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Research Article

Antifungal Activity of *Aegle marmelos* (L.) Corrêa and *Cassia alata* L. Extracts Against *Candida krusei* and *Candida parapsilosis*

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ABSTRACT

Candidiasis is a fungal infection caused by several *Candida* species, including *Candida krusei* and *Candida parapsilosis*. It poses a significant threat to human health, particularly in immunocompromised individuals and those with underlying medical conditions. The rapid development of microbial resistance against antibiotics and the emergence of new strains are burdens to the global health community. Medicinal plants could be an excellent source for their potential antimicrobial effects. Therefore, this study aims to assess the antifungal action of *Aegle marmelos* and *Cassia alata* extracts on the growth and morphological changes of *Candida krusei and Candida parapsilosis.* The pathogens were cultured on media containing each extract individually. Itraconazole was used as a positive control, whereas dimethyl sulfoxide (DMSO) was used as a negative control. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined by the microdilution method. All samples were processed for microscopy observations using a scanning electron microscope (SEM). The MIC of methanolic extract indicated that the leaf and unripe fruit of *A. marmelos* and *C. alata* against *C. parapsilosis* and *C. krusei* were similar at 75 mg/mL. Results of the MFC showed both pathogens were killed at a concentration of 150 mg/mL for both plant extracts. The MIC and MFC results of the methanolic extract indicated that the unripe fruit of *A. marmelos* exhibited fungistatic activity, while the leaves of both *C. alata* and *A. marmelos* demonstrated both fungicidal and fungistatic effects against *C. parapsilosis* and *C. krusei.* SEM observations showed significant changes in the morphology of *C. krusei and C. parapsilosis* compared to the control, in which the fungi were in normal form. The findings indicate the potential use of *A. marmelos* and *C. alata* methanolic extract as antifungal agents for candidiasis treatment. The extracts showed comparable inhibitory action to the commercial fungicide itraconazole.

Key words: *Aegle marmelos*, Antimicrobial, candidiasis, *Cassia alata*, methanolic, yeast

INTRODUCTION

Various species of *Candida* are significant pathogens in immunocompromised patients. Candidiasis is a fungal infection caused by various *Candida* species (Turner & Butler, 2014). Treating and controlling these infections is particularly challenging due to their inherent resistance to many conventional antifungal treatments. The genetic variants linked to antifungal drug resistance in clinical isolates have also been recorded (Zurita & Cuomo, 2023). The rapid spread of antibiotic resistance among pathogenic microorganisms and the emergence of novel strains pose difficulties in treating the infection. Therefore, alternative treatments involving plant extracts need to be explored to combat the candidiasis pathogen, offering promising potential for a more natural and sustainable approach to the disease.

Medicinal plants have been used for centuries in traditional medicine, offering a rich source of bioactive compounds with potential antimicrobial properties. *Aegle marmelos (A. marmelos)* and *Cassia alata (C. alata)*, known for their medicinal benefits in various cultures, are promising candidates for antifungal research (Monika *et al*., 2023). Devi *et al*. (2020) found that silver nanoparticles of methanolic extracts of *A. marmelos* have antimicrobial activities against *Bacillus cereus*, *Pseudomonas aeruginosa (P. aeruginosa)* and *Escherichia coli (E. coli)*. The detailing of *A. marmelos* fruit mash phytofibricated intervened silver nanoparticles also have antimicrobial activity on *Candida albicans* (*C. albicans)* (Patil & Muthusamy, 2020). *C. alata* has been reported to have different bioactive compounds in the leaves; for instance, anthraquinones, aloe-emodin, chrysophanol, emodin as well as the flavonoid and kaempferol (Adelowo, 2017; Fatmawati *et al*., 2020). Their extracts may provide new, effective options for managing *Candida* infections, particularly those resistant to standard treatments. Investigating these plant extracts could lead to the development of more effective and less toxic antifungal therapies.

A significant knowledge gap exists regarding the antifungal potential of *A. marmelos* and *C. alata,* particularly against *Candida krusei (C. krusei)* and *Candida (C. parapsilosis)*. Previous studies have highlighted the antimicrobial properties of these plants; however, their effects on *Candida* species have not been thoroughly explored. Markedly, the root and stem of *C. alata*

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showed significant antimicrobial efficacy against *S. aureus* (Toh *et al*., 2023). The impacts of fungi make it necessary for us to understand fungal biology. Exploring the effects of plant extracts on the morphology and growth of fungal cells is important. Since cell structure, function, and composition are interconnected, understanding fungal cell biology requires insights into various aspects of both structure and function.

This study aims to evaluate the antifungal effects of *A. marmelos* and *C. alata* extracts against *C. krusei* and *C. parapsilosis*. This involves assessing the growth inhibition and morphological changes in the *Candida* species when exposed to the extracts. Additionally, we aim to compare the efficacy of these extracts with established antifungal agents, such as itraconazole.

