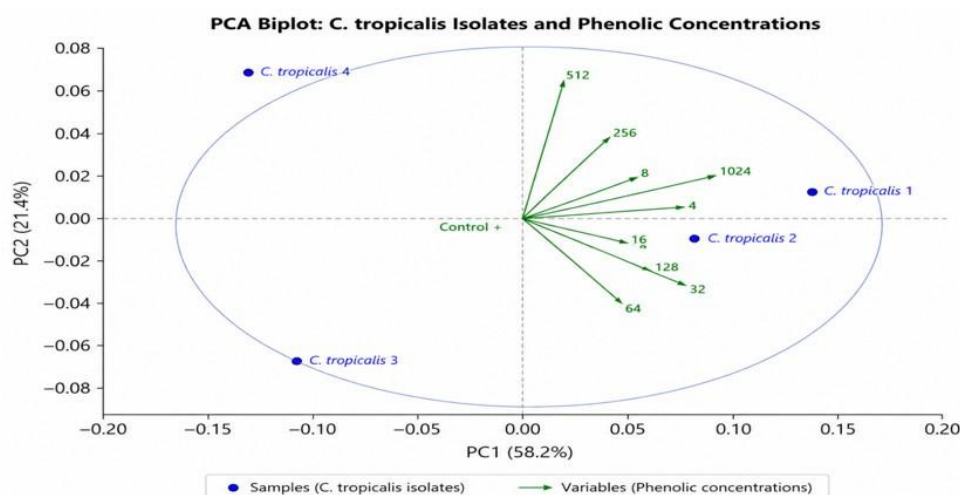


Figure 10: This paper presents an evaluation of the inhibitory effects of quercetin on *Candida tropicalis* isolates through a principal component analysis / minimum inhibitory concentration (PCA/MIC) approach. The study demonstrated significant separations among several isolates using a PCA plot suggesting differing sensitivities of these isolates toward varying levels of quercetin.

A considerable difference exists in the level of sensitivity/behavior of *C. tropicalis* yeast isolates to phenolic concentrations. Isolates 1 and 2 demonstrate a high degree of susceptibility to phenolic compounds especially when exposed to higher levels of quercetin. Conversely, isolates 3 and 4 display little to no sensitivity to phenolic compounds as well as minimal or no inhibition of growth similar to that seen in the negative control.

DISCUSSION

The data presented within this study aligns with the experimental design utilized in this investigation. Chemical composition of the plant extracts was identified via High-Performance Liquid Chromatography (HPLC), biological activity was assessed using inhibition zone assays and responses of individual yeast isolates were analyzed using Principal Component Analysis (PCA) plots. Chemistry First/Biology Second methodology has been suggested as one method for evaluating phenolic rich plant extracts against *Candida* (Chaharmiri Dokhaharanai et al. 2024; Susanti et al. 2024).

Statistical analyses used to interpret the data in the text included Mean $\pm$ Standard Deviation (SD) values, Maximum Inhibition Zone (MIz) measurements and Tukey Grouping classifications illustrated in Tables 3 & 4.

It was clear that the largest effect occurred with the ethanol extracts. A broad range of phenolics was found in the ethanol extract of *C. lancifolius* -- specifically rutin and gallic acid, whereas an even greater number of phenolics were detected in the ethanol and ethyl acetate extracts of *H. helix* including rutin, gallic acid, quercetin, apigenin, and kaempferol. Phenolic acids and flavonoids can cause alterations in membrane permeability of fungi, disorganization of membranes, inactivation of enzymatic processes, generation of reactive oxygen species (ROS), and disruption of biofilm formation (Shariati et al. 2022; Zhang et al. 2022; Alharbi et al. 2026).

These mechanisms help to explain why *C. lancifolius* ethanol extract exhibited strong antifungal activity against *C. albicans* and *P. kudriavzevii* (Tables 3 & Fig. 3) as well as why *H. helix* ethanol extract had strong antifungal activity against *C. albicans*, *C. parapsilosis*, and *C. glabrata* (Tables 4 & Fig. 5). Differences in susceptibility among *Candida* spp. are probably due to variations in cell wall architecture, efflux capabilities, stress response pathways and biofilm behavior (Dantas et al. 2025).

