Original Article

The influence of *Azadirachta indica*, *Melaleuca alternifolia*, and *Cocos nucifera* on *Candida albicans* strain in tissue conditioner at varying time intervals

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Abstract

Aim: The search for alternative therapies for oral candidiasis is a necessity and the use of medicinal plants seems to be one such promising solutions. Incorporation of phytotherapeutic agents, *Azadirachta indica* (neem oil), *Melaleuca alternifolia* oil (tea tree oil), and *Cocos nucifera* oil (coconut oil), were tested for their efficacy as antifungal agents against *Candida albicans*. Next, the efficacy of these three antifungal agents when incorporated in a soft relining material at minimum inhibitory concentration (MIC) was evaluated.

Settings and Design: Evaluative - *In-vitro* study design.

Materials and Methods: The MIC against *C. albicans* ATCC 24433 was calculated for *M. alternifolia* oil, *A. indica* oil, and *C. nucifera* oil using the broth microdilution method. Based on the preliminary screening results for MIC, tissue conditioner samples were prepared to evaluate the zone of inhibition (ZOI) and MIC. Antifungal activity of the MIC of the three oils was assessed and compared by measuring the mean ZOI. Antifungal activity of the three oils was assessed using one-way analysis of variance (ANOVA) and *post hoc* test.

Statistical Analysis Used: Oneway ANOVA and post hoc Tukey honestly significant difference test.

Results: Inhibition against *C. albicans* was exhibited when 20% v/v, 25% v/v, and 15% v/v of *C. nucifera* oil, *M. alternifolia* oil, and *A. indica* oil were used, respectively. The results of ANOVA and *post hoc* test at the end of 48 h and 7 days suggested that all three oils were significantly different from each other ($P = 0.000$) and *A. indica*/neem oil with 15% concentration had the best antifungal activity at the end of 48 h and 7 days.

Conclusion: The antimycotic activity of *M. alternifolia*, *C. nucifera,* and *A. indica* mixed with the Visco-gel tissue conditioner can be used as an alternative therapy for denture stomatitis.

Keywords: Antifungal, *Candida albicans*, *Cocos nucifera* oil, denture stomatitis, *Melaleuca alternifolia* oil and *Azadirachta indica* oil

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INTRODUCTION

Denture stomatitis is the most common oral opportunistic infection on the palate in elderly denture wearers. It is also the most common mucosal lesion associated with removable prostheses, which is often recurrent and characterized by inflammation or erythema on the oral mucosa of denture-bearing mucosa. In the majority of these cases, the denture wearer is unaware of any underlying problem.[1-5]

The onset and severity of *Candida*-associated denture stomatitis is of multifactorial origin.[1,6-8] It is influenced by local and systemic factors such as salivary flow, denture cleanliness, age of prosthesis, denture base material, denture trauma, continuous denture wearing, smoking, medication, endocrinopathies, immunosuppression, and metabolic and nutritional intake. *Candida*-associated denture stomatitis occurs when the conditions of the oral environment are favorable for the growth and adhesion of the yeast and also when systemic factors of the host bring about depression of the mechanism of defense.[9-13]

Denture-induced stomatitis is primarily caused by the opportunistic fungal pathogen *Candida albicans*. At least 65% of elderly denture wearers carry *Candida*, and these yeasts have been isolated from 93% of patients with denture stomatitis. There is evidence, indicating that *Candida* is able to adhere to acrylic resin dentures.[14-16] This is the first step that may lead to the development of the infectious process and that may ultimately result in varying degrees of denture stomatitis of the adjacent mucosa.[11,17,18] *Candida* adheres directly or via a layer of denture plaque to denture base (polymethylmethacrylate).[11,19] Without this adherence, microorganisms would be removed from the oral cavity when saliva or food is being swallowed.