MATERIALS AND METHODS

Plant materials

Aegle marmelos leaves and unripe fruits were collected from Sri Subramaniam Temple, Serdang, Selangor, Malaysia located at GPS coordinate 2°05'59.3"N 101°042'05.6"E. *Cassia alata* leaves were collected at Botanical Garden, Department of Biology, Faculty of Science, UPM, Selangor, Malaysia at GPS coordinate 3°00'05.9"N 101°042'20.3"E. The authentication vouchers for *A. marmelos* (AM001) and *C. alata* (AM 002) have been deposited in the Department of Biology Herbarium. The fresh leaves of *A. marmelos* and *C. alata* were washed using running tap water and dried using an oven at 50°C for 3 days. The unripe fruits of *A. marmelos* were spread on the plate and dried in the oven at 60°C for 4 days (Bhanot, 2018). All plant samples were ground into powder form using a Waring commercial mechanical grinder (Torrington, CT, USA) and kept at 4°C until further use.

Fungal cultures

Two strains of fungi, *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 2209) were provided by the Laboratory of Bacteriology, Faculty of Veterinary Medicine, UPM. The fungal strain was cultured on Sabouraud dextrose agar (SDA) (HKM, China) and incubated for 24 - 48 hr at 37 ± 20°C for further study.

Methanolic extraction

Both plant leaves and fruits were weighed and combined with methanol at a ratio of 1:10 and 250 grams of fruits, respectively. Using a conical flask lined with aluminum foil and parafilm, 1 L of methanol was used to absorb *A. marmelos*. The extraction was carried out in a room temperature incubator (Psycrotherm, New Brunswick Scientific, USA) for 72 hr while being continuously shaken at 110 rpm. The extract was then filtered via Whatman No.1 filter paper. When the filtration process was finished, the extracts were dried in a vacuum at 40°C using a rotary evaporator (Buchi, Switzerland) to achieve a gel-like consistency. When not being tested for antibacterial properties, the concentrates were stored hermetically at 4°C in an airtight container (Balakumar *et al*., 2011).

Aqueous extraction

For cold extraction, 50 g powdered leaf of *A. marmelos* was soaked with 500 mL distilled water, while 100 g powdered leaves of *C. alata* were soaked with 600 mL distilled water. The 150 g powder of unripe fruits of *A. marmelos* was mixed in with 600 mL distilled water. The herb aqueous was incubated in a shaker at room temperature for 72 hr at 120 rpm following the procedure by Iraqui *et al*. (2019). The supernatant of each plant separated was filtered through Whatman No. 1 filter paper and concentrated in a rotating evaporator at 40°C. The filtrate was collected. The concentrated extract was then immersed in liquid nitrogen and freeze-dried for 72 hr in a freeze dryer (Thermo Fisher Scientific, Denmark) with a drying chamber pressure of 2×10^{-1} mbar and cryo temperature of -50°C. The three lyophilized extracts of each plant were placed in a freezer at -20°C before antimicrobial activity was conducted (Bhuyan *et al*., 2017).

Agar well diffusion method

The agar well diffusion test was performed using SDA as mentioned by the Clinical and Laboratory Standard Institute. The inoculum was prepared using culture from a 48-hr-old Sabouraud Dextrose Broth (SDB) culture. The turbidity of the suspension was adjusted with 0.5 McFarland standard (1×10^6 CFU/mL) (Berkow *et al*., 2020). The inoculum was evenly spread across the entire surface of the plate using a swab, with the Petri dish rotated approximately 60 degrees after each of the three applications. Finally, it was swabbed all around the fringe of the agar surface. Five wells with 4 mm in diameter were punched using an 8 mm cork borer at an equivalent distance and a 100 µL volume containing different concentrations of extract (150 mg/mL, 75 mg/mL & 37.5 mg/mL) was filled into three wells and diffused at room temperature for 2 hr. Wells loaded with DMSO solvent filled in as negative and Itraconazole (30 µg/mL) served as a positive control. The plate was then incubated at 37°C for 48 hr and the diameter of the zone of inhibition (ZOI) of the tested microorganism was measured (Boregowda *et al*., 2019). The mean of the triplicate result was calculated.

Minimum Inhibitory Concentration (MIC)

Plant extract that gave a positive result for the agar well diffusion assay was used to determine MIC and MFC for *Candida* species by microdilution method (Clinical & Laboratory Standards Institute, 2004). The plant extract was two-fold serially diluted with SDB to achieve the final concentration ranging from 1.17 mg/mL to 150 mg mL^{-1} in 96 well microplates. Then, the 100 µL prepared fungal inoculum that was adjusted to 0.5 McFarland was added to each well to make a total volume of 200 µL in each well. Itraconazole was used as a standard antifungal agent at concentrations of 0.18-30 µg/mL. The DMSO (Sigma-Aldrich) was filled in as a negative control. The microtiter plates were incubated at 37°C and for 48 hr. Fungal growth inhibition was evaluated by adding 40 µL of the 2,3-bis (2-methoxy-4-nitro-5-sulphophenyl)- 2H-tetrazolium-5-carboxanilide (MTT) (Sigma-Aldrich) to each well that was read after incubation at 37°C for 2 hr. A concentration of 1.25 mg/mL MTT was prepared in sterile distilled water and was added to the mixture. The MTT solution served as an indicator, with a change to purple indicating growth

or new cells were present while remaining clear or yellow coloration signified no growth. The minimum inhibitory concentration (MIC) was determined as the lowest concentration showing no visible growth. The experiment was conducted independently in triplicate and repeated twice (Appiah *et al*., 2017).