Chemical characterization of the extracts using HPLC (Figs. 1 & 2) confirmed the presence of multiple peaks at different retention times demonstrating chemical heterogeneity of each extract. Variability in inhibition vs. concentration curve shape in the antifungal assays (Figs. 3 – 6) may be attributed to limitations inherent in agar diffusion techniques, variable solubility of compounds in solution, potential competitive interactions between compounds within crude mixtures and/or tolerance among isolates as described in similar studies (Sateriale et al. 2025; Fakhim et al. 2025). These hypotheses were supported by PCA plots (Figs. 7 – 10), which separated isolates based upon their response profiles to gallic acid and quercetin with a few outlier isolates exhibiting characteristics indicative of increased resistance to these compounds.

The results of this study support many previous studies concerning the anti-*Candida* and antibiofilm activities of various phenolic/flavonoid compounds. Gallic acid has been reported to induce apoptosis-like cell death and mitochondrial dysfunction in *C. albicans* (Liberato et al. 2022; Valderrama et al. 2024; Zheng et al. 2024) and is consistent with the potent antifungal activity displayed by the gallic acid-rich ethanol extract of *C. lancifolius*. Quercetin has also been reported to inhibit adherence/invasion/biofilm formation by *C. albicans* (Tan et al. 2023; Rahbari et al. 2025), providing additional evidence for the relevance of the quercetin-containing fractions derived from *H. helix*. Generally, ethanol extracts will contain a wider variety of phenols compared to polar/non-polar or water-based extracts thereby resulting in a larger overall inhibitory spectrum (Maželienė et al. 2025; Jaiswal et al. 2022). Any discrepancies with past studies are most likely due to differences in fungal species being evaluated, inoculum density, extraction methods/preparative procedures and source material/plant parts.

In summary, this study fills a void within the scientific literature by establishing direct correlations between the phenolic content and antifungal activity in two plant species that have historically received limited comparison (Salim et al. 2025; Qabaha et al. 2025). Demonstrated inhibitory action against clinical fungal isolates utilizing both extracts combined with identification of the specific phenolics contributing to this activity addresses the primary objective posed within this research effort. The greatest activity was associated with the ethanol extracts particularly against *C. albicans* and other *Candida* spp. (Alharbi et al. 2026; Dantas et al. 2025). Practically speaking, the combination of plant/solvent systems identified in this study represent the top candidate for subsequent confirmatory MIC determinations, purification/identification of active components, cytotoxicity evaluations and potential formulation development.

CONCLUSION

Ethanol extracts from *Conocarpus lancifolius* and *Hedera helix* exhibit a wide array of phenolic compounds and produce species-specific antifungal activities against clinical yeast isolates with *Talaromyces* showing complete resistance across all isolates examined. Therefore, it appears that ethanol represents the best option for continued research with both plant species and that gallic acid and quercetin represent the first priority phenolics for subsequent confirmatory MIC evaluations, cytotoxicity assessments and examinations into mechanisms of action prior to consideration for therapeutic applications

