In-vitro Antifungal Activity of Carica papaya Leaf Extract Against Candida albicans and it's Synergy with Clotrimazole.

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Abstract:

Natural products, have shown potential antifungal properties, but their efficacy and possible synergy with existing antifungal drugs remain under-explored.This study aims to evaluate the anti-fungal activity in increasing concentration of ethanolic extract of Carica papaya leaf against Candida albicans and assess its interaction with clotrimazole. Ethanolic Carica papaya leaf extract was prepared by Soxhlet extraction. Antifungal activity against C. albicans was assessed using agar well diffusion (zone of inhibition) and minimum inhibitory concentration (MIC) assays. The extract was tested alone at increasing concentrations and in combination with clotrimazole 1% mouth paint.The ethanolic extract of Carica papaya leaf demonstrated significant antifungal activity against Candida albicans, although its efficacy remained lower than that of clotrimazole 1%. When combined with clotrimazole, the overall antifungal activity decreased, suggesting a possible antagonistic interaction.Carica papaya leaf extract exhibits promising antifungal properties, but its combination with clotrimazole did not enhance efficacy against Candida albicans but reduced it. Further theoretical and in vivo studies are recommended before recommending C.papaya as an adjunct to clotrimazole therapy.

Keywords: Carica papaya leaf, *Candida albicans*, Clotrimazole.

Introduction

Plant-derived natural products or chemicals have demonstrated a significant medicinal benefit and integrating traditional knowledge with modern assay methods increases the chances of discovering new effective therapeutics[1].The papaya, or Carica papaya Linn., is a member of the Caricaceae family and is widely recognized worldwide for its nutritional and medicinal qualities. The plant's antibacterial, antioxidant, antipyretic, insecticidal, antifungal, and anti-mollusk properties are due to bioactive components like proteolytic enzymes (papain, chymopapain), alkaloids (carpain, carpasemine), sulphurous chemicals (benzyl isothiocyanate), flavonoids, triterpenes, organic acids, and oils which have all been identified in the leaves and seeds of Carica papaya [2-4].

Carica papaya is increasingly studied for its antifungal effect, especially against *Candida albicans,*the most common fungus in the oral cavity.Oral candidiasis, an opportunistic infection of the oral cavity is caused by *Candida albicans* [5]. The dorsum of the tongue is where oral

candidiasis lesions are commonly found but also appear on the buccal mucosa, labial mucosa, hard palate, ventral surface of the tongue, gingiva, corners of the lips, soft palate, lateral borders of the tongue, and floor of the mouth.It commonly occurs when the normal oral flora is disrupted or when the immune system is weakened. Standard treatment involves use of antifungal agents. Azoles are widely used class of antifungals. Clotrimazole,a first-generation azole, is used mainly as a topical therapeutic optionin the form of creams, powders, lozenges[6].However, the emergence of antifungal resistance represents a major challenge in clinical practice. [6-7]. To improve antifungal activity, lower the dosage, and postpone the development of resistance, clotrimazole is being investigated in combination with natural plant extracts. The present study aims to (i) evaluate the antifungal activity of ethanolic *Carica papaya* leaf extract against *C. albicans* and (ii) assess its in-vitro interaction with clotrimazole 1% mouth paint, using agar well diffusion and MIC/NIC assays

Materials and methods

Plant material and Extraction

50 gm of driedC. papaya leaf powder was weighed after sieving and then mixed with 500 ml of solvent (Absolute Ethanol) in the thimble and heated at 70-80°C. After all solvent has evaporated, the sample extracts left in the thimble was collected separately and transferred into weighing beakers.The extract was completely dried in the oven at 45°C. Extract was collected in micro centrifuge tube and stored at 4°C[8]. The percentage recovery of the dried ethanolic extract was calculated from the weights of initial plant powder and final dried extract (Table 1)

