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Original Research Article

In vitro evaluation of antifungal property of ethanolic extract of *Syzygium aqueum* fruit in leucorrhoea using zone of inhibition

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ABSTRACT

Background: Leucorrhoea, characterized by abnormal vaginal discharge, is commonly associated with *Candida albicans* infection. Conventional antifungal agents face resistance, creating the need for plant-based alternatives. *Syzygium aqueum* (water apple) is known to possess antimicrobial activity.
Methods: Ethanolic extract of *Syzygium aqueum* fruit was prepared by cold maceration. Antifungal activity was evaluated against *Candida albicans* using agar well diffusion on potato dextrose agar medium. Extract concentrations (10, 5, 2.5, 1.25 mg/ml) were tested. DMSO served as negative control, and ketoconazole (50 mg/ml) as positive control. Zones of inhibition were measured after incubation at 37°C for 24 hours.
Results: Extract exhibited concentration-dependent antifungal activity with maximum inhibition zone of 20 mm at 10 mg/ml, while ketoconazole showed 31 mm inhibition.
Conclusions: *Syzygium aqueum* demonstrated moderate antifungal efficacy against *Candida albicans*, supporting its potential as a natural antifungal option in leucorrhoea management.

Keywords: Antifungal, Ethanolic extract, Leucorrhoea, Syzygium aqueum, Zone of inhibition

INTRODUCTION

Leucorrhoea, or abnormal vaginal discharge, is a frequent gynecological complaint, often linked with *Candida albicans* infection. Current antifungal drugs like azoles are effective but limited by increasing resistance and side effects. The search for natural antifungal agents has gained momentum.[1,2] Emerging evidence reports rising rates of azole-resistant *Candida* strains in patients with vaginal candidiasis, presenting challenges in effective clinical management and underscoring the need for alternative therapies.[3]

Plant-based compounds are increasingly explored for antimicrobial activity due to their lower toxicity profiles and traditional use in folk medicine.[4] *Syzygium aqueum* (water apple), belonging to the family Myrtaceae, is widely consumed in South and Southeast Asia. It has been reported to exhibit antimicrobial, antioxidant, and anti-inflammatory activities. However, its antifungal efficacy against *Candida albicans* in leucorrhoea remains underexplored. This study aimed to evaluate the antifungal activity of ethanolic extract of *Syzygium aqueum* fruit against *Candida albicans* using agar well diffusion assay.[5]

METHODS

This study was conducted in the department of pharmacology, ACS Medical College and Hospital, Chennai, Tamil Nadu, India, during May 2025 - June 2025.

Test organism and strain confirmation

Candida albicans (ATCC number: 90028). The identification was confirmed by germ tube test.

Inoculum preparation and standardization

Colonies from 24 hr growth on potato dextrose agar were suspended in sterile saline and adjusted to 0.5 McFarland (~1-5×10^6 CFU/ml) using a densitometer.

Plant material and authentication

Fresh fruits of *Syzygium aqueum* were collected, authenticated, and subjected to cold maceration.

Extraction procedure

Fifty grams (50 gm) of fresh water apple sample was weighed and soaked in 100 ml of 95% ethanol (1:2 w/v) for 24 hours at room temperature with intermittent agitation. The macerate was filtered (Whatman No.1) using sterile filter paper and the filtrate concentrated under reduced pressure at ≤40°C (rotary evaporator), then dried to constant weight and stored in amber vials at 4°C. Extraction yield (% w/w) was calculated as:

From 50 g fresh fruit, the dried extract weighed 2 gm (4% w/w).

Agar well diffusion assay

PDA (manufacturer, lot) was prepared per label instructions and sterilized (121°C, 15 minutes). Plates were swab-inoculated with standardized yeast suspension. Six-millimeter wells were punched; 20 µL of extract solutions (10, 5, 2.5, 1.25 mg/ml) were dispensed per well (corresponding to 200, 100, 50, 25 µg/well). DMSO (20 µl) served as negative control; ketoconazole 50 mg/ml (20 µl) as positive control. Plates were incubated 24 h at 37°C (inverted). Zones of inhibition (outer clear edge) were measured with a digital calliper.