The treatment of *Candida*-associated denture stomatitis is complex because of its multifactorial etiology.[9,20] The therapeutic strategy adopted ranges from meticulous denture cleaning to use of systemic as well as topical antifungal agents.[20-22] Poor response to topical antifungal drugs is common, due to the diluent effect of saliva, swallowing, and tongue movements. Multiple topical applications are required; hence, patient compliance is important. The widespread use of systemic medications has resulted in toxicity, drug interactions, and the development of resistant species.[22-24]

One method of overcoming these shortcomings was by incorporating medication into the denture liners.[22] Incorporation of antifungals in the tissue conditioners or denture liners is different from conventional topical antifungals, as there is gradual release of antimicrobials through the tissue conditioners to achieve an effective therapeutic concentration in infected sites, even under the diluent effects of saliva.[8,17] In addition to the added advantage of tissue recovery through the antifungals, the tissue conditioners also minimize trauma and cushion the underlying infected tissues.

Therefore, it is necessary to search for new compounds to act against these microorganisms, but in a selective and low toxicity way. Thus, several studies have been conducted on the efficacy of medicinal plants and their extracts, against these microorganisms. The use of phytomedicines can acquire meaning in therapeutic treatments. In addition, these medicinal plants may play a very important role in the treatment of denture stomatitis.[21,23,25,26]

Medicinal plants are a rich source of unique, complex, and diverse chemical structures, which warrants their thorough investigation as a potential source of novel antifungal agents as there is limited literature regarding their use intraorally. Several studies using plant essential oils have been carried out. Essential oils such as tea tree oil, lemongrass oil, citronella oil, and some of their constituents have been tested against the *in vitro* growth of *C. albicans* and found to be effective against *Candida*.[14] This study was undertaken to ascertain the antimicrobial effect of *Cocos nucifera* (coconut oil), *Azadirachta indica* (neem oil), and *Melaleuca alternifolia* (tea tree oil) and to determine the minimal inhibitory concentration required to eradicate *C. albicans* formed *in vitro* and to study the effects of these oils in tissue conditioners.

MATERIALS AND METHODS

Calculation of minimum inhibition Concentration (MIC)

Lyophilized culture of *C. albicans* was inoculated in Tryptone Soya Broth and was incubated overnight at 37°C. The turbidity of the culture was adjusted to 0.5 McFarland standard at 600 nm. *M. alternifolia* oil, *A. indica,* and *C. nucifera* oils (medical grade; Sigma-Aldrich) were primarily screened for their antifungal activities by calculating the minimum inhibitory concentration (MIC) against *C. albicans* ATCC 24433 using the broth microdilution method. In a 96-well plate, 150 µl of media was added, to which 1.5 µl of culture and 7.5 µl of different concentrations of the oils were added in the pattern as stated below [Table 1 and Figure 1].

The results were observed after 48 h of incubation at 37°C. Absorbance was calculated at 600 nm. The lowest

Table 1: Coding of samples

Composition	Coding for *Melaleuca alternifolia* oil	Coding for *Azadirachta indica* oil	Coding for *Cocos nucifera* oil
0% oil + 150 µl media + 1.5 µl culture	M-positive control	A-positive control	C-positive control
5% oil + 150 µl media + 1.5 µl culture	M-1	A-1	C-1
10% oil + 150 µl media + 1.5 µl culture	M-2	A-2	C-2
15% oil + 150 µl media + 1.5 µl culture	M-3	A-3	C-3
20% oil + 150 µl media + 1.5 µl culture	M-4	A-4	C-4
25% oil + 150 µl media + 1.5 µl culture	M-5	A-5	C-5
30% oil + 150 µl media + 1.5 µl culture	M-6	A-6	C-6
35% oil + 150 µl media + 1.5 µl culture	M-7	A-7	C-7
40% oil + 150 µl media + 1.5 µl culture	M-8	A-8	C-8
40% oil + 150 µl media + 0 µl culture	M-negative control	A-negative control	C-negative control

