

Studying The Anti Candidal-Activity of Different Herbal Oils Incorporated into Tissue Conditioner: (A Comparative study)

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ABSTRACT

This in vitro study was undertaken with the aim of testing the efficacy of the tissue conditioner mixed with four different commercially available herbal oils (Origanum oil, (Nigella sativa) Black seeds oil, Garlic oil, and Ginger oil) against *Candida albicans*. Control groups tested with antifungal test discs included fluconazole, flucytosine, and tissue conditioner discs (Acrosoft). The susceptibility test of plant extracts alone was also conducted. The combination groups tested were Acrosoft mixed with ginger oil, Acrosoft mixed with origanum oil, Acrosoft mixed with black seed oil, and Acrosoft with garlic oil. Test discs were completely embedded in the tissue conditioner mixed with plant extracts and gently placed on the agar plates. The plates were incubated at 35 °C for 72 hours. After incubation, inhibiting diameters of various groups were noted. There was complete resistance of *Candida albicans* to both fluconazole and flucytosine, and there was no inhibition zone observed regarding the susceptibility of ginger oil, origanum oil, black seed oil, and garlic oils. Similarly, there was no inhibition of *Candida albicans* observed in Acrosoft material. For the combination of tissue conditioner with plant extracts, results showed no inhibition in black seed oil and ginger oil combined with tissue conditioner. In contrast, the combination of garlic oil and origanum oil with tissue conditioner showed a zone of inhibition, and the inhibition diameters ranged from 5-9 mm.

Keywords: Antifungal, Herbal Oil, Tissue Conditioner.

INTRODUCTION

There are several methods for replacing missing natural teeth, including complete removable dentures, implants, and overdenture implants, with complete removable dentures being the most common treatment for edentulous patients. However, the oral cavity's microflora plays a crucial role in maintaining the health of gums and throats in individuals with natural teeth or dentures. Denture-related stomatitis (DRS) is a pathological reaction of the denture-bearing mucosa caused by trauma from ill-fitting dentures. If the yeast *Candida* is involved, the term denture stomatitis is used with the prefix *Candida*-

associated.^(1,2) *Candida albicans* is a diploid asexual fungus that reproduces via budding in culture and tissues, forming pseudohyphae in the process. It can exist in either yeast (blastospore) or mycelial form (pseudohyphae). Denture wearers with normal mucosa typically have the yeast form, while DRS patients exhibit the mycelial form. *Candida albicans* is a gram-positive yeast and the primary etiological factor in denture stomatitis.⁽³⁾

The insertion of a removable prosthesis in the oral cavity induces significant changes in the oral environment, potentially affecting the integrity of oral tissues.⁽⁴⁾ Denture-associated stomatitis, one of the most common clinical manifestations of oral candidiasis, affects 24-60% of otherwise healthy denture wearers. Although *Candida albicans* is the primary cause, other contributing factors, such as bacterial causes, mechanical irritation, and allergies,

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are also present, making the condition a complex entity.^(6, 2)

While denture stomatitis is typically asymptomatic, it can cause symptoms such as burning, bleeding, and an unpleasant taste.⁽⁷⁾ Fungal infections, including denture stomatitis, are often challenging to cure compared to bacterial infections due to the slow development of fungal organisms. Treatment for denture stomatitis may focus on the oral mucosa, and various successful antifungal medications, both topical (e.g., Amphotericin B and Nystatin) and systemic (e.g., azoles like fluconazole and ketoconazole), have been used. However, systemic antifungal therapy may face challenges related to taste and frequent dosages, impacting patient compliance.⁽⁵⁾

Amphotericin B and Nystatin are commonly used as topical antifungal drugs, while azoles like fluconazole and ketoconazole serve as systemic antifungal therapy. However, the unpleasant taste and the need for frequent dosages associated with these medications have been linked to poor patient compliance.⁽³⁾