Minimum Fungicidal Concentration (MFC)

The minimum fungicidal concentration (MFC) of methanol extract of leaves and fruit *A. marmelos* and leaves of *C. alata* against *C. krusei* and *C. parapsilosis* was determined by sub-culturing 20 µL as the lowest concentration of extract that showed no fungal growth upon the addition of MTT (1.25 mg/mL) into Petri dish containing SDA. Then, the plate was incubated for 48 hr at 37°C. The MFC was determined by whether the fungi exhibited growth or cell death (Freire *et al*., 2017). Each experiment was performed in triplicate two times.

Fungistatic and fungicidal determination

The MFC/MIC proportion was measured to determine the fungicidal (MFC/MIC ≥ 4) or fungistatic (MFC/MIC <4) activities/ effects of a plant extract (Siddqui *et al*., 2013).

Scanning Electron Microscope (SEM)

This micrography observation was conducted in the Microscope Unit, Institute of Bioscience, UPM, to determine the effect of the plant extract on the morphology of the fungi. The ultrastructure study of the morphological characteristics of the fungi was observed according to the standard protocols by Su *et al*. (2015). Sabouraud dextrose agar (SDA) with MIC concentration of 75 mg/mL, leaf, and unripe fruit of *A marmelos* and *C. alata* were dropped onto inoculated agar with *C. krusei* and *C. parapsilosis* were further incubated at 37 ± 2°C for 2 days. The leaf of *A. marmelos* at MIC concentration of 37.5 mg/mL was dropped onto inoculated agar with *C. parapsilosis* and further incubated for 2 days at the same temperature. The DMSO-treated plates were filled in as a control. A small fungal block was removed from the inhibition zone and prefixed with 2.5% glutaraldehyde for 4-6 hr at 4°C. Then, the sample was washed with 0.1 M sodium cacodylate buffer three times for 10 min each and was post-fixed in aqueous 1% osmium tetroxide for 2 hr at 4°C. Then, the samples were washed again with the same buffer three times for 10 min each and dried out in a ranged series from 35% to 100% acetone. The samples were moved into a sample basket and put into critical drying with CO_2 (CPD 030, Bal-TEC, Switzerland) for 90 min. The samples were mounted onto a stub using double-sided tape and placed into a sputter coater for gold coating. The mounted samples were observed in a scanning electron microscope (JEOL JSM-6400, Japan).

Statistical analysis

All the data on the antimicrobial activity of active extract were subjected to statistical analysis. The statistical difference of the mean zone of inhibition of the extract for fungi was carried out by employing One-way ANOVA followed by Tukey HSD post hoc test was used for comparison within groups and with different groups. The statistical significance level was established at $p<0.05$. All statistical analysis was performed by SPSS statistical software 21 (SPSS Inc. Chicago, USA).

RESULTS AND DISCUSSION

Antimicrobial Activities of *A. marmelos* and *C. alata*

Agar well diffusion and disc diffusion methods were effectively used for screening the antimicrobial activity of *A. marmelos* methanolic extract against selected pathogenic yeast cultures. Methanol extract of leaves, unripe fruit of *A. marmelos,* and leaves of *Cassia alata* showed good antifungal activities in agar well diffusion against the *C. krusei* and *C. parapsilosis* at three concentrations: 150 mg/mL, 75 mg/mL and 37.5 mg/mL per well (Figures 1 & 2).

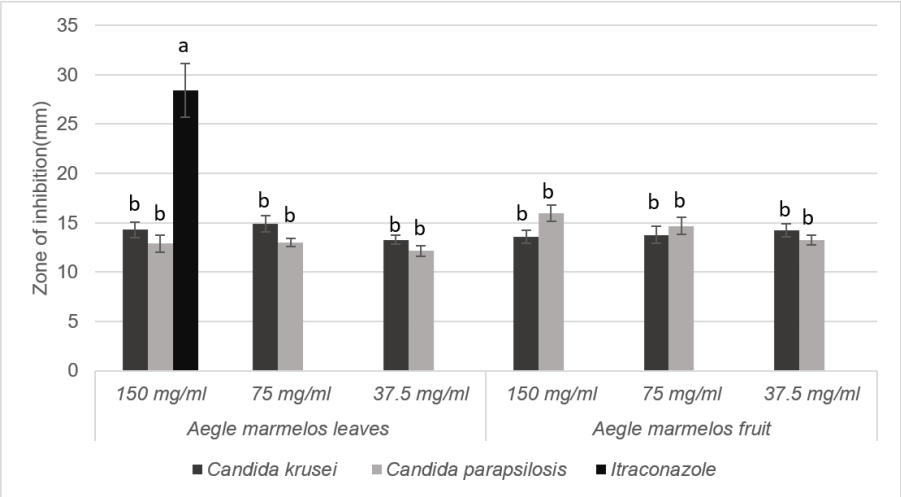

Fig. 1. Effect of different concentrations of methanol extract from leaf, unripe fruit of *Aegle marmelos* against *C. krusei* and *C. parasilosis*. The concentration used for plant extract was mg/mL and for Itraconazole was µg/mL. Samples were incubated at 37 ± 2°C for 48 hr. The data were expressed as the mean ± SE (n=6). The same letter shows no significant difference ($p<0.05$).