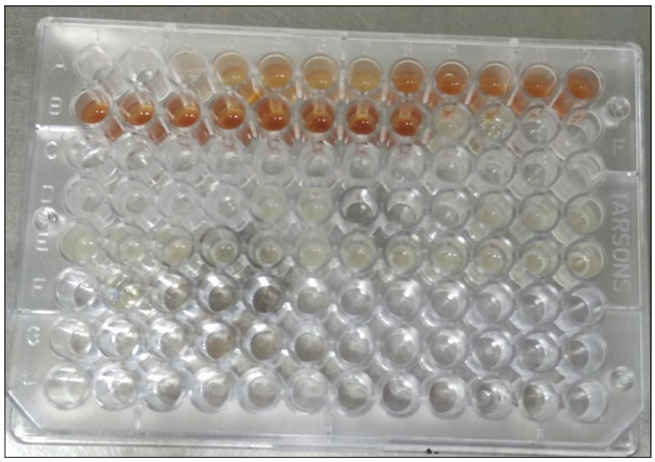

Figure 1: Experimental setup. A1, A2: A-positive control; A3, A4: A-1; A5, A6: A-2; A7, A8: A-3; A9, A10: A-4; A11, A12: A-5; B1, B2: A-6; B3, B4: A-7; B5, B6: A-8; B7, B8: A-Negative control; B9, B10: M-Positive control; B11, B12: M-1; C1, C2: M-2; C3, C4: M-3; C5, C6: M-4; C7, C8: M-5; C9, C10: M-6; C11, C12: M-7; D1, D2: M-8; D3, D4: M-Negative Control; D5, D6: C-Positive control; D7, D8: C-1; D9, D10: C-2; D11, D12: C-3; E1, E2: C-4; E-3, E-4: C-5; E-5, E-6: C-6; E-7, E-8: C-7; E-9, E-10: C-8; E-11, E-12: C- Negative Control; F1, F2: Blank

concentration of the oil needed to inhibit microbial growth compared to the positive control culture was defined as MIC.

Based on the preliminary screening results for MIC, tissue conditioner samples were prepared to evaluate the zone of inhibition (ZOI) and MIC.

Preparation of tissue conditioner samples

Samples were prepared using commercially available heat-cured acrylic resin (Trevalon) and medical-grade *M. alternifolia* oil, *A. indica* oil, and *C. nucifera* oil (Sigma-Aldrich Enterprises) for experimental groups.

A standard amount of 2.2 g of polymer to 1.8 g of liquid was used. Liquid monomer without the oils (control) and with eight different concentrations (5%, 10%, 15%, 20%, 25%, 30%, 35%, and 40%) of three oils (*M. alternifolia*, *A. indica*, and *C. nucifera*) was then mixed with the powder in the ratio suggested by the manufacturer to formulate the autopolymerized tissue conditioner samples (Visco-gel). Customized flask was designed and constructed. This flask consisted of six disc spaces which had the desired dimensions of 5 mm depth and 30 mm diameter, in which the heat-polymerized acrylic resin samples were made. The designed flask consisted of an upper member which had disk spaces with a measurement of 3 mm depth and 30 mm diameter, in which the autopolymerizing tissue conditioner and the modified tissue conditioner were incorporated and allowed to cure. Tissue conditioner samples each with a thickness of 3 mm were then applied to one side of the heat-cured denture base resin.

Zone of inhibition

Inoculation of agar plates with Candida albicans

Diluted *C. albicans* solution (0.5 ml) was dropped on each sterile Tryptone Soya agar plate, and a lawn culture was made. Three wells (7-mm deep, 5 mm in diameter) were created in each agar plate for all concentrations of materials to be tested. Pure *M. alternifolia* oil, *A. indica* oil, and *C. nucifera* oil (as antifungal agents) mixed in Visco-gel tissue conditioner and coated on heat-cured denture base resin were used in this study. Control disc was soaked in sterile distilled water.