REFERENCES

- [1] Abdulla, N.Q.F.; Ismail, H.M. (2023). The efficiency of antifungal drugs and plant extracts against *Candida albicans* derived from women with vulvovaginitis. *Iraqi Journal of Science*. 64(2): 560–572. <https://doi.org/10.24996/ij.s.2023.64.2.6>
- [2] Akhter, N.; Mannan, M.A.; Pandey, D.; Sarkar, A.; Sharma, H.; Kumar, M.; Ghosh, A. (2023). Gallic acid is a strong inhibitor of *Candida auris* through *Sarcochlamys pulcherrima*. *BioTechnologia*. 104(2): 105–119. <https://doi.org/10.5114/bta.2023.127202>
- [3] Akwongo, B.; Kakudidi, E.K.; Nsubuga, A.M.; Andama, M.; Namaganda, M.; Tugume, P.; Asiimwe, S.; Anywar, G.; Katuura, E. (2024). Antifungal activity of plant extracts used to treat candidiasis in Pader District of Northern Uganda. *Tropical Medicine and Health*. 52: Article 84. <https://doi.org/10.1186/s41182-024-00628-x>
- [4] Alharbi, H.O.A.; Sarwar, T.; Rahmani, A.H. (2026). Gallic acid: Therapeutic potential in pathogenesis management — A narrative review. *International Journal of Molecular Sciences*. 27(3): 1536. <https://doi.org/10.3390/ijms27031536>
- [5] Bhairavi, V.A.; Vidya, S.L.; Sathishkumar, R. (2023). Identification of effective plant extracts against candidiasis using in silico and in vitro approaches. *Future Journal of Pharmaceutical Sciences*. 9: Article 38. <https://doi.org/10.1186/s43094-023-00489-x>
- [6] Centers for Disease Control and Prevention. (2024). Candidiasis basics. <https://www.cdc.gov/candidiasis/about/index.html>
- [7] Chaharmiri Dokhaharani et al. (2024). Potent antifungal activity of Mazuj and Ghalghaf gall extracts against three *Candida* species and compositional analysis using HPLC-DAD and LC-ESI-MS/MS. *Journal of Medicinal Plants and Byproducts*. 13(Special): 747–758. <https://doi.org/10.22034/jmpb.2023.363209.1589>
- [8] Cho et al. (2026). Synergistic effects of botanical extracts on the growth inhibition of *Candida albicans*. *PLOS ONE*. 21(1): e0340665. <https://doi.org/10.1371/journal.pone.0340665>
- [9] Crisóstomo et al. (2024). Cell wall-mediated antifungal activity of the water extract of *Hedera helix* leaves against *Diplodia corticola*. *Antibiotics*. 13(12): 1116. <https://doi.org/10.3390/antibiotics13121116>
- [10] Dantas et al. (2025). Bioactive compounds of plants as alternatives to antifungals in *Candida* strains. *Pharmaceutics*. 17(6): 687. <https://doi.org/10.3390/pharmaceutics17060687>
- [11] El Baakili et al. (2023). Ultrasonic-assisted extraction of phenolic compounds and antioxidant activity of Moroccan *Retama sphaerocarpa* L. leaves: Optimization of extraction parameters by response surface methodology and characterization of phenolic compounds by HPLC/ESI-MS analysis. *Heliyon*. 9: e17168. <https://doi.org/10.1016/j.heliyon.2023.e17168>
- [12] Fakhim et al. (2025). Polyphenolic compounds inhibiting fluconazole-sensitive and resistant *Candida* strains. *Iranian Journal of Microbiology*.
- [13] Jaiswal et al. (2022). Phenolic compounds from plant sources: Review on their extraction and biological activities. *Molecules*. 27(6): 1935.
- [14] Khursheed et al. (2024). Ultrasound assisted extraction of phyto constituents: Comprehensive review. *Ultrasonics Sonochemistry*. 104: 106832. <https://doi.org/10.1016/j.ultsonch.2024.106832>