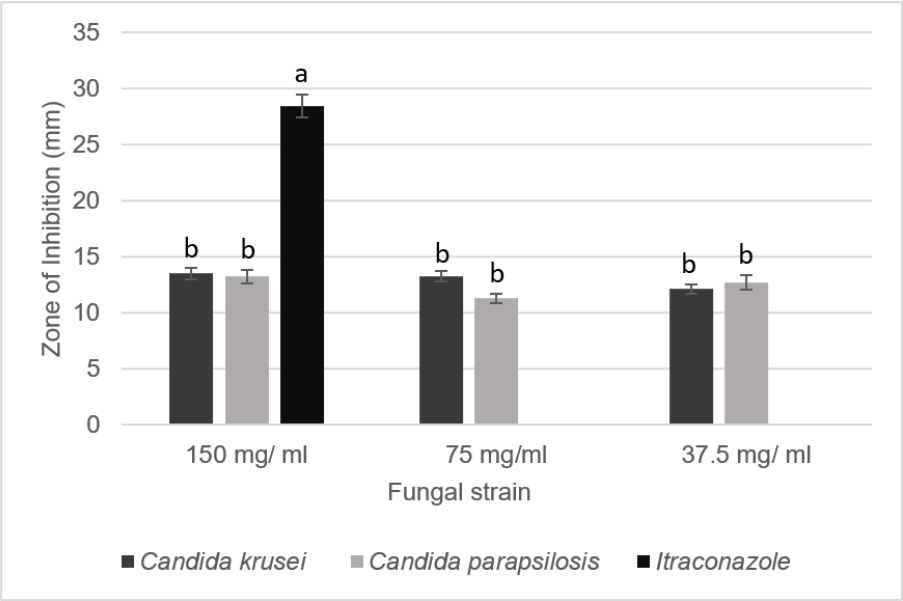

Fig. 2. Effect of different concentrations of methanol extract from *C. alata* against *C. krusei* and *C. parasilosis*. The concentration used for plant extract was mg/mL and for Itraconazole was µg/mL. Samples were incubated at 37 ± 2°C for 48 hr. The data were expressed as the mean ± SE (n=6). The same letter shows no significant difference (p<0.05).

Results showed that the methanol extracts had an inhibitory effect against *C. krusei* and *C. parapsilosis*. However, the aqueous extract of *A. marmelos* had no antimicrobial activities against the tested fungi. This analysis demonstrated that the leaf of *A. marmelos* affected *C. krusei* and *C. parapsilosis*, with inhibition zones of 14.30 ± 0.79 mm at 150 mg/mL and 75 mg/mL and 13.26 ± 0.45 mm at 37.5 mg/mL of the methanol extract, respectively, The inhibition zones were 12.89 ± 0.89 mm at 150 mg/mL; 12.99 ± 0.40 mm at 75 mg/mL and 12.16 ± 0.53 mm at 37.5 mg/mL of the methanol extract, respectively (Figure 2). The unripe fruit of *A. marmelos* was most effective against *C. krusei* and *C. parpasilosis*, with zone of inhibition of 13.59 ± 0.67 mm and 15.96 ± 0.81 mm at 150 mg/mL, 14.22 ± 0.66 mm and 14.66 ± 0.87 mm at 75 mg/mL, and 13.77 ± 0.85 mm and 13.21 ± 0.50 mm at 37.5 mg/mL of the methanol extract, respectively. On the other hand, the aqueous extract of *A. marmelos* showed no zone of inhibition against *C. krusei* and *C. parapsilosis* at concentrations of 150 mg/mL, 75 mg/mL, and 37.5 mg/mL. The largest inhibition zone was seen at 150 mg/mL concentration of unripe fruit *of A. marmelos* against *C. parapsilosis*, followed by 75 mg/mL concentration of *A. marmelos* leaves against *C. krusei*.

The methanolic extract of *C. alata* showed significant inhibition by agar well diffusion. Studies have shown that *C. alata* extracts possess significant antifungal properties due to the presence of anthraquinones and other phyto-pigments. These compounds have been effective in inhibiting the growth of *C. albicans, C. krusei*, and *C. parapsilosis*. Inhibition zones for *C. krusei* were 13.48 ± 0.48 mm at 150 mg/mL, 13.13 ± 0.54 mm at 75 mg/mL, and 13.26 ± 0.48 mm at 37.5 mg/mL, whereas *C. parapsilosis*, they were 13.23 ± 0.63 mm at 150 mg/mL, 11.31 ± 0.41 at 75 mg/mL and 12.68 ± 0.67 at 37.5 mg/mL.

This result showed that the methanol extract of *C. alata* displayed antifungal activity due to the presence of secondary metabolites (Adelowo, 2017). The antifungal capabilities of *C. alata* in this study are consistent with the findings of Wuthi-udomlert *et al*. (2005) who reported the antimicrobial activities of the aqueous extract of *C. alata* on *Candida* spp. Abubacker *et al*. (2008) discovered that the aqueous flower extract of *C. alata* is a strong inhibitor of aflatoxin producers, plants, and human pathogenic organisms. Additionally, the methanolic fraction of *C. alata* leaves was shown to be active against *T. mentagraphytes* at 50 mg/mL, with no activity against *C. albicans* (Villasenor *et al*., 2002). Moreover, *C. alata* leaves had a significant effect on healing burns (Nasution *et al*., 2019) and were effective against clinical isolates of Gram-positive and Gram-negative bacteria like *V. cholerae, B. subtilis, S. aureus, Streptococcus* species, and *E. coli*.