Melaleuca alternifolia oil mixed with Visco-gel

Different concentrations of the oil mixed in the tissue conditioner and coated on the heat-cured denture base resin were placed into the punch holes (5-mm diameter) in the inoculated petri plates. Plates were incubated at 37°C for 7 days. Mean inhibition diameter for each test punch hole was measured in millimeters across the punch hole at 48 h and on day 7 using a metal graduated ruler.

Azadirachta indica oil mixed with Visco-gel

A similar procedure was followed for combinations of *A. indica* oil with Visco-gel in different concentrations.

Cocos nucifera oil mixed with Visco-gel

A similar procedure was followed for combinations of *C. nucifera* oil with Visco-gel in different concentrations.

The MIC in each of the three groups of oils was assessed and verified. Antifungal activity of the MIC of the three oils was assessed and compared by measuring the mean ZOI as the parameter at 48 h and on day 7 while carrying out the monitoring every day.

RESULTS

The MIC for the *C. nucifera* oil, *M. alternifolia* oil, and *A. indica* oil was calculated using the broth microdilution method. It was found that inhibition against *C. albicans* was exhibited when 20% v/v, 25% v/v, and 15% v/v of *C. nucifera*/coconut oil, *M. alternifolia* oil, and *A. indica* oil was present in the media, respectively [Figures 2-4].

Melaleuca alternifolia/tea tree oil

To test the susceptibility of *C. albicans* against the most effective concentration of *M. alternifolia* oil mixed in the tissue conditioner, the mean ZOI of different concentrations (% v/v) of *M. alternifolia* oil in tissue conditioner (n = 20 each) was recorded and analyzed. It was observed that the percentage increase in the mean ZOI from 20% to 25% concentration was the highest (137.50%). The analysis of variance (ANOVA) followed by *post hoc* test showed that at 48 h, 25% concentration was significantly (P = 0.000) more effective than all other concentrations tested [Table 2]. At the end of 7 days, the mean ZOIs of different concentrations were once again analyzed. It was noted that though there was a decrease in the mean ZOI of respective concentrations, it was maintained within the same range, suggesting that the antifungal activity was present even at the end of 7 days. Furthermore, the percentage increase in the mean ZOI from 20% to 25% concentration was again found to be the highest (134.45%). The ANOVA followed by *post hoc* test showed that 25% concentration was significantly (P = 0.000) more effective than all other concentrations tested [Table 3].

Table 2: The percentage increase in the mean zone of inhibition of *M. alternifolia* oil At 48 hrs

Concentration of *M. alternifolia* oil (%)	Mean (mm)	Percentage of increase
5	5	100.00
10	5	100.00
15	5.2	104.00
20	6.4	123.08
25	8.8	137.50
30	10.6	120.45
35	13	122.64
40	15.35	118.08

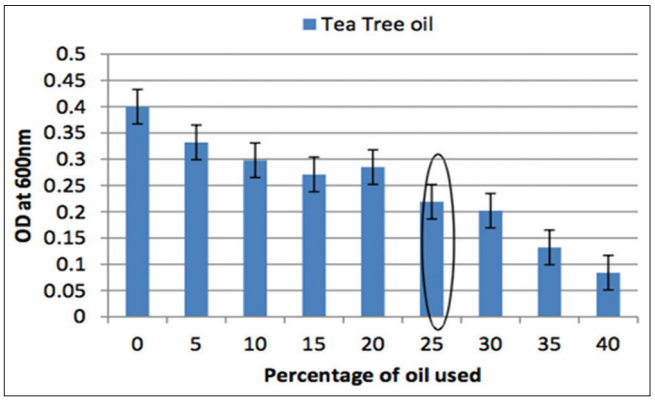

Figure 2: Minimum inhibitory concentration against *Candida albicans* was observed when 25% tea tree oil is present in the media

