

To study the effect of *Cocos nucifera* oil when incorporated into tissue conditioner on its tensile strength and antifungal activity: An *in vitro* study

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Abstract

Aim: This study aims to test the tensile strength and growth of *Candida albicans* on Viscogel tissue conditioner when incorporated with coconut oil (CCO) and to compare its efficacy with other antifungal agents.

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

Materials and Methods: Fifty dumbbell-shaped samples ($n = 10$) of Viscogel tissue conditioner were fabricated according to ASTM standard and were classified into 5 groups (10% CCO, 30% w/w tea tree oil, 5% w/w fluconazole, 0.03% w/w silver nanoparticles, and plain tissue conditioner). These samples were compared and evaluated for their tensile strength. Further to test the antifungal activity, a total of 60 samples ($n = 15$) were fabricated, each group ($n = 15$) was further divided into three subgroups ($n = 5$), namely 24-h, 3-day, and 5-day period, which were inoculated in sabouraud dextrose agar plate to test for the growth of *C. albicans*. **Statistical Analysis Used:** Oneway ANOVA and *post hoc* Tukey honestly significant difference test.

Results: 10% w/w CCO yielded a mean tensile strength of 20.06 as compared to the plain tissue conditioner which showed a mean tensile strength of 17.81. Similarly, 10% w/w CCO incorporated into Viscogel tissue conditioner showed a significant reduction in the colonization of *C. albicans* on the 5th day.

Conclusions: 10% w/w of CCO when mixed with Viscogel tissue conditioner showed a significant reduction in the growth of *C. albicans*, and addition of the same increased the tensile strength of the tissue conditioner.

Keywords: Antifungal agents, *Candida albicans*, coconut oil, tensile strength, tissue conditioner

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INTRODUCTION

As the access to dental care is improving, people are retaining their natural teeth for a longer period of time, but the incidence of edentulousness still remains significant. Removable dental prosthesis has been and remains the

basic treatment for patients with complete or partial edentulousness. The need for long-term use of removable prosthesis can bring about profound changes in the oral cavity, and one such commonly encountered sequel of wearing removable prosthesis is denture stomatitis.^[1]

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Successful treatment plan for denture stomatitis includes elimination of the local irritating factors, treatment of the abused tissue, diet counseling, systemic evaluation, replacement of the old prosthesis, establishment of proper occlusal scheme and correction of any occlusal prematurities, meticulous maintenance of oral hygiene, and use of soft tissue liners.

Lately, tissue conditioners have been used as a drug delivery tool for elderly patients suffering from denture stomatitis caused due to *Candida* infection, as the success of topical application of drugs in the oral cavity may be compromised by lack of patient compliance.^[2,3] Furthermore, maintaining the regular and optimal dose of topical antifungal agents is challenging in geriatric denture wearers due to reduced motor skills, cognitive impairment, and memory loss.^[4] To overcome these difficulties, antifungal agents have been incorporated into soft tissue liners and are checked for their effectiveness against *Candida albicans*.

There are many published reports on antifungal agents such as nystatin, azole group derivatives, chlorhexidine, metallic oxide powders, photocatalysts, and silver nanoparticles incorporated into tissue conditioners, which have been reported to have varying degree of success.^[1,5,6-15] However, due to the resistance developed by *Candida* species toward these drugs, therapeutic effects of plant oils that have antifungal properties are being explored.

A number of studies have been carried out which have investigated the effect of natural and herbal antimicrobials against *C. albicans* by incorporating them into tissue conditioners, for example, tea tree oil (TTO), lemongrass oil, thai herbs, and origanum oil.^[16-21]

One such herbal oil that has both antifungal and antimicrobial property is *Cocos nucifera* or commonly known as coconut. Coconut oil (CCO) has been proven to have antimicrobial and antifungal property against *Candida* species including *C. albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei*.^[22]

What makes CCO unique as compared to other oils is the presence of medium chain fatty acids as compared

to the long chain fatty acids present in other edible oils. CCO has 92% saturated fatty acids, of which 50% is lauric acid. It has been postulated that lauric acid along with other medium chain fatty acids has the capacity to modify the cell wall of microorganisms, disrupt their cellular mechanism by infiltrating the cell membrane, and obstruct the enzymes involved in energy production and nutrient transfer, leading to the death of the microorganism.^[23]

Thus, the purpose of this *in vitro* study was to analyze the effect of CCO on *C. albicans* by incorporating it into tissue conditioners and compare its efficacy with TTO, silver nano particles (SNPs), and fluconazole (FLU).