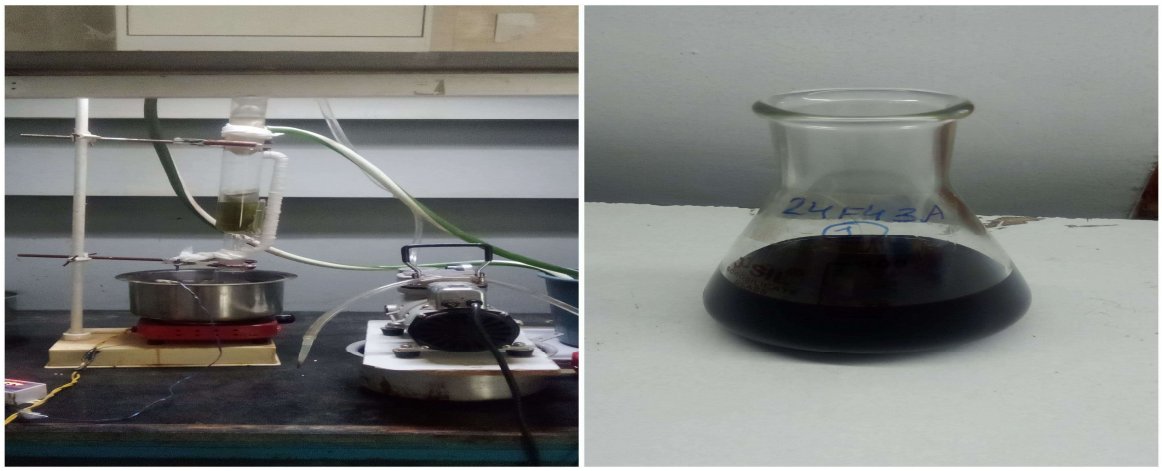

Figure 1: Ethanolic Extraction of Carica papaya

Table 1: Extract obtained from the sample

S. No.	Sample	Weight of Powder (g)	Weight of Extract(mg)	% Recovery
1	Carica papaya	50g	1313mg	2.626%

Fungal strain and inoculum preparation

Candida albicans(MTCC 854) was obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The organism was cultured on Sabouraud dextrose agar (SDA) plates.Inoculum adjusted to 0.5 McFarland standard (~1.5 × 10^8 CFU/mL).

Reagents and materials

Materials used included Sabouraud dextrose agar (SDA) plates (SRL Chem, Cat no. 19427), the solvent dimethyl sulfoxide (DMSO; SRL Chem, 28580), Clotrimazole 1% mouth paint (positive control), and sterile 96 well microplates. The amount loaded per well was 50 µl.

Antifungal activity assay

Antifungal activity of the ethanolic *Carica papaya* leaf extract alone and in combination with clotrimazole was evaluated using the agar well diffusion method (zone of inhibition) adapted from the Kirby–Bauer technique. The SDA plates were inoculated by spreading with 100 µl of Fungal culture, *Candida albicans* (Inoculum was prepared by adjusting 0.5 McFarland Unit - Approx cell density (1.5 X10 8 CFU/mL from Sabouraud dextrose broth) and followed by the wells containing 10 µl of different concentration (0 to 500 mg/ml). One well in each plate was loaded with solvent alone which served as vehicle control and Clotrimazole 1% well (10 µl) was taken as positive control.The plates of *Candida albicans* were incubated (Basil Scientific Corp. India- Incubator) at 37 °C for 24 hrs. The clear zones created around the well were measured and recorded. Each test condition was repeated in two and three plates as shown in figure 2 and 3.