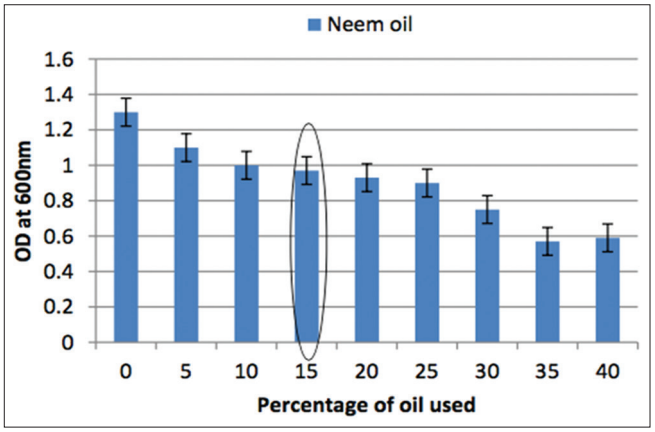

Figure 3: Minimum inhibitory concentration against *Candida albicans* was observed when 15% neem oil is present in the media

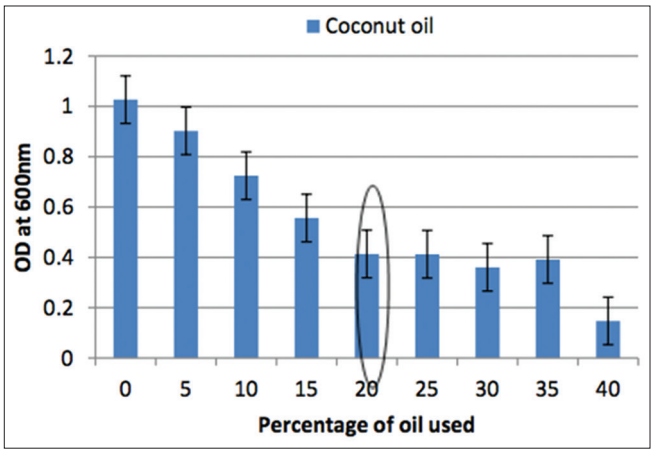

Figure 4: Minimum inhibitory concentration against *Candida albicans* was observed when 20% coconut oil is present in the media

Azadirachta indica/neem oil

Similarly, the mean ZOI for different concentrations of *A. indica*/neem oil was measured and analyzed. It was observed that the percentage increase in the mean ZOI from 10% to 15% concentration was the highest (146.11%). The ANOVA followed by *post hoc* test showed that at 48 h, 15% concentration was significantly ($P = 0.000$) more effective than all other concentrations of *A. indica* oil tested [Table 4].

A. indica oil group also presented with significant antifungal activity at the end of 7 days, though there was a decrease in the mean ZOI of respective concentrations. The mean ZOIs of different concentrations were once again analyzed. The percentage increase in the mean ZOI from 10% to 15% concentration was still observed to be the highest (147.40%). The ANOVA followed by *post hoc* test showed that at the end of 7 days, 15% concentration was significantly ($P = 0.000$) more effective than all other concentrations tested [Table 5].

Cocos nucifera/coconut oil

Similarly, the percentage increase in the mean ZOI from 15% to 20% concentration was the highest (152.78%). The ANOVA followed by *post hoc* test showed that at 48 h, 20% concentration of *C. nucifera*/coconut oil was significantly ($P = 0.000$) more effective than all other concentrations tested [Table 6].

The *C. nucifera* group also presented with antifungal activity at the end of 7 days. The percentage increase in the mean ZOI from 15% to 20% concentration was observed to be the highest (149.23%). The ANOVA followed by *post hoc* test showed that even at the end of 7 days, 20% concentration was significantly ($P = 0.000$) more effective than all other concentrations tested [Table 7].

To compare the antifungal activity of the three oils mixed with the tissue conditioner, the mean ZOI of the MIC of each oil was taken as the parameter at 48 h [Table 8] and on day 7 [Table 9] while carrying out the monitoring every day. The results of ANOVA and *post hoc* test at the end of 48 h and 7 days suggested that all three oils were significantly different from each other ($P = 0.000$) and *A. indica*/neem oil with 15% concentration had the best antifungal activity at the end of 48 h and also 7 days [Table 10].