Douglas and Walker proposed a method of combining tissue conditioner with antifungal medications, specifically mixing Nystatin with tissue conditioner, finding it to be fungicidal to varying degrees. However, reinfection occurred 10 days after treatment discontinuation, prompting further investigation into different antifungal drugs. Tissue conditioners, known for softening the denture-bearing mucosa affected by ill-fitting dentures, act as a cushion beneath dentures, reducing stress on the denture-bearing mucosa.⁽⁵⁾

Tissue conditioners are resilient compounds frequently used to soften the denture-bearing mucosa caused by ill-fitting dentures. They operate as a cushion beneath the dentures, reducing the stresses on the denture-bearing mucosa.^(6,7)

Many advantages of employing tissue conditioners as a therapy strategy include no requirement for patient compliance, simultaneous treatment of damaged denture-bearing tissue and candidal infection, and patient convenience because the denture does not need to be

removed for an extended period.^(8, 9, 10)

Given the advantages of using antifungal compounds in tissue conditioners, a range of medications has been evaluated utilizing various tissue conditioners. The most often used antifungal agent was Nystatin, followed by chlorhexidine, amphotericin, and azole group antifungals such as fluconazole, ketoconazole, miconazole, and itraconazole.⁽⁷⁾

Various chemical substances, including silver zeolite, silver nanoparticles, and magnesium oxide, have been investigated for their antifungal properties in tissue conditioners. Silver zeolite-incorporated tissue conditioners have been shown to manage denture plaque efficiently while remaining unaffected by human saliva.^(11,12) In the case of silver nanoparticles, similar outcomes for controlling denture plaque have been observed.⁽¹³⁾

The contemporary natural health trend has contributed to an increase in interest in commercially accessible naturopathic remedies. Medicinal plant extracts have been employed as alternative treatments for health concerns in developing nations.⁽¹⁴⁾

The primary benefits of natural medicinal plant extracts as antimicrobial agents are increased safety and stability with no adverse effects, which are lacking in both organic and inorganic antimicrobial agents. As a result, natural and herbal products, such as herbal oils, have been investigated for their antifungal properties in addition to organic and inorganic substances. Tea tree oil has been studied for its antifungal activity both in vitro and in vivo.⁽¹⁴⁾ Tea tree oil-modified tissue conditioners exhibited inhibitory and fungicidal action against *Candida albicans* and are beneficial in treating denture stomatitis. Furthermore, its efficacy was equivalent to that of organic antifungals such as fluconazole.⁽¹⁵⁾

MATERIALS AND METHODS

Culture methods

Candida albicans test organisms were cultured on Sabouraud dextrose agar plates prepared with Sabouraud Broth base. Muller Hilton agar and 2 percent glucose with

0.5 µg/ml Methylene blue dye (GMB medium) were prepared and used for the sensitivity test.⁽¹⁶⁾ The fungal strains were obtained from the fungal laboratory of the Medical Microbiology Department.

Antifungal assay (Susceptibility test procedure)

GMB medium containing Muller Hilton agar and 2% glucose with 0.5 µg/ml Methylene blue dye was used to prepare 50 plates.⁽¹⁶⁾

Although ginger oil, origanum oil, black seed oil, and garlic oil are commercially available, they were synthesized in the laboratory for this investigation. Each plant extract stock solution was prepared at a final concentration of 50 µg/ml. The 25 µg per disc concentration was achieved by applying 20 µl of the prepared stock solutions on 6 mm paper discs and allowing them to air dry at room temperature. Subsequently, they were stored in a refrigerator at 4°C.⁽¹⁷⁾ Adjusting the inoculum density of the test fungus using 0.5 McFarland standard tubes, the suspension was then evenly spread onto GMB medium in three directions using a sterile swab. A swab dipped in a standardized inoculum solution was used to streak evenly over plates. The test agents were applied to the surfaces of infected plates using discs. Inverted plates were incubated at 35°C for 72 hours.⁽¹⁸⁾