Fungistatic and fungicidal activities

The methanol extracts of *A. marmelos* and *C. alata* showed significant antifungal potential against *C. krusei* and *C. parapsilosis*. This study is the first to specifically report the antifungal activity of *A. marmelos* against *C. krusei* and *C. parapsilosis*, highlighting the methanol extract as a particularly potent antifungal agent. Since the methanol extracts of unripe fruit and leaves of *A. marmelos* and *C. alata* were the most active *in vitro* experiment, showing significant antimicrobial activity against *C. krusei* and *C. parapsilosis*, they were further subjected to broth microdilution. Nalini *et al*. (2017) reported that *C. alata* extracts exhibit antifungal activity by producing zones of inhibition against *C. albicans* cultures, highlighting their potential as natural antifungal agents. In this study, the MIC value for the methanol extract of *A. marmelos* and *C. alata* against the tested *Candida* species ranged from 37.5 mg/mL to 150 mg/mL, with the respective MFC values ranging from 75 to 150 mg/mL. The MIC and MFC values for Itraconazole ranged from 15 mg/mL and 30 mg/mL, respectively as shown in Table 1.

Table 1. The MIC and MFC of methanol extract of *Aegle marmelos* and *Cassia alata* leaves (mg/mL)

Fungal	Itraconazole (µg/mL)			*A. marmelos* leaves			*C. alata* leaves			Unripe fruits of *Aegle marmelos*		
	MIC	MFC	MFC/ MIC	MIC	MFC	MFC/ MIC	MIC	MFC	MFC/ MIC	MIC	MFC	MFC/ MIC
Candida krusei (ATCC 6258)	15	30	2	75	150	2	75	150	2	75	150	2
Candida parapsilosis (ATCC 22019)	15	30	2	37.5	150	4	75	150	2	75	150	2

The MFC/MIC ratio was used to determine whether the methanol extract of *Aegle marmelos* and *Cassia alata* has fungistatic (MFC/MIC≥4) or fungicidal activity (MFC/MIC<4) following protocol by Siddqui *et al*. (2013). The leaves of *A. marmelos* were a predominately fungistatic agent against *C. parapsilosis* while the leaves of *C. alata* and *A. marmelos* fruit and leaves were determined as fungicidal against both *Candida* species. It has been suggested that fungicidal drugs are often preferred over drugs with fungistatic activity. For instance, fungicidal therapy for invasive candidiasis and candidaemia is associated with a higher probability of persistent recurrent infection (Kumar *et al*., 2018). Likewise, dermatophyte infection recurs more often when a fungistatic rather than the fungicidal drug has been used (Kyle & Dhal, 2004).

A. marmelos and *C. alata* have significant potential as fungistatic or fungicidal agents due to their rich phytochemical profiles such as phenolics, flavonoids, alkaloids, terpenoids, and tannins. These compounds can disrupt fungal membranes and inhibit fungal spore germination, and hyphal growth. Further research on dosage optimization, toxicity, and application methods will help to understand their full potential in medicine and agriculture.

Morphological Changes of Pathogens Treated with Plant Extract

The action of methanol extract of *A. marmelos* and *C. alata* on *C. parapsilosis* and *C. krusei* morphology was evaluated *via* SEM. Figures 3 and 4 demonstrated that the methanol extracts caused changes in cell structure.

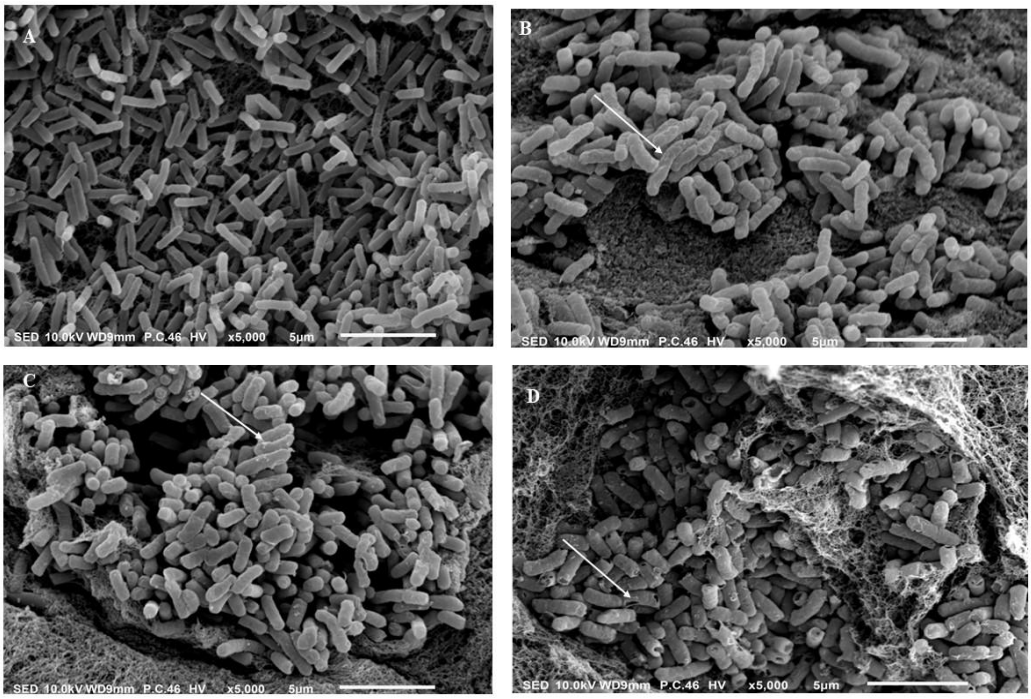

Fig. 3. SEM analysis of the effect of *A. marmelos* (leaves and fruits) and *C. alata* (leaves) extract on the morphology of *C. parapsilosis.* Control cell (A), morphology changes in the cell including cell wall deformities and cell wall damage are indicated by the arrow in the cell treated with the leaves *A. marmleos* (B), and protuberance is indicated by the arrow in the cell treated with *C. alata* leaves (C) and hole is indicated by the arrow in the cell treated with unripe fruit of *A. marmelos* (D). Magnification X5000. The screening was performed in at least 20 fields.