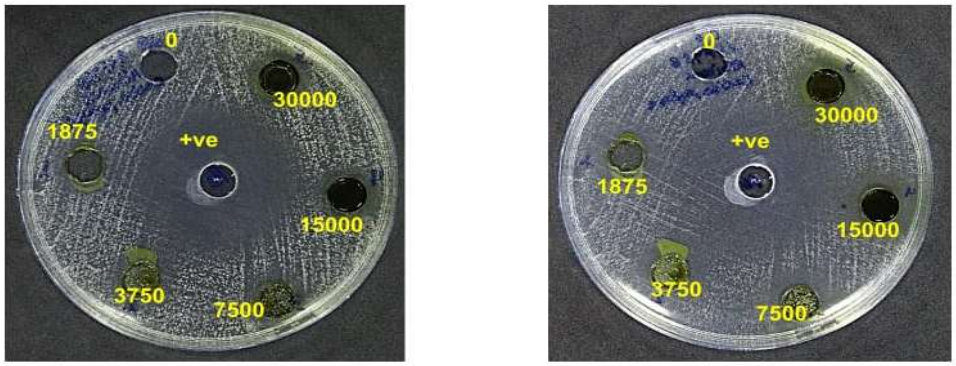

Figure 2: Agar diffusion assay of Carica papaya

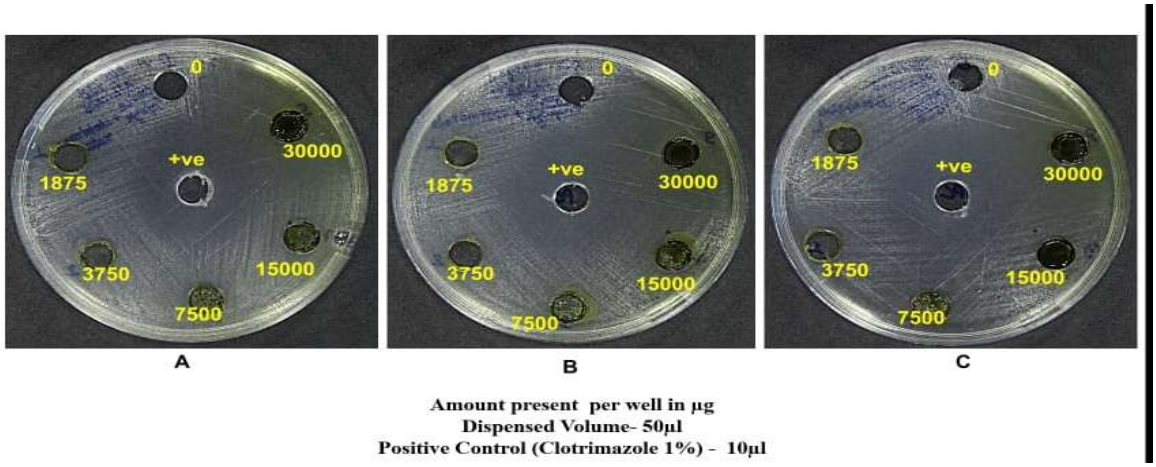

Figure 3: Agar diffusion assay of Carica papaya and clotrimazole 1%

Minimum Inhibitory Concentration (MIC)

Anti-microbial activity assay

0.5 Mc farland Standard dilution of microbes to be used for the study. 100 µl diluted log cultures of test organism (*C. albicans*– MTCC 854) was added to the micro centrifuge tube and added with 5 µl of prepared treatment dilutions of different concentrations to the defined tubes and incubated for the 24 hours. After Incubation all content was transferred to the 96 well plate and turbidity reading was taken by Elisa Plate Reader (iMarkBiorad) at 630 nm. Clotrimazole (1 %) was used as Positive Control. MIC and NIC values were estimated by using software Graph Pad Prism -6 and by using Gompertz model.