Out of the 18 plates, two served as indicators of pure *C. albicans* development. Antifungal test discs containing fluconazole (flu) and flucytosine (AFY) were evenly distributed on two more plates using sterile tweezers. Two additional plates were used to assess the susceptibility of the tissue conditioner. ACROSOF TC1 tissue conditioner was created by mixing powder and liquid in a sterile dish according to the manufacturer's suggested ratio, and sterile discs were thoroughly embedded in the mixture. The discs were carefully placed on the agar plate. The susceptibility of plant extracts alone was examined using four plates after achieving the final concentration of 16 µg/ml for each ginger oil, origanum oil, black seed oil, and garlic oil.⁽¹⁹⁾

The remaining eight plates were used with Acrosoft mixed with ginger oil, Acrosoft mixed with origanum oil,

Acrosoft mixed with black seed oil, and Acrosoft mixed with garlic oil. Test discs were thoroughly embedded in the tissue conditioner mix with plant extract and gently placed on the agar plates. The plates were then incubated at 35 °C for 72 hr.⁽²⁰⁾

Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Science (SPSS version 22, Chicago, Illinois, USA). Mean, Standard Deviation (SD), and inferential statistics including Shapiro-Wilk, One-way Analysis of Variance (ANOVA), and Tukey's Honestly Significant Difference (Tukey's HSD) were employed, with a significance level set at p-value < 0.05.

RESULTS:

The in vitro study results revealed that the tissue conditioner Acrosoft alone did not exhibit inhibition of *C. albicans* growth. Additionally, there was complete resistance of *Candida* to both fluconazole and flucytosine as antifungal agents (Figure 1). No inhibition zones were observed for ginger oil and garlic oil. In contrast, origanum oil and black seed oil showed a non-significant zone of inhibition of 1mm (Figure 5). Table 1 provides the mean and standard deviation for the readings of each type of oil used in this study.

For the combination of tissue conditioner with a plant extract, there was no inhibition observed in the case of black seed oil combined with tissue conditioner. Ginger oil combined with tissue conditioner showed a non-significant zone of inhibition of 1mm (Figure 5).

The study results indicated that the combination of tissue conditioner with origanum oil (Figure 2,3) showed a significant zone of inhibition ranging from 5-9 mm ($p < 0.05$). Similarly, combining tissue conditioner with garlic oil (Figure 4) resulted in a significant zone of inhibition of 9 mm ($P < 0.005$) (Table 1). The overall results of the zone of inhibition for plant extracts and tissue conditioner are presented in Figure 5 and Table 2.

Table (1): Descriptive and statistical test of (the mean of zone of inhibition of *Candida albicans*) among groups of herbal oil mixed with tissue conditioner and tissue conditioner alone that had been tested using One-way Analysis of Variance (ANOVA).

Groups of herbal oil mixed with tissue conditioner that had been tested	Mean of zone of inhibition	F	P value
Garlic oil+ Acrosoft tissue conditioner	8±1.732	143.8523	0.0000 Sig.
Black seed oil+ Acrosoft tissue conditioner	0		
Ginger oil+ Acrosoft tissue conditioner	1±0.354		
Organium oil+ Acrosoft tissue conditioner	7.75±1.693		
Acrosoft tissue conditioner	0		

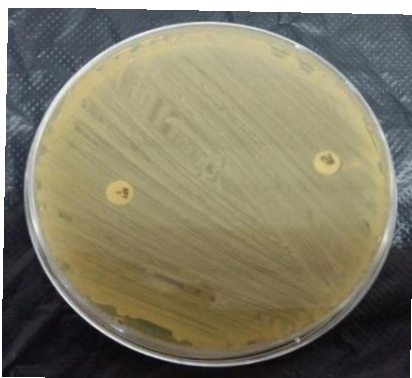


Figure (1): Antifungal susceptibility test for *Candida albicans* against fluconazole and flucytosine



Figure(3): Antifungal susceptibility test for *Candida albicans* against Acrosoft tissue conditioner mixed with Black seed oil (zone of inhibition 5mm)