The observation of morphological changes showed that untreated cells maintained a normal morphology with smooth surfaces. Meanwhile, *Candida* cultures treated with methanol extract after 48 hr showed unmistakable primary changes due to the interaction with the active plant extract. Untreated *C. parapsilosis* cells appeared in clusters and pseudohyphae with no extracellular material seen (Figure 4). A similar morphology was observed for untreated *C. krusei*, with polar bud scars and no filamentous structure (Figure 4). Additionally, many cells were of normal size, oval and homogenous with smooth surfaces. However, *C. parapsilosis* treated with *Aegle marmelos* leaves (MIC = 37.5 mg/mL) and *C. alata* as well as unripe fruit of *A. marmelos* (MIC = 150 mg/mL), showed cell wall damage, distortions, ruffles, bulges, holes on the surface and a raisin-like

appearance (Figure 3B-3D). A similar effect was observed with *A. marmelos* and *C. alata* at a concentration MIC (75 mg/mL) after 48 hr, showing a significant morphological change in *C. krusei*, which appeared asymmetrical in shape with wrinkles, breaks, roughness, and holes on the surface (Figure 4B-4D), demonstrating cell lysis.

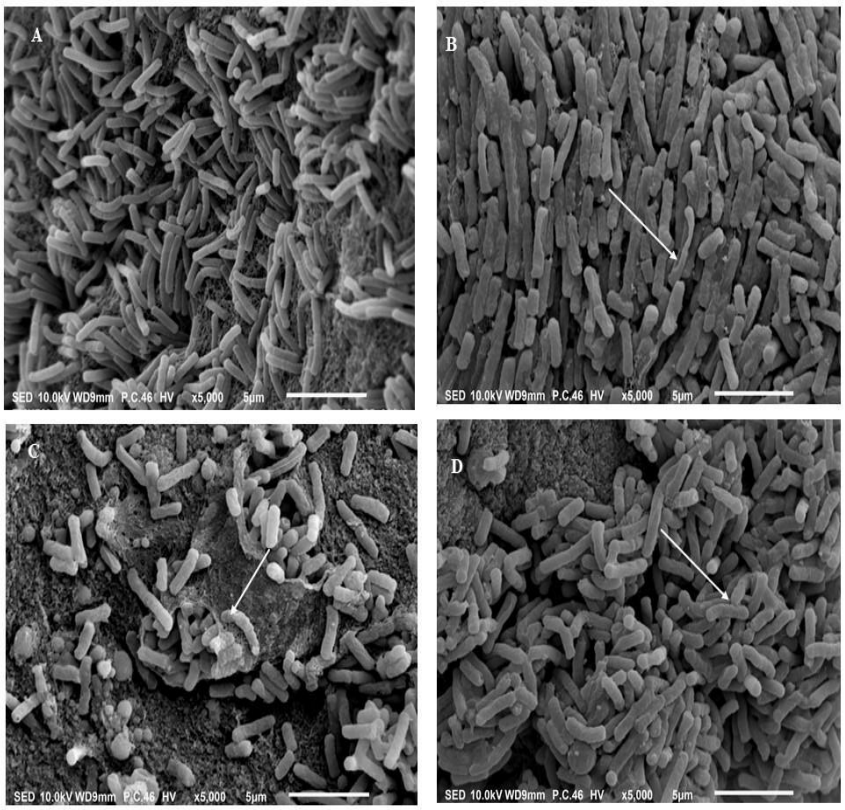

Fig. 4. Scanning electron micrograph analysis of the effect of *A. marmelos* (fruit and leaves) and *C. alata* (leaves) extract on the morphology of *C. krusei*. Control cell (A), morphology alteration in the cell including hole and cell wall damage are indicated by the arrow in the cell treated with the leaves *A. marmelos* (B), wrinkles and protuberance are indicated by the arrow in the cell treated with *C. alata* leaves (C) cell wall deformities and raisin-like appearance indicated by the arrow in the cell treated with unripe fruit of *A. marmelos* (D). Magnification X5000. The screening was performed in at least 20 fields.

The morphological changes, including cell wall damage, cell wall disfigurement, ruffles, and raisin-like appearance, were attributed to the leakage of intracellular material due to treatment with methanol plant extract. Some cells displayed surface protuberance due to shrinkage, leading to cell death. The high antifungal efficacy of the methanol extract of *A. marmelos* and *Cassia alata* on the tested *Candida* strains resulted in the development of holes, ultimately causing cell death. This damage could play a role in reducing fungal pathogenesis *in vivo*. These distinct changes in fungal strain strongly suggest that the methanol extracts of both plants induced osmotic imbalance and perturbation in cell membrane structure in *Candida* species, leading to collapse and cell death by lysis.