Ethical approval

As the study was entirely in vitro and did not involve human or animal subjects, formal ethical clearance was not required.

RESULTS

The ethanolic extract of *Syzygium aqueum* produced a distinct concentration-dependent antifungal effect against Candida albicans as demonstrated by the agar-well diffusion assay. Figures 1-3 depict the stages of extract preparation. Figure 1 shows the freshly collected S. aqueum fruits used for the study, Figure 2 illustrates the cold-maceration process in ethanol, and Figure 3 presents the clarified filtrate obtained after evaporation. From 50 gm of fresh fruit, 2 gm of dry extract (4% w/w) was obtained, yielding a light-amber residue suggestive of phenolic and flavonoid constituents responsible for antimicrobial activity.

Table 1: Zone of inhibition of *Syzygium aqueum* extract against *Candida albicans*.

Concentration (mg/ml)	Zone of inhibition (mm)
10	20
5	16
2.5	12
1.25	10
DMSO	-
Ketoconazole (50 mg/ml)	31

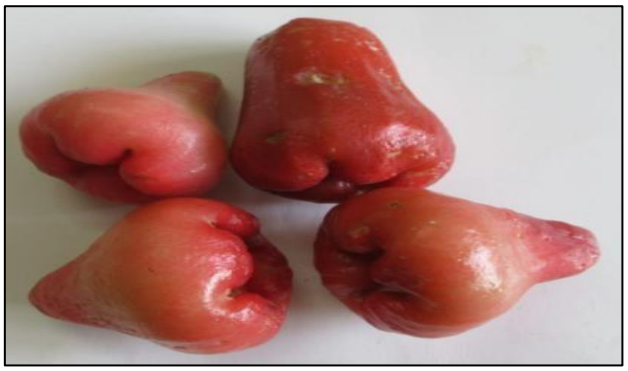

Figure 1: Fresh fruits of *Syzygium aqueum* (water apple) used for ethanolic extract preparation.

Figure 2: Cold maceration of *Syzygium aqueum* fruit pieces in ethanol for extract preparation.

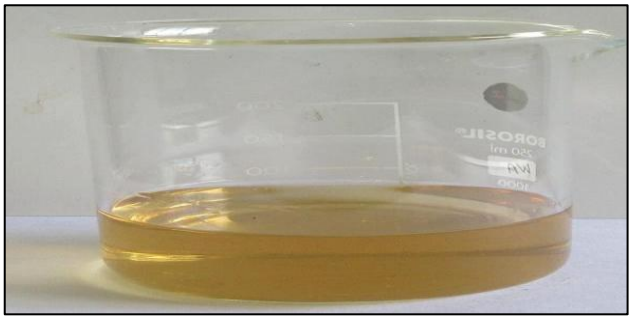

Figure 3: Filtered ethanolic extract of *Syzygium aqueum* obtained after maceration.

When tested against C. albicans, the extract showed progressive inhibition with increasing concentration. At 10 mg/mL, the mean inhibition zone measured 20 mm, decreasing to 16, 12 and 10 mm at 5, 2.5 and 1.25 mg/ml respectively (Table 1). The negative control (DMSO) exhibited no inhibition, confirming the absence of solvent interference, whereas the positive control ketoconazole (50 mg/ml) produced a 31 mm zone. The agar-well diffusion plate (Figure 4) visually confirms these findings, showing clear, circular inhibition zones surrounding wells containing higher extract concentrations.

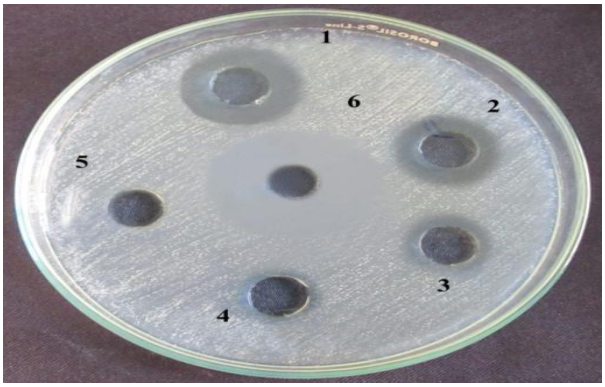

Figure 4: Agar well diffusion plate on PDA showing antifungal activity of ethanolic extract of *Syzygium aqueum* fruit against *Candida albicans*.