Statistical analysis

Melaleuca alternifolia oil

At 48 hours

The statistical tool one-way ANOVA was used. To verify the MIC, the percentage increase in the mean ZOI was calculated and the results are given in Table 2.

Table 3: The percentage increase in the mean zone of inhibition of *M. alternifolia* oil At 7 days

Concentration of *M. alternifolia* oil (%)	Mean (mm)	Percentage of increase
5	5	100
10	5	100
15	5	100.00
20	5.95	119.00
25	8	134.45
30	9.75	121.88
35	12.55	128.72
40	14.75	117.53

Table 4: The percentage increase in the mean zone of inhibition of *A indica* oil at 48 hrs

Concentration of *A.Indica* oil (%)	Mean (mm)	Percentage of increase
5	6.35	127.00
10	8.35	131.50
15	12.20	146.11
20	13.60	111.48
25	15.20	111.76
30	17.00	111.84
35	19.20	112.94
40	20.10	104.69

Table 5 :The percentage increase in the mean zone of inhibition of *A indica* oil at 7 days

Concentration of *A.Indica* oil (%)	Mean (mm)	Percentage of increase
5	6.10	122.00
10	7.70	126.23
15	11.35	147.40
20	12.90	113.66
25	14.60	113.18
30	15.95	109.25
35	18.75	117.55
40	19.15	102.13

Table 6 :The percentage increase in the mean zone of inhibition of *C. nucifera* oil at 48 hrs

Concentration	Mean (mm)	Percentage of increase
5	5.00	100.00
10	5.20	104.00
15	7.20	138.46
20	11.00	152.78
25	12.30	111.82
30	13.75	111.79
35	14.40	104.73
40	16.55	114.93

Since the maximum percentage of increase was noted for the concentration level 25%, it was verified that the MIC for *M. alternifolia* oil is 25%.

After 7 days

To verify the MIC, the percentage increase in the mean inhibition zone was calculated and the results are given in Table 3.

Table 7: The percentage increase in the mean zone of inhibition of oil at *C nucifera* oil 7 days

Concentration	Mean (mm)	Percentage of increase
5	5	100.00
10	5	100.00
15	6.5	130.00
20	9.7	149.23
25	11.8	121.65
30	13.3	112.71
35	13.15	98.87
40	16.2	123.19

Table 8: Mean zone of inhibition of minimum inhibitory concentration at 48 h

Oil (%)	Mean ZOI (mm)
C.Nucifera oil (20%)	11.000
A.Indica oil (15%)	12.200
M. alternifolia oil (25%)	8.800

ZOI: Zone of inhibition

Table 9: Mean zone of inhibition of minimum inhibitory concentration at 7th day

Oil (%)	Average (mm)
C.Nucifera oil (20%)	9.7
A.Indica oil (15%)	11.35
M. alternifolia oil (25%)	8.000

Table 10: The anti fungal activity of the minimum most effective concentration of the three oils at the end of 7 days

Oil	Mean difference	
	A.Indica oil	*M. alternifolia* oil
C.Nucifera oil	−1.650*	1.700*
A.Indica oil		3.350*

*Significant at 5% level

Since the maximum percentage of increase was noted for the concentration level 25%, it was verified that the MIC level for *M. alternifolia* oil after 7 days was 25%.

Azadirachta indica oil

At 48 hours

To verify the MIC, the percentage increase in the mean inhibition zone was calculated and the results are given in Table 4.

The maximum hike occurred from 10% to 15% (146.11%), verifying that the MIC for *A. indica* oil is 15%.

After 7 days

To verify the MIC, the percentage increase in the mean inhibition zone was calculated and the results are given in Table 5.

The maximum hike occurred from 10% to 15% (147.40%), verifying that the minimum most effective level of concentration for *A. indica* oil at the end of 7 days was 15%.