In a recent study, SEM analysis was used to decide the effect of the medicinal balm of *O. gratissimum* and revealed morphological changes in the cell wall and the morphology of some subcellular organelles of *C. albicans, C. parapsilosis,* and *C. krusei* (Alyousef, 2021). Additionally, another study reported that the fungistatic effect was caused by the inhibition of β-glucan synthase, while the fungicidal effect was caused by changes in the cell wall integrity and loss of mechanic resistance, leading to cell destruction by osmotic pressure (Gutierrez *et al*., 2018). The essential oil from the *A. marmelos* leaves might disrupt the Ca^{2+} dipicolonic acid metabolism pathway and inhibit spore germination. Ca^{2+} ion uptake is crucial for spores' germination and dormancy. Thus, *A. marmelos* may display antifungal activity by reducing the vegetative fungal body inside the host or on a solid medium. Similarly, other authors have reported that the essential oil from *A. marmelos* leaves has significant antifungal activity (Cox *et al*., 2000). Essential oil of plant extract possesses antifungal activity at different growths (Lamia *et al*., 2018). Additionally, the essential oil of *A. marmelos* leaves showed significant inhibition of *A. niger* and *F. oxysporum* spores at various concentrations (Ibrahim *et al*., 2015).

CONCLUSION

The findings of this study highlight the potential of these medicinal plants in treating candidiasis and demonstrate the strong antifungal activity of methanol extracts from *A. marmelos* and *C. alata* against non-*Candida albicans* species. Both extracts showed significant fungistatic and fungicidal activity, with inhibitory effects comparable to itraconazole. Microscopy confirmed significant morphological damage to the fungi, suggesting these plant extracts could offer alternative treatment options for candidiasis.

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ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Abubacker, M.N., Ramanathan, R. & Senthil K.T. 2008. In vitro antifungal activity of *Cassia alata* flower extract. Indian Journal of Natural Products and Resources, 7(1): 6-9.
Adelowo, F. 2017. An overview of the phytochemical analysis of bioactive compounds in *Senna alata*. Advances in Biochemistry, 5(5): 102-109. https://doi.org/10.11648/j.ab.20170505.14
Alyousef, A.A. 2021. Antifungal activity and mechanism of action of different parts of *Myrtus communis* growing in Saudi Arabia against Candida species. Journal of Nanomaterials, 2021: 1-10. https://doi.org/10.1155/2021/3484125
Appiah, T., Boakye, Y.D. & Agyare, C. 2017. Antimicrobial activities and time-kill kinetics of extracts of selected Ghanaian mushrooms. Evidence-Based Complementary and Alternative Medicine, 2017: 4534350. https://doi.org/10.1155/2017/4534350
Balakumar, S., Rajan, S., Thirunalasundari, T. & Jeeva, S. 2011. Antifungal activity of *Aegle marmelos* (L.) Corrêa (Rutaceae) leaf extract on dermatophytes. Asian Pacific Journal of Tropical Biomedicine, 1(4): 309-312. https://doi.org/10.1016/S2221-1691(11)60049-X
Berkow, E.L., Lockhart, S.R. & Luis, O.Z. 2020. Antifungal susceptibility testing: Current approaches. Clinical Microbiology Review, 33(3): 1-30. https://doi.org/10.1128/CMR.00069-19
Bhanot, M. 2018. Development and sensory evaluation of unripe and ripe Bael fruit powder (*Aegle marmelos*), Journal of Pharmacognosy and Phytochemistry, 7(4): 2894-2897.
Bhuyan, D.J., Vuong, Q.V., Chalmers, A.C., van Altena, I.A., Bowyer, M.C. & Scarlett, C.J. 2017. Phytochemical, antibacterial and antifungal properties of an aqueous extract of *Eucalyptus microcorys* leaves. South African Journal of Botany, 112: 180-185. https://doi.org/10.1016/j.sajb.2017.05.030
Boregowda, R.S., Murali, N., Udayashankar, A.C., Niranjana, S.R., Lund, O.S. & Prakash, H.S. 2019. Antifungal activity of *Eclipta alba* metabolites against sorghum pathogens. Plants, 8(3): 72. https://doi.org/10.3390/plants8030072
Clinical and Laboratory Standards Institute, 2024. Performance standards for antimicrobial susceptibility testing. 34th Ed. CLSI supplement M100. Wayne, PA, USA
Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R. & Wyllie, S.G. 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (Tea tree oil). Journal of Applied Microbiology, 88(1): 170-175. https://doi.org/10.1046/j.1365-2672.2000.00943.x
Devi, M., Devi, S., Sharma, V., Rana, N., Bhatia, R.K. & Bhatt, A.K. 2020. Green synthesis of silver nanoparticles using methanolic fruit extract of *Aegle marmelos* and their antimicrobial potential against human bacterial pathogens. Journal of Traditional and Complementary Medicine, 10(2): 158-165. https://doi.org/10.1016/j.jtcme.2019.04.007
Fatmawati, S., Yuliana, Purnomo, A.S. & Abu Bakar, M.F. 2020. Chemical constituents, usage and pharmacological activity of *Cassia alata*. Heliyon, 6(7): 04396. https://doi.org/10.1016/j.heliyon.2020.e04396
Freire, J.C.P., Júnior, J.K.D.O., Silva, D.D.F., Sousa, J.P. De, Guerra, F.Q.S. & De Oliveira Lima, E. 2017. Antifungal activity of essential oils against *Candida albicans* strains isolated from users of dental prostheses. Evidence-Based Complementary and Alternative Medicine, 2017: 1-9. https://doi.org/10.1155/2017/7158756
Gutiérrez, J.A., Caballero, S., Díaz, L.A., Guerrero, M.A., Ruiz, J. & Ortiz, C.C. 2018. High antifungal activity against candida species of monometallic and bimetallic nanoparticles synthesized in nanoreactors. ACS Biomaterials Science and Engineering, 4(2): 647-653. https://doi.org/10.1021/acsbiomaterials.7b00511
Ibrahim, N.A., El-Sakhawy, F.M., Mohammed, M.M.D., Farid, M.A., Abdel-Wahed, N. A.M. & Deabes, D.A.H. 2015. Chemical composition, antimicrobial and antifungal activities of essential oils of the leaves of *Aegle marmelos* (L.) Corrêa growing in Egypt. Journal of Applied Pharmaceutical Science, 5(2): 1-5. https://doi.org/10.7324/JAPS.2015.50201
Ïraqui, P., Chakraborty, T., Das, M.K. & Yadav, R.N. 2019. Herbal antimicrobial gel with leaf extract of *Cassia alata* L. Journal of Drug Delivery and Therapeutics, 9(3): 82-94. https://doi.org/10.22270/jddt.v9i3.2527
Kumar, A., Zarychanski, R., Pisipati, A., Kumar, A., Kethireddy, S. & Bow, E.J. 2018. Fungicidal versus fungistatic therapy of invasive Candida infection in non-neutropenic adults: a meta-analysis. Mycology, 9: 116-128. https://doi.org/10.1080/21501203.2017.1421592
Kyle, A.A. & Dahl, M.V. 2004. Topical therapy for fungal infections. American Journal of Clinical Dermatology, 5: 443-451. https://doi.org/10.2165/00128071-200405060-00009
Lamia, S.S., Shimo, M.S., Sakib, S., Rashed, B., Prima, A.A. & Mony, A.T. 2018. Phytochemistry and pharmacological properties of *Aegle marmelos* L. (Rutaceae): A review. International Journal of Research in Pharmacy and Pharmaceutical Sciences, 3(3): 45-54.
Monika, S., Thirumal, M. & Kumar, P.R. 2023. Phytochemical and biological review of *Aegle marmelos* Linn. Future Science OA, 9(3): FSO849. https://doi.org/10.2144/fsoa-2022-0068
Nalini, G.R., Azad S.A.K. & Cherryl, D.M. 2017. Documentation and comparison of antifungal sensitivity of *Cassia alata* leaf and flower extracts against Candida albicans. Human Jounal, 8(2): 191-197.
Nasution, S.L.R., Putri, M., Hulu, W., Girsang, E. & Nasution, A.N. 2019. Optimization of *Senna alata* leaves extract for healing