The null hypothesis was that there will be no antifungal activity exhibited toward *C. albicans* and no change in the tensile strength of Viscogel tissue conditioner when incorporated with CCO.

MATERIALS AND METHODS

Viscogel tissue conditioner incorporated with CCO, TTO, SNPs, and FLU were used as samples [Table 1].

Methodology to determine the optimum concentration of coconut oil

A pilot study was performed to determine the optimum concentration of CCO, at which the antifungal testing could be carried out without altering the physical properties of the tissue conditioner. The pilot study involved incorporating four different concentrations of CCO into the tissue conditioner and evaluating its effect on its physical property. The concentration which yielded the maximum tensile strength under maximum load was preferred for the antifungal study.

Preparation of mold

A total of 12 wax samples ($n = 3$) were fabricated using modeling wax (Hindustan Modeling Wax No. 2, The Hindustan Dental Products, Hyderabad, India) according to the ASTM standard (ASTM D412) with a cross-sectional area of 20 mm × 6 mm × 3 mm. These wax samples were invested in the lower part of the flask with type IV dental stone or die stone (Kalrock; Kalabhai Karson Pvt, Ltd), flaked, and dewaxed to form molds.

Table 1: Materials used in the study

Type	Material	Concentration (%)	Code	Manufacturer
Tissue conditioner	Viscogel tissue conditioner	-	-	DENTSPLY DeTrey GmbH, Germany
Antifungal agent	Coconut oil	10	CCO	Falcon, Bangalore, India
Antifungal agent	Tea tree oil	30	TTO	Falcon, Bangalore, India
Antifungal agent	Fluconazole	5	FLU	Aurobindo Pharma Ltd, Hyderabad, India
Antifungal agent	Silver nanoparticles	0.03	SNP	Aadarsh innovations, Pune, India

Preparation of samples to test tensile strength

One control group of tissue conditioner with no CCO incorporated and three test groups with varying concentrations of CCO (10% w/w, 20% w/w, and 30% w/w) were added to 3 g of tissue conditioner powder and 2.2 ml of liquid, hand mixed in a plastic cup with a mixing spatula as per manufacturer's recommendation. The tissue conditioners were homogeneously manipulated and loaded into the lower part of the mold, flaked, and allowed to gel. The set samples were retrieved, and excess was trimmed with a Bard-Parker Knife [Figure 1].

The 12 samples ($n = 3$) thus obtained were categorized into 4 groups and stored in distilled water in 4 separate plastic beakers at 37°C for 7 days, which were changed regularly.

The samples were grouped as follows:

- Group 1: Control group
- Group 2: 10% CCO
- Group 3: 20% CCO
- Group 4: 30% CCO.

On the 7th day, the maximum tensile strength evaluation was carried out for all the groups using an Instron Universal testing machine (Model 3366, Instron Corp., Canton, Mass.) at a rate of 40 mm/min. The tissue conditioner with 10% w/w of CCO exhibited a mean tensile strength of 23.31 as compared with the 20% w/w and 30% w/w concentration of CCO which showed a mean tensile strength of 18.78 and 14.00, respectively. Hence, 10% w/w of CCO was preferred for further antifungal testing.

Similarly, tensile strength evaluation and comparison of 10% w/w CCO with 5% w/w FLU, 30% w/w TTO, and 0.03% w/w SNP were carried out using the same method. A total of 50 samples ($n = 10$) were made, which were grouped as follows:

- Group A: 10% w/w CCO
- Group B: 30% w/w TTO

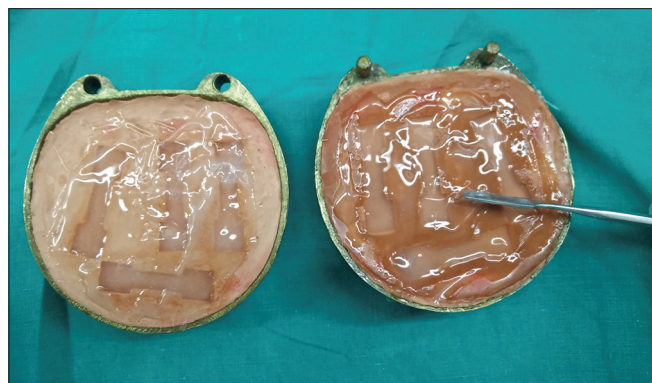


Figure 1: Preparation of samples to test tensile strength

- Group C: 5% w/w FLU
- Group D: 0.03% w/w SNP
- Group E: plain tissue conditioner which acted as a control group.