Cocos nucifera oil

At 48 hours

To verify the MIC, the percentage increase in the mean inhibition zone was calculated and the results are given in Table 6.

The percentage increase in the mean minimum inhibitory zone from 15% to 20% was the highest (152.78%) and was occurring for 20%. Hence, it was verified that the MIC level for *C. nucifera* oil is 20%.

After 7 days

To verify the MIC, the percentage increase in the mean inhibition zone was calculated and the results are given in Table 7.

The percentage increase in the mean minimum inhibitory zone from 15% to 20% was the highest (149.23%) and was occurring for 20%. Hence, it was verified that the minimum most effective concentration level for *C. nucifera* oil at the end of 7 days was 20%.

To compare the antifungal activity of the three oils mixed with the tissue conditioner, the mean ZOI of the MIC of each oil was taken as the parameter at 48 h and on day 7 while carrying out the monitoring every day. ANOVA was performed and it shows that there was a significant difference in the measurement level because the significance value (*P* value) is 0.000, which is less than the level of significance 0.05.

The mean ZOI of these three oils at their MIC level at 48 h is given in Table 8.

Table 8 shows that *A. indica*/neem oil with 15% concentration can be considered to have the best antifungal activity at the end of 48 h.

The mean ZOI of these three oils at their MIC level at day 7 is given in Table 9.

The results of ANOVA and post hoc test at the end of 48 hrs and 7 days suggested that all three oils were significantly different from each other and *Azadirachta indica* / Neem oil with 15% concentration had the best anti fungal activity at the end of 48 hours and also 7days

DISCUSSION

A successful therapy can be defined as "a suitable agent prescribed to treat the right organism at appropriate dosage." One of the most promissory sources to the research of new agents is found in plants, which have compounds with antimicrobial properties that is being studied by diverse researchers, but many of these compounds are not known yet. The investigation of these active principles, once they have different targets that were found in the antifungal in use, is a potential area that must be studied.[26] Hence, this study was undertaken to identify if *C. nucifera*, *A. indica,* and *M. alternifolia* have any antifungal potential.

The results of the present *in vitro* study revealed that the tissue conditioner, Visco-gel, alone did not demonstrate inhibition of *C. albicans* growth. This is consistent with the studies done by Kulak-Ozkan *et al.*[13] and Catalan.[31] Most tissue conditioners have maximum effect between 24 and 72 h. It has been reported that tissue conditioners continue to flow for 7 days and suggested that they are clinically effective throughout this period. Therefore, study time parameters of 48 h and day 7 were chosen,[27,28] and Visco-gel, which is a commonly used tissue conditioner, was used in the study.[28]

The plant *M. alternifolia* has been used as an antiseptic remedy for decades. The essential oil of *M. alternifolia*, termed tea tree oil, contains almost a hundreds of components, the majority of which are monoterpenes and related alcohols. It has a minimum content of 30% of terpinen-4-ol and a maximum content of 15% of 1,8-cineole. Terpinen-4-ol is a major *M. alternifolia* component and exhibits strong antimicrobial and anti-inflammatory properties. In preliminary trials, de Campos Rasteiro *et al.*[28] suggest that *M. alternifolia* formulations may be effective in the treatment of acne, fungal infections, pustules, recurrent herpes labialis, and in bacterial pathogen decolonization protocols. The antimicrobial activity of *M. alternifolia* is attributed to its ability to denature proteins and alter the properties and function of the cell wall membrane, leading to the loss of intracellular components and eventually cell death.[29,30] Tea tree oil can be used as an alternative to or in combination with conventional drugs (including antibiotics and chemotherapeutic agents).[30] The findings of this study suggest that *M. alternifolia* oil demonstrated a strong and inhibitory fungicidal activity of *C. albicans* and this is in accordance with the studies conducted by Sharma and Hegde, Mertas *et al.*, Catalán *et al.*, and Rawat *et al.*[28,30-32]

The MIC of tea tree oil that showed significant antifungal activity was found to be at 25% v/v tea tree oil at 48 h and at 7 days.