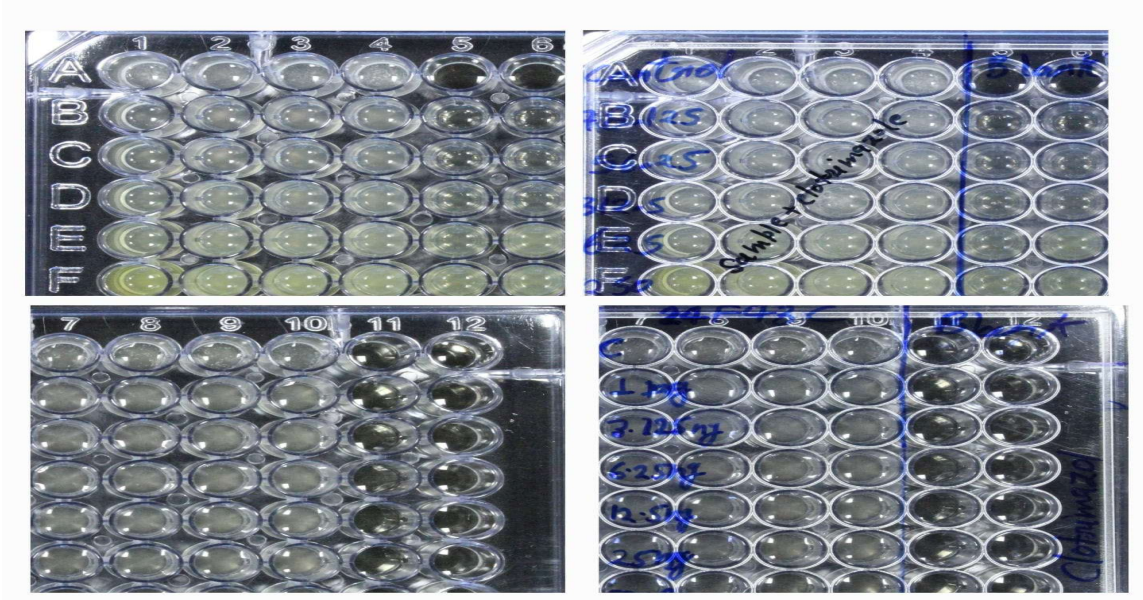

Figure 4: Elisa plate reader with different dilutions

Results

Antifungal activity of *Carica papaya* extract alone (agar diffusion)

The ethanolic *C. papaya* leaf extract exhibited concentration-dependent antifungal activity against *C. albicans* in the agar well diffusion assay (Table 2). At low doses, zones of inhibition were small or absent, whereas higher doses produced larger zones. However, the zones produced by the extract remained consistently smaller than those produced by clotrimazole 1% positive control.

Table 2:Antifungal Activity of Carica papaya against *Candida albicans*

Amount (µg/well)	Plate A	Plate B	Average	SD	SEM
PC	32	31	31.5	0.7071067812	0.5
0	0	0	0	0	0
1875	0	12	6	8.485281374	6
3750	10	15	12.5	3.535533906	2.5
7500	12	16	14	2.828427125	2
15000	14	16	15	1.414213562	1
30000	15	17	16	1.414213562	1