Preparation of samples to test antifungal effect

Preparation of mold

Thirty circular wax patterns 8 mm in diameter and 1.2 mm in cross-section were fabricated using modeling wax (Hindustan Modeling Wax No. 2, The Hindustan Dental Products, Hyderabad, India) and arranged on a glass slab. An impression of the arranged wax patterns was made using putty impression material (GC Flexceed). The obtained putty mold was utilized for making a stone model. A vacuum forming thermoplastic sheet (TJ dental, Mumbai, India) was placed over the stone mold and molded under vacuum, using a vacuum-forming unit (Dunaform, Al Dente). The formed thermoplastic sheet was retrieved and subsequently used as a mold to prepare the samples for microbiological testing.

Preparation of the samples

Mixing coconut oil with Viscogel tissue conditioner

10% w/w of CCO was hand mixed with Viscogel tissue conditioner according to manufacturer's instructions in a plastic mixing cup. With the help of a mixing spatula, the tissue conditioner mixture was applied over the thermoplastic sheet mold and allowed to gel. A total of 15 samples were made [Figure 2].

The set samples were placed into 2 ml cryovials which were labeled as follows:

- 1CCO1–1CCO5 = 5 samples for the 1st day culture
- 3CCO1–3CCO5 = 5 samples for the 3rd day culture
- 5CCO1–5CCO5 = 5 samples for the 5th day culture.



Figure 2: Preparation of samples for microbiological study

Similarly, 30% w/w of TTO, 5% w/w of FLU, and 0.03% SNP were mixed homogeneously with Viscogel tissue conditioner according to manufacturer's instructions and allowed to gel. The obtained samples were placed separately in 2 ml cryovials and labeled accordingly.

Therefore, a total of 60 samples ($n = 15$) were made, which were stored in 2 ml cryovials and kept for ultraviolet sterilization.

Preparation of inoculum

Standard strain of ATCC 24433 *C. albicans* was inoculated into Sabouraud Dextrose broth (Himedia Laboratories) and incubated at 37°C. After 8 h, *C. albicans* suspension was standardized by dilution with sterile broth to a density visually equivalent to barium sulfate standard 0.5 McFarland.

Inoculation of samples with *Candida albicans*

In 2 ml cryovials, 1 ml of Sabouraud dextrose agar broth was filled using the P1000 micropipette, and 10 µl of diluted *C. albicans* was dropped into each vial using a P10 micropipette. These vials were then stored in an incubator under 37°C for 1, 3, and 5 days.

Inoculation of agar plate using spot inoculation method 24-h inoculation

The agar plate was divided into a total of 20 boxes, i.e., 5 columns – one for each sample and 4 rows – one for each group of antifungal agent. 10 µl of *C. albicans* Sabouraud Dextrose Agar broth solution was pipetted out from the cryovials labeled 1CCO1–1CCO5, 1FLU1–1FLU5, 1SNP1–1SNP5, and 1TTO1–1TTO5 using a P10 micropipette and spot inoculated into each box [Figure 3]. The agar plate was sealed using Parafilm M sealing tape to prevent cross contamination and stored in an incubator

at 37°C for 24 h. The plate was examined for growth of *C. albicans* after 24 h.

Similar procedure was carried out for the 3rd and 5th day inoculation by obtaining the inoculum from the labeled 3rd-day and 5th-day cryovials, respectively. Both the agar plates were examined after 24 h interval to evaluate the growth of *C. albicans*.

The 24-h, 3rd-day, and 5th-day agar plates were further compared for the effective antifungal property of each agent against *C. albicans*.

RESULTS

Study demonstrated 10% w/w of CCO to have the mean tensile strength of 23.31 as compared with the 20% w/w and 30% w/w concentration of CCO which showed a mean tensile strength of 18.78 and 14.00, respectively. Hence, 10% w/w of CCO was preferred for further antifungal testing.