Wells 1-5: extract at 10, 5, 2.5, and 1.25 mg/ml plus DMSO (negative control); Well 6: ketoconazole 50 mg/ml (positive control). Zones measured after 24 hours at 37°C.

The increase in zone diameter with concentration demonstrates a clear dose-response relationship, indicating that active phytochemicals diffuse efficiently through the medium. The consistency of results across replicate plates supports the reproducibility of the assay. The extract exhibited significant antifungal activity, validating the traditional use of *S. aqueum* and suggesting that its bioactive compounds could act synergistically to disrupt fungal growth.

DISCUSSION

The ethanolic extract of *Syzygium aqueum* fruit demonstrated a concentration-dependent inhibitory effect against *Candida albicans*, with a maximal zone of inhibition of 20 mm at 10 mg/ml. This finding supports previous reports highlighting the antifungal potential of *Syzygium* species and related medicinal plants. In a comparative study, *S. aromaticum* (clove) extract exhibited strong antifungal activity with inhibition zones ranging from 22 mm to 30 mm against *C. albicans*, attributed primarily to eugenol, which exerts membrane-disruptive and protein-denaturing effects.[6]

Similarly, *S. cumini* extracts demonstrated substantial inhibition against *C. albicans* and *Aspergillus niger*, showing minimum inhibitory concentrations as low as 31.25 µg/ml. These consistent results across *Syzygium* species suggest that their antifungal effects may be linked to shared phytochemical constituents including flavonoids, terpenoids, and phenolic compounds.[7-9] The antifungal efficacy observed in *S. aqueum* aligns with a recent phytochemical assessment demonstrating that this species contains high levels of ellagic acid, myricetin, and gallic acid, compounds known to inhibit fungal ergosterol biosynthesis and increase membrane permeability.[10] This bioactive likely act synergistically to suppress fungal proliferation and adhesion. In a 2024 study by Al-Hashimi et al, aqueous and ethanolic extracts of *S. aromaticum* produced inhibition comparable to nystatin and fluconazole, confirming the genus-wide antifungal capability.[8]

When compared with other herbal extracts recommended for leucorrhoea- such as *Clitoria ternatea*, *Lavandula angustifolia*, and *Punica granatum*- the antifungal activity of *S. aqueum* remains moderate but clinically relevant.[11] Lavender and chamomile extracts produced inhibition zones of up to 32 mm, reflecting potent antifungal potential with established safety in gynecological formulations. However, many of these extracts require higher concentrations or formulated delivery systems to match the efficacy of standard antifungals.[3] The antifungal mechanism of *S. aqueum* may be attributed to multiple bioactive groups acting in concert. Flavonoids and phenolic acids interfere with fungal oxidative metabolism and inhibit germ tube formation, while terpenes and saponins alter membrane lipid composition, leading to cytoplasmic leakage and cell death.[12] The detected presence of tannins further enhances the antifungal effect by denaturing fungal proteins and inhibiting hyphal elongation. This multi-target action confers a broad-spectrum fungistatic property that may help counteract resistance issues associated with azole antifungals.[13-15]

This study has some limitations. This study was done only in vitro using Candida albicans isolates and does not show how the extract will act in vivo.

Only one fungal strain was tested, so the results may not apply to other Candida species that cause leucorrhoea. The exact active chemical compounds responsible for the antifungal effect were not identified in this study.

The safety of the extract on human tissues was not tested, so further studies are needed before clinical use.

CONCLUSION

The study establishes that the ethanolic extract of *Syzygium aqueum* fruit exhibits promising in vitro antifungal activity against Candida albicans, supporting its potential as a natural agent for managing leucorrhoea-associated fungal infections. The underlying activity is likely due to synergistic phytochemicals such as flavonoids, tannins, terpenoids, and phenolics. Nevertheless, as these findings are based on in vitro assays, their clinical translation requires caution. Future work should focus on in vivo

toxicity evaluation, pharmacokinetic assessment, and formulation studies, followed by randomized clinical trials to validate therapeutic efficacy and safety.

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Ethical approval: Not required

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