burns. Journal of Education and Sport 9(5): 127-136.

Patil, S. & Muthusamy, P. 2020. A bio-inspired approach of formulation and evaluation of *Aegle marmelos* fruit extract mediated silver nanoparticle gel and comparison of its antibacterial activity with antiseptic cream. European Journal of Integrative Medicine, (33): 101025. https://doi.org/10.1016/j.eujim.2019.101025

Siddiqui Z.N., Farooq F., Musthafa T.N.M., Ahmad, A. & Khan, A.U. 2013. Synthesis, characterization and antimicrobial evaluation of novel halopyrazole derivatives. Journal of Saudi Chemical Society, 17(2): 237-43. https://doi.org/10.1016/j.jscs.2011.03.016

Su, P.W., Yang, C.H., Yang, J.F., Su, P.Y. & Chuang, L.Y. 2015. Antibacterial activities and antibacterial mechanism of *Polygonum cuspidatum* extracts against nosocomial drug-resistant pathogens. Molecules, 20(6): 11119-11130. https://doi.org/10.3390/molecules200611119

Toh, S.C., Lihan, S., Bunya, S.R. & Leong, S.S. 2023. In vitro antimicrobial efficacy of *Cassia alata* (Linn.) leaves, stem, and root extracts against cellulitis causative agent *Staphylococcus aureus*. BMC Complementary Medicine and Therapies, 23: 85. https://doi.org/10.1186/s12906-023-03914-z

Turner, S.A. & Butler, G. 2014. The Candida pathogenic species complex. Cold Spring Harbor Perspective in Medicine, 4(9): a019778. https://doi.org/10.1101/cshperspect.a019778

Villasenor, I.M., Canlas, A.P., Pascua, M.P.I., Sabando, M.N. & Soliven, L.A.P. 2002. Bioactivity studies on *Cassia alata* L. leaf extracts. Phytotherapy Research 16(S1): 93-96. https://doi.org/10.1002/ptr.768

Wuthi-udomlert, M., Prathanturarug, S. & Soonthomchareonnon, N. 2005. Antifungal activity of *Senna alata* extracts using different methods of extraction. Acta Horticulturae, 597: 205-208. https://doi.org/10.17660/ActaHortic.2003.597.28

Zurita, A.M. & Cuomo, C.A. 2023. Genome-wide identification of variants associated with antifungal drug resistance. Methods in Molecular Biology, 2658: 81-103. https://doi.org/10.1007/978-1-0716-3155-3_7