Descriptive statistical analysis of mean tensile strength and standard deviation of each group in Table 2 demonstrates SNP to have a mean tensile strength of 21.63, followed by CCO which shows a mean tensile strength of 20.06, FLU showing 18.55 and plain tissue conditioner exhibiting a mean tensile strength of 17.81. TTO exhibited the least mean tensile strength of 10.58.

Hence, adding 10% w/w of CCO to Viscogel tissue conditioner increases its tensile strength as compared to that of plain Viscogel tissue conditioner.

One-way ANOVA analysis followed by *post hoc* Tukey honestly significant difference test was carried out between the groups for assessing the significant difference between any of the five groups [Tables 3 and 4]. The significance level was set at $P < 0.05$. There was no statistical difference



Figure 3: Inoculation of agar plate using spot inoculation method

Table 2: Descriptive statistics of tensile strength and standard deviation of each group

Group	n	Mean	SD	SE	Minimum	Maximum
A	10	20.0600	2.03740	0.64428	17.44	23.01
B	10	10.5800	0.80954	0.25600	9.52	11.72
C	10	18.5590	2.47627	0.78306	16.00	22.55
D	10	21.6350	2.60470	0.82368	18.62	25.50
E	10	17.8170	0.94701	0.29947	16.85	19.35
Total	50	17.7302	4.26789	0.60357	9.52	25.50

SD: Standard deviation, SE: Standard error

Table 3: One-way ANOVA analysis

	Sum of squares	df	Mean square	F	Significance
Between groups	724.952	4	181.238	48.669	0.000

Table 4: Post hoc Tukey honestly significant difference analysis to statistically compare the tensile strength between Groups A, B, C, D, and E

Group (I)	Group (J)	Mean difference (I-J)	SE	P
A	B	9.48000*	0.86301	0.000
	C	1.50100	0.86301	0.421
	D	-1.57500	0.86301	0.372
	E	2.24300	0.86301	0.088

* $P < 0.05$ significance difference. SE: Standard error

between the test group, i.e., 10% CCO with 5% w/w FLU and 0.03% w/w SNP ($P > 0.05$), whereas there was a significant difference between the 10% CCO and 30% w/w TTO ($P < 0.05$).

The microbiological study carried out to determine the antifungal activity of Viscogel tissue conditioner against *C. albicans* when incorporated with 10% w/w of CCO, displayed a gradual and steady decrease in the growth of *C. albicans* when incubated for 24 h, 3 days, and 5 days [Figures 4-6].

DISCUSSION

Denture stomatitis is an opportunistic infection related to an inflammatory process which compromises the mucosal surface beneath the dentures. Although the etiology is multifactorial, *C. albicans* is the only microorganism with an established pathogenic role.

A possible initial step in the treatment of denture stomatitis is advising the patient to wear dentures only for few hours in a day; however, this is not practical for the patient. Due to its multifactorial etiology, the management of Candida-associated denture stomatitis is also intricate. Topical application of antifungal drugs can be ineffective as it depends on patient compliance. Hence, tissue conditioners incorporated with antifungal agents have been into use as an antifungal drug delivery system to treat the abused tissue under the denture-bearing area. This can be a promising method of drug delivery system to overcome the obstacles of other therapies.

The most commonly used combination of antifungal agent and tissue conditioner in literature is nystatin and Viscogel. Nystatin when incorporated into tissue conditioners has shown antifungal and antimicrobial activity, but its effect was comparatively less as compared with the azole groups.^[1,7,10,12]

Another commonly used antifungal agent is Amphotericin B. Although it is a good and potent antifungal agent, when incorporated into tissue conditioners, it is completely

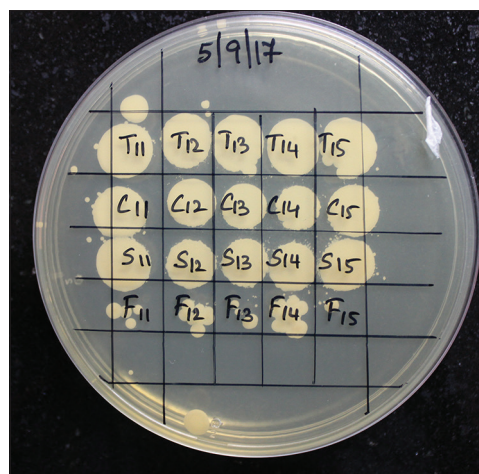


Figure 4: Evaluation of growth of *Candida albicans* after 24 h of inoculation

ineffective.^[14,24] This could be due to the inability of the compound to be stable or to convert its chemistry when incorporated into tissue conditioners.