- [15] Lee et al. (2024). Quercetin induces membrane damage and apoptosis-like cell death in *Candida albicans*. *Journal of Microbiology*. 62: 233–244.
- [16] Liberato et al. (2022). Gallic acid inhibited biofilm formation and induced oxidative stress-related mitochondrial damage in *Candida* species. *Frontiers in Cellular and Infection Microbiology*. 12: Article 823238.
- [17] Mažlienė, Ž.; Bakutis, B.; Kaškonienė, V.; Kazlauskaitė, J.A.; Ramanauskienė, K.; Bendikienė, V. (2025). Comparative antifungal activity of ethanolic, hydroethanolic and aqueous extracts of selected medicinal plants against clinical *Candida* strains. *Molecules*. 30(5): 1024.
- [18] Naorungroj, S.; Bahtiar, A.A.; Pinweha, S.; Gonwong, S. (2025). Global trends in *Candida* epidemiology and clinical antifungal susceptibility over the last decade. *Journal of Fungi*. 11(4): 220.
- [19] Qabaha, K.; Abbadi, J.; Al-Rimawi, F. (2025). Molecular insight into the anti-inflammatory efficacy and HPLC analysis of *Hedera helix* leaf extract. *African Health Sciences*. 25(2): 330–342. <https://doi.org/10.4314/ahs.v25i2.39>
- [20] Rahbari, F.; Shams-Ghahfarokhi, M.; Baheiraei, N.; Razzaghi-Abyaneh, M. (2025). Effects of quercetin nanoemulsions encapsulated with beta-cyclodextrin/succinate on biofilm formation and *als3* gene expression in drug resistant *Candida albicans*. *Journal of Drug Delivery Science and Technology*. 114(Part A): Article ID 107451.
- [21] Salim, A.; Arasteh, A.A.; Sahrish, R.; Labash, D.; El-Keblawy, A.A.; Gad, H.A.; Ashmawy, N.S. (2025). Metabolic profiling and biological evaluation of essential oils from *Conocarpus* species: Antidiabetic activity, antioxidant properties and antibacterial potential. *Plants*. 14(3): 464. <https://doi.org/10.3390/plants14030464>
- [22] Sateriale, D.; Forgione, G.; Raucci, S.; Jabbar, A.; Imperatore, R.; Germinario, C.; Pagliuca, C.; Colicchio, R.; Vitiello, M.; Mercurio, M.; Paolucci, M.; Salvatore, P.; Pagliarulo, C. (2025). Sustainable strategies to combat biofilms: An eco-friendly approach using agri-waste derived pomegranate extract as a natural antifungal agent against emerging *Candida* pathogens. *Frontiers in Microbiology*. 16: Article ID 1724685. <https://doi.org/10.3389/fmicb.2025.1724685>
- [23] Shariati, A.; Didehdar, M.; Razavi, S.; Heidary, M.; Soroush, F.; Chegini, Z. (2022). Natural compounds: Promising agents for the treatment of *Candida* biofilms. *Frontiers in Pharmacology*. 13: 917787. <https://doi.org/10.3389/fphar.2022.917787>
- [24] Susanti, I.; Pratiwi, R.; Rosandi, Y.; Hasanah, A.N. (2024). Separation methods of plant extract phenolic compounds as candidate antioxidant agents. *Plants*. 13(7): 965. <https://doi.org/10.3390/plants13070965>
- [25] Tan, Y.; Lin, Q.; Yao, J.; Zhang, G.; Peng, X.; Tian, J. (2023). Quercetin's effects on *Candida albicans* planktonic cells and biofilm cells in vitro and vulvovaginal candidiasis in vivo. *Phytomedicine*. 113: 154800. <https://doi.org/10.1016/j.phymed.2023.154800>
- [26] Valderrama, V.; Sanchez, P.; Delso, M.; Diaz-Dosque, M.; Escobar, A.; Budini, M.; Catalán, M.; Vivar, R.; Lopez-Munoz, R.; Jara Ja.; Molina-Berrios, A. (2024). Triphenylphosphonium derivatives TPP+ C10 and TPP+ C12 of gallic acid inhibit mitochondrial function in *Candida albicans* exerting antifungal and antibiofilm activities. *Journal of Applied Microbiology*. 135(1): lxad316. <https://doi.org/10.1093/jambio/lxad316>
- [27] World Health Organization (WHO). (2022). WHO fungal priority pathogen list to guide research development and public health action. <https://www.who.int/publications/i/item/9789240060241>
- [28] Yusoff, I.M.; Mat Taher, Z.; Rahmat, Z.; Chua, L.S. (2022). Review of ultrasound-assisted extraction method for plant-based bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. *Food Research International*. 157: 111268. <https://doi.org/10.1016/j.foodres.2022.111268>
- [29] Zhang, Y.; Cai, P.; Cheng, G.; Zhang, Y. (2022). Brief overview of identified phenolic compounds from plants and their extraction, analysis and biological activity. *Natural Product Communications*. 17(1). <https://doi.org/10.1177/1934578X211069721>
- [30] Zheng, Y.; Geng, Y.; Hou, W.; Li, Z.; Cheng, C.; Wang, X.; Yang, Y. (2024). Study on antifungal activity of gallic acid and its azole derivatives against *Fusarium graminearum*. *Molecules*. 29(9): 1996. <https://doi.org/10.3390/molecules29091996>