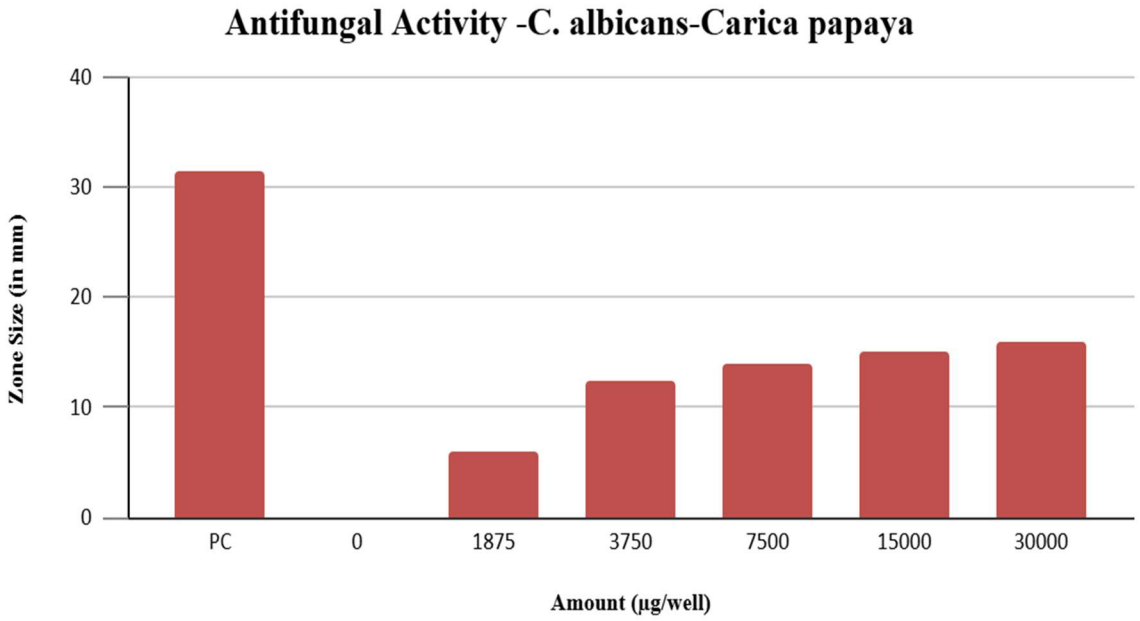

Figure 5: Antifungal activity of Carica papaya against *Candida albicans*

Inference:*Carica papaya* extract exhibits **concentration-dependent antifungal activity** against *C. albicans*, but its efficacy is **lower than the standard antifungal agent** used as the positive control

Antifungal activity of *Carica papaya* + clotrimazole combination (agar diffusion)

When *C. papaya* extract was combined with clotrimazole 1%, the zones of inhibition showed a variable pattern (Table 3). At most tested doses, the combination produced zones of inhibition smaller than those obtained with clotrimazole alone.

Table 3: Antifungal Activity of Carica papaya and Clotrimazole1% combination against *Candida albicans*

Amount (µg/well)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	32	32	32	32	0	0
0	0	0	0	0	0	0
1875	0	10	0	3.333333333	4.714045208	2.72165527
3750	10	10	10	10	0	0
7500	10	12	10	10.66666667	0.9428090416	0.544331054
15000	0	12	20	10.66666667	8.219218671	4.745368112
30000	33	14	33	33.33333333	0.4714045208	0.272165527

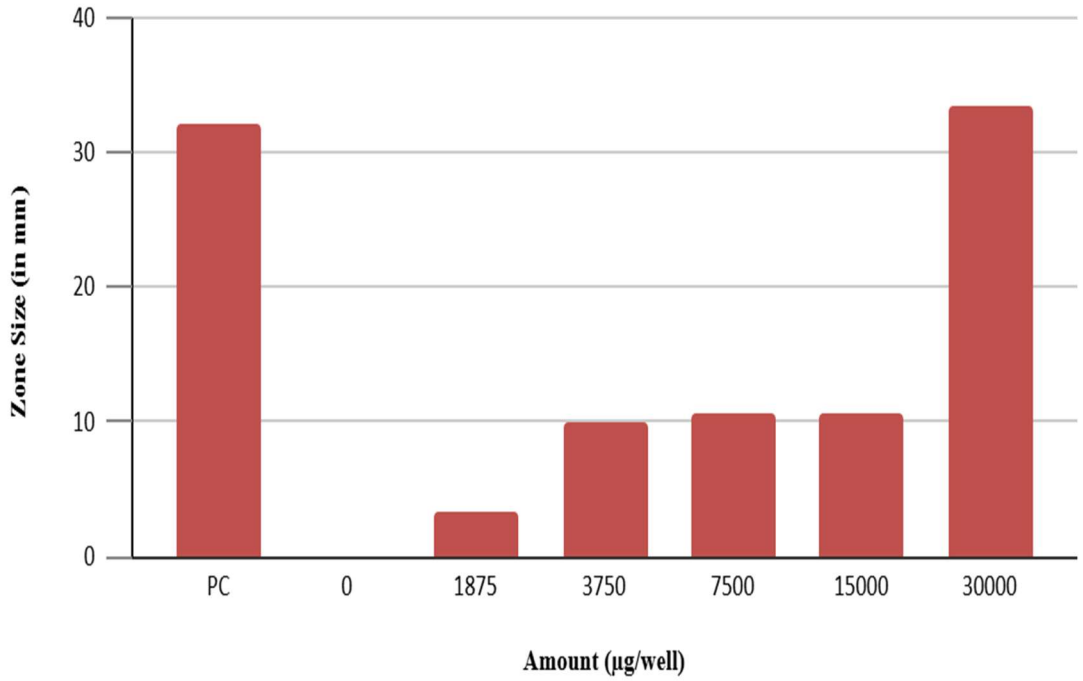

Figure 6: Antifungal activity of Carica papaya and clotrimazole combination against *C. albicans*

Inference: Although a large zone was observed at the highest tested combination dose (30,000 µg/well), overall, the combination did not consistently exceed the activity of clotrimazole alone and was often less effective, suggesting antagonistic interaction rather than clear synergy.