Among the organic antimicrobial agents, azole group derivatives were found to be the most effective and potent against *C. albicans*.^[1,7,10,12] Although they are found to be effective, prolonged usage may cause drug resistance, hepatotoxicity, and renal toxicity.^[5,6-10] Furthermore, topical application of the same can result in unpleasant taste and poor patient compliance due to loss of memory or manual dexterity of the aged patient.

To avoid the complications of organic antimicrobial agents, researchers started incorporating inorganic compounds such as SNPs, magnesium oxide, photocatalysts, and silver zeolite into tissue conditioners, to test its antifungal activity against *C. albicans*.^[15-18] It has been proven that silver zeolite when incorporated into soft liners has substantial effect against plaque-forming bacteria and also is not affected by human saliva.^[18]

The advancement in nanotechnology has brought into use nanoparticles such as silver and gold. Due to their increased surface area and enhanced reactivity, they have been considered as potent antimicrobial agents.^[19] SNPs have been incorporated into tissue conditioners, and it has been proven to prevent colonization of *C. albicans*.^[19] However, the disadvantages of using SNPs include their high cost, discoloration of tissue conditioner when incorporated with the same, and metallic taste.

Photocatalyst like photocatalytic titania has been proven to have an antifungal effect when used as an antifungal coating on tissue conditioner.^[20] However, according to

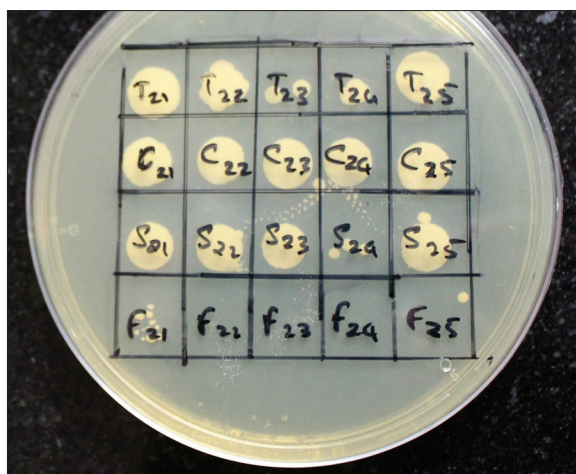


Figure 5: Evaluation of growth of *Candida albicans* on 3rd day of inoculation

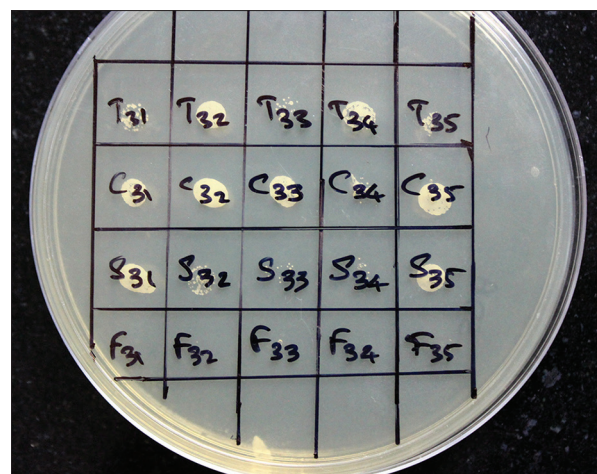


Figure 6: Evaluation of growth of *Candida albicans* on 7th day of inoculation

Uchimaru *et al.*, there has been a reduction in the antifungal activity after incorporating photocatalyst (Photohap) in tissue conditioner.^[15]

In addition to these, a number of studies have been carried out which have investigated the effect of natural and herbal antimicrobial agents against *C. albicans* by incorporating them into tissue conditioners, which have shown good antifungal and antimicrobial activity.^[22,23,25,26] The main advantage of using natural and herbal medicaments over organic and inorganic compounds is its ready availability, minimal or no side effects, and cost-effectiveness.