It was observed that the percentage increase in the mean ZOI from 20% to 25% concentration was the highest. At the end of 7 days, it was noted that though there as a decrease in the mean ZOI of respective concentrations, it was maintained within the same range, suggesting that the antifungal activity was present even at the end of 7 days.

The results of the present microbiologic *in vitro* study suggest that 20% v/v coconut oil when mixed with Visco-gel showed substantial antifungal activity, resulting in complete inhibition of *C. albicans* growth after 24 h ($P < 0.001$). Thus, 20% coconut oil in Visco-gel was the MIC and was considered for further comparison. This was in accordance with the study conducted by Ogbolu *et al.*,[33] who studied the antimicrobial properties of coconut oil; Shino *et al.*,[34] who studied the effect of coconut oil on early childhood caries and concluded that it has shown antifungal activity that is comparable to that of ketoconazole; and Kannan and Mohammed,[35] who studied the antifungal activity of coconut oil in the oral cavity. An important finding of the study was that the anti fungal activity continued unabated even at the end of 7 days,and 20 % concentration was significantly ($P = 0.000$) more effective than all other concentrations tested, implying that it remains active within its tissue conditioner polymeric structure for at least 7 days.

The results of the present study are in accordance with the study conducted by Krishnamoorthy *et al.*,[36] which revealed that there was a gradual reduction in the colonization of *C. albicans* in all the antifungal agents, when compared between the 24-h incubation period and the 5th-day incubation period. This clearly proves that leaching of tissue conditioner when incorporated with antifungal agents is slow, consistent, and gradual, which was in accordance with the study by Graham *et al.*,[37] who have stated in their *in vitro* study that tissue conditioners flow for a period of 7 days and that they are clinically effective throughout this period.

A. indica (Neem), a tropical tree in the Indian subcontinent, has been used for its medicinal properties in the Ayurvedic medicine for more than 4000 years.[38,39]

In another study, the antibacterial activity of natural extracts such as neem leaf and grape extracts were compared with sodium hypochlorite and it was concluded that neem leaf extracts and grape seed extracts showed

zones of inhibition suggesting that they had antimicrobial properties.[37,39]

This is in accordance with the results of our study. Yerima *et al.* studied the effect of bacteria isolated from the mouth using different parts of *A. indica* (neem) and they concluded that bark and leaf extracts showed antibacterial activity against all the test bacteria used.[40] This is in accordance with the results of our study, where neem bark extract (Sigma-Aldrich) was used for evaluating the antifungal activity. Tyagi *et al.*[41] and Hegde and Kesaria[42] in a study on root canal irrigants concluded that neem leaf extract has a significant effect against *C. albicans*. Kumar *et al.*[43] confirmed the findings of this study and stated soft lining materials incorporated with antifungal agents have the potential to act against *C. albicans*.

When all the three oils were compared, it was found that neem oil had the best antifungal activity at 48 h and 7 days. The difference in MIC between this study and others could be attributed to the microbiological method used for evaluating the oils and whether an aqueous or ethanol extract was used to conduct the study.

The present study was performed under controlled laboratory conditions; therefore, *in vivo* studies are suggested for affirming the results. Further studies should be conducted to test the effect of these antifungals on the physical and mechanical properties of the tissue conditioner. Further drug development programs could be undertaken to investigate the bioactivity, mechanism of action, and pharmacokinetics of these compounds.

CONCLUSION

Adding fungicidal compounds directly to the tissue conditioners could be studied *in vivo* as a method for treating *Candida*-associated denture stomatitis. The antimycotic activity of *M. alternifolia*, *C. nucifera,* and *A. indica* mixed with the Visco-gel tissue conditioner can be used as an alternative therapy for denture stomatitis. Future research should be performed to determine changes that can occur in the physical properties of tissue conditioners.

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Conflicts of interest

There are no conflicts of interest.

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