Table4:Zone of Inhibition of the samples

S. No.	Sample Id	Amount loaded	Max. - ZI - Average (inmm)
1.	Clotrimazole (PC)	10µl	32
2.	Carica papaya	1875 µg	6
3.	Carica papaya + Clotrimazole 1%	1875 µg	3.33

MIC and NIC values

Table 5:MIC and NIC of clotrimazole 1% mouth paint alone and in combination with *Carica papaya* leaf extract

SAMPLE	MIC	NIC
Clotrimazole 1% Mouth Paint	55.42 ng/mL	20.6 ng/mL
Clotrimazole 1% + Carica papaya Leaf Extract	359.7 µg/mL	7.733 µg/mL

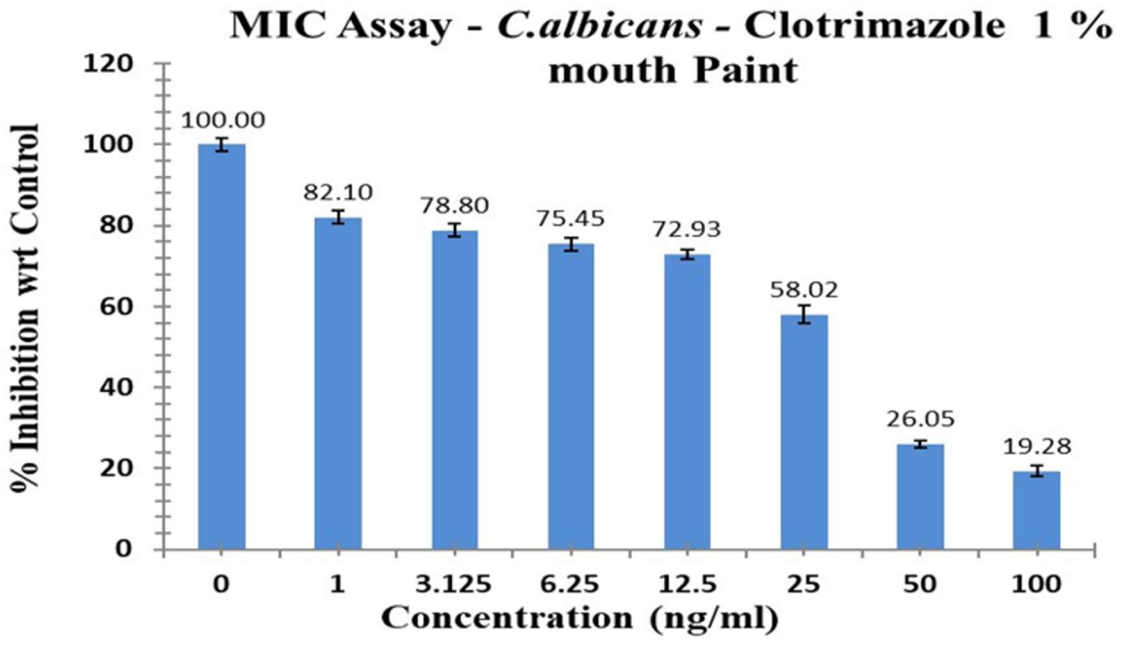

Figure 7:MIC assay of antifungal activity of Clotrimazole 1% mouth paint

Inference:Clotrimazole 1% mouth paint alone shows highest antifungal activity against *C. albicans* at lower concentrations (1–12.5 ng/ml), with inhibition decreasing significantly at higher concentrations.