One such herbal oil that has antifungal and antimicrobial property but has not been incorporated into tissue conditioner to check its effectiveness against *Candida*-associated denture stomatitis is *C. nucifera* or commonly known as coconut.

It has been proven that 5% w/w CCO has substantial antifungal activity against *C. albicans* biofilm isolated from clinical samples, which showed a marked reduction under scanning electron microscopic examination.^[27] Furthermore, in another study carried out by PS Kumar, it has been substantiated that incorporation of 20% v/v of CCO reduced the growth of *C. albicans*.^[28] However, as incorporation of any substance into the polymeric structure of tissue conditioner can affect its physical property, in this study, various concentrations of virgin CCO (10% w/w, 20% w/w, and 30% w/w) were incorporated into Viscogel tissue conditioner and tested for its effect on the tensile strength of the tissue conditioner.^[29]

The tensile strength of a relining material provides us with the information on the ultimate tensile strength of

the material in tension.^[30] Subjecting a rubber material to compression and shear stress does not provide us a guide to determine quality of the material; whereas, the tensile strength of a rubber material is a measure to determine its quality to resist rupture under wet environmental conditions. Soft liners especially loose their adhesion to denture base surface, loose resiliency, and also have accumulation of plaque and debris.

The results exhibited 10% w/w CCO to have the maximum mean tensile strength as compared with the 20% w/w and 30% w/w concentrations of CCO.

The descriptive statistical analysis between the groups demonstrated SNP to have the maximum mean tensile strength of 21.63 followed by CCO which showed a mean tensile strength of 20.06. It was also proved statistically that incorporating TTO into Viscogel tissue conditioner reduced its mean tensile strength drastically to 10.58 from 17.81.

All the samples were stored in distilled water simulating the wet environment provided by saliva. The wet environment allows the ethanol and esters to get leached into water, which is then absorbed by the polymeric phase of the gel. This may cause changes in the viscoelastic properties of tissue conditioner.^[30]

For evaluation of antifungal activity against *C. albicans*, spot inoculation technique has been employed. Although disc diffusion and agar punch well methods have been used in previous studies, it can cause dehydration of the agar medium, as they have to be kept incubated at 37°C for 7 days. This could give a false-positive result of inhibition of growth of *C. albicans* on the agar medium. Furthermore,

C. albicans requires continuous supply of nutrition for its growth. This condition was fulfilled by the spot inoculation technique used in this *in vitro* study, as the samples were inoculated in SDA (Sabouraud Dextrose Agar) broth along with diluted *C. albicans*, where the microorganism received continuous nutrition supply for its growth. Therefore, the chances of false-positive inhibition of *C. albicans* growth were minimal or negligible.

The results of the present microbiological study 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. Furthermore, FLU displayed a complete inhibition of growth of *C. albicans* on day 5. This clearly proves that leaching of tissue conditioner when incorporated with antifungal agents is slow, consistent, and gradual which was in accordance with Graham *et al.* 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.^[29]

Furthermore, results of tensile strength evaluation proved that Viscogel tissue conditioner when incorporated with CCO, SNP, and FLU showed significant increase in its tensile strength as compared with the plain Viscogel tissue conditioner; on the other hand, TTO when incorporated into Viscogel tissue conditioner demonstrated a drastic decrease in its tensile strength.

Since only one tissue conditioner was used in the study, the results obtained here may therefore not apply to other tissue conditioners incorporated with CCO. Studies by incorporating the same into different brands of tissue conditioner should be carried out, and further *in vivo* studies are required using the combination of CCO and tissue conditioners to treat denture stomatitis. Furthermore, additional studies are required to determine the rate of release of these antifungal agents from tissue conditioner.

CONCLUSIONS

10% CCO was effective in producing significant antifungal activity on *C. albicans*, and the antifungal activity improved gradually from 24 h to day 5. At the same time, incorporation of the same statistically increased the tensile strength of Viscogel tissue conditioner when tested, after 7 days. Thus, we suggest CCO when mixed with Viscogel tissue conditioner can be used against *C. albicans* resistant to conventional therapies and also that this association showed an increase in tensile strength of Viscogel.

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Conflicts of interest

There are no conflicts of interest.

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