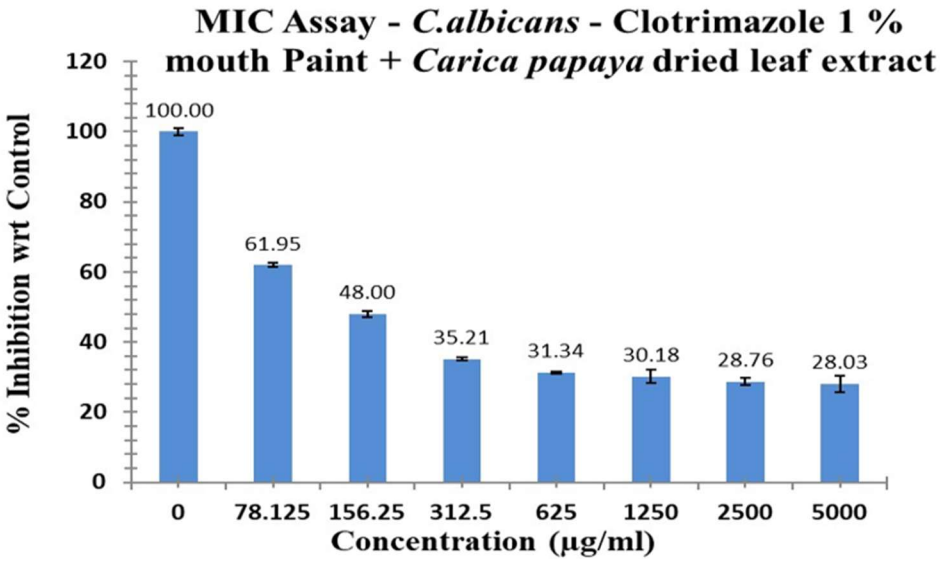

Figure 8: MIC assay of antifungal activity of Carica papaya and Clotrimazole 1% mouth paint

Inference: Clotrimazole 1% + *Carica papaya* leaf extract shows lower overall antifungal activity, with maximum inhibition of only 61.95% at 78.125 µg/ml, decreasing steadily with higher concentrations.

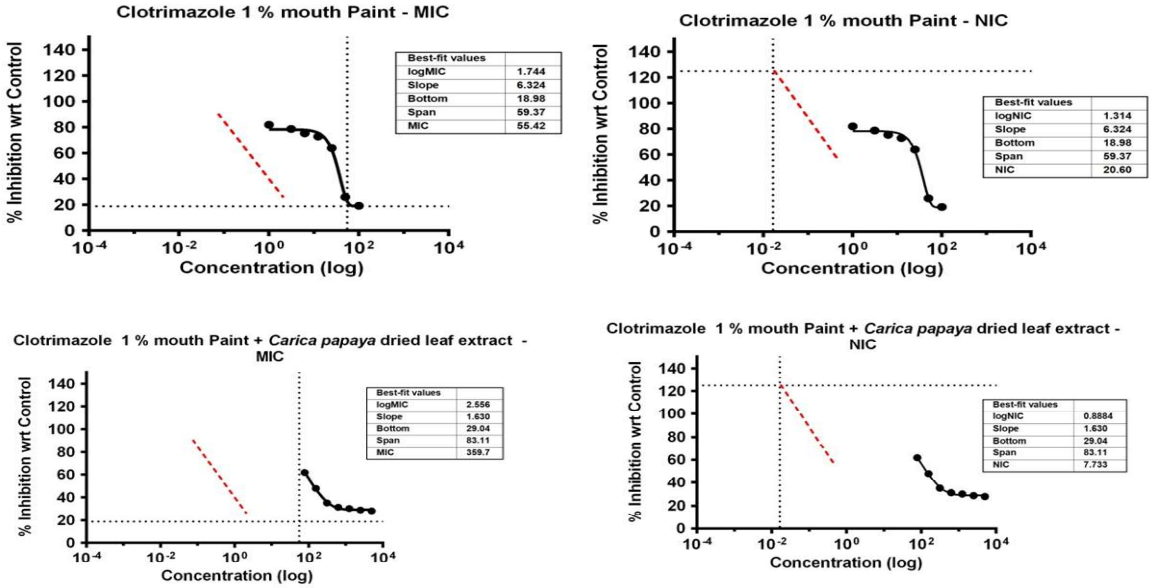

Figure 9: MIC and NIC analysis of comparative antifungal activity of clotrimazole 1% mouth paint with and without C. papaya leaf extract

Inference: MIC and NIC values of the combination is higher than Clotrimazole alone, suggesting the combination require higher concentration to achieve similar inhibition and a slight modulation in non-inhibitory threshold.

Discussions

In this study the antifungal property of ethanolic extract of Carica papaya in different concentration is demonstrated and compared with a standard drug Clotrimazole. The results were measured using Minimum Inhibitory Concentration (MIC) and Non- Inhibitory Concentration (NIC) parameters. Our study showed that the extract effectively suppressed the candidal growth and this effect increased with higher concentration of the extract. A research performed by Ana Medawati et al, in the year 2023 about the active components of *Carica papaya* showed that Papaya leaves have active compounds, such as flavonoids, alkaloids, saponins, and tannins, which can inhibit the growth of *Candida albicans* fungus through various mechanism, such as inhibition of cell wall formation, disruption of cell membranes, fungal mitochondrial dysfunction, inhibition of protein synthesis, inhibition of RNA/DNA synthesis, and inhibition of cell division of the efflux pump in the oral cavity. The increasing activity with increasing concentrations may be due to the presence of more amount of active principle in higher concentration of the ethanolic extract of Carica papaya. However, when compared with standard antifungal drug Clotrimazole it showed less efficacy. The results revealed a marked difference in the antifungal response between the two formulations. The antifungal activity of clotrimazole alone was high, with a low MIC of 56.42 ng/ml and NIC of 20.60 ng/ml. Even at low concentrations, the steep slope of the dose-response curve demonstrated a quick and effective inhibition of fungal growth, demonstrating Clotrimazole's strong efficacy and dependability as a common antifungal medication.

Synergistic antifungal activity of combination of clotrimazole and Carica papaya extract when studied, showed that the antifungal activity of clotrimazole substantially decreased. The MIC sharply increased to 359.7 µg/ml and the NIC to 7.733 µg/ml, indicating that much higher concentrations were needed to achieve similar inhibitory effects; additionally, the dose-response slope was significantly flatter, suggesting a less responsive and weaker antifungal effect; the results suggest a possible antagonistic effect between clotrimazole and phytochemicals of Carica papaya, which may interfere with clotrimazole’s mechanism of action or decrease its bioavailability. Therefore, the combination should be approached with caution. The findings of this study contradictedwith previous research on antifungal and antimicrobial activity of Clotrimazole cream incorporated with biotic material conducted by Anand P. et al, in the year 2025, who found that incorporation of Papayaseed extract into the clotrimazole formulation slightly improved its anti- fungal activity[9].The conflicting result may be due to difference in method of obtaining the extract or concentration of the extract used.Recent researches have also explored the synergistic potential of papaya leaf extract when combined with established antifungal agents. In particular, one study conducted by Nilofer Noorie et al in the year 2020, assessed the effects of papaya leaf extract used along with Fluconazole and found that the combination led to a significantly greater inhibition of fungal growth compared to fluconazole alone[10]. This suggest that certain phytochemical in papaya leaves may enhance the antifungal activity of conventional drugs opening avenues for integrated therapeutic approaches and reduce drug resistance.Additional investigations could usefully clarify the potential for papaya leaf

extract in combination therapies with standard antifungal agents such as clotrimazole and fluconazole.

From this study, we found that Carica papaya leaf extract has antifungal activity, when combined with clotrimazole its efficacy decreased. Overall, clotrimazole 1% mouth paint alone remains a more effective antifungal treatment against *Candida albicans.* Further, in-vivo studies with other fungal agent will assess the potential use of these compounds for extended therapeutic applications.

Conclusion

Although Carica papaya leaf extract alone exhibited antifungal activity against *Candida albicans*, its combination with Clotrimazole demonstrated a decreased efficacy as showed by reduced zone of inhibition andincreased MIC. These findings indicate a likely antagonistic interaction between *C. papaya* leaf extract and clotrimazole.Thus,Carica papaya cannot be assumed as a synergistic adjunct in antifungal therapy without further evidence and the combination must be used with caution. Further studies with refined concentrations and in vivo models are recommended.

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