Figure (2): Antifungal susceptibility test for *Candida albicans* against Acrosoft tissue conditioner mixed with Organium oil (zone of inhibition 9 mm)

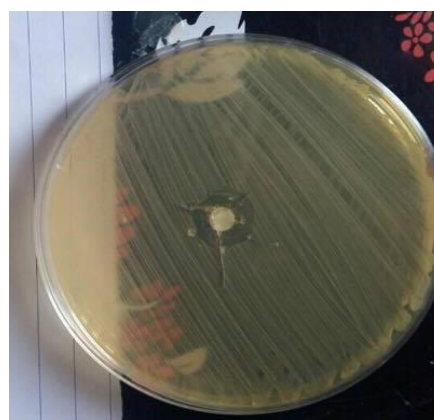


Figure (4)): Antifungal susceptibility test of candida albicans against acrosoft mixed with Garlic oil (zone of inhibition 9mm)



Figure (5): Antifungal susceptibility test for *Candida albicans* against Acrosoft mixed with Ginger oil (zone of inhibition 1mm)

Table (2): Multiple pairwise comparisons of (the mean of zone of inhibition of *candida albicans*) among groups of herbal oil mixed with tissue conditioner and tissue conditioner that had been tested using Tukey's HSD

Groups of herbal oil mixed with tissue conditioner and tissue conditioner that had been tested		Mean difference	P value
Garlic oil + Acrosoft tissue Conditioner	Black seed oil + Acrosoft tissue conditioner	8.00*	0.000
	Ginger oil + Acrosoft tissue conditioner	7.00*	0.000
	Organum oil + Acrosoft tissue conditioner	0.25	0.9859
	Acrosoft tissue conditioner	8.00*	0.000
Black seed oil + Acrosoft tissue Conditioner	Ginger oil + Acrosoft tissue conditioner	-1.00	0.2629
	Organum oil + Acrosoft tissue conditioner	-7.75*	0.000
	Acrosoft tissue conditioner	0	----
Ginger oil+ Acrosoft tissue conditioner	Organum oil + Acrosoft	-6.75*	0.000
	Acrosoft tissue conditioner	1	0.2629
Organuim oil +Acrosoft tissue Conditioner	Acrosoft tissue conditioner	7.75*	0.000

DISCUSSION

Tissue conditioner is a soft relining substance used therapeutically in the wound healing process. Prolonged use of prostheses has made the site susceptible to microbial buildup. *C. albicans*, a prominent *Candida* species isolated from the oral cavity, is closely associated with tissue inflammation known as Candidiasis. Therefore, the incorporation of synthetic or herbal antifungal agents into the tissue conditioner aims to counteract this disadvantage or reduce the risk of acquiring fungal infections.⁽²¹⁾

Medicinal herbs are particularly interesting in developing nations due to their excellent antimicrobial action, safety, and affordability. The recent trend of incorporating extracts of medicinal plants into biomaterials has proven to be a natural alternative with effective antifungal properties.^(22,23)

It has been discovered that the methanolic extract of black seeds (*Nigella sativa*) effectively inhibits fungal development. The antifungal activity of thymoquinone obtained from black seed oil extract was investigated, and the water extract showed substantial action against *Candida albicans*.⁽²⁴⁾

In this study, origanum oil, ginger oil, black seed oil, and garlic oil were added to Acrosoft TC1 tissue conditioner material and evaluated for their fungicidal activity.

Although garlic oil is known for its ability to eliminate a wide variety of pathogens, including fungi, no usage of garlic oil combined with tissue conditioners has been recorded for treating denture stomatitis. The current in vitro investigation indicated that the tissue conditioner Acrosoft did not suppress the development of *Candida albicans*, which is consistent with the findings of Kulak and Kazazoglu, who observed that the presence of conditioners promoted the adhesion, proliferation, and hyphae production of *C. albicans*.⁽²⁵⁾

The tissue conditioner containing both black seed oil and ginger oil did not prevent yeast growth. This unexpected and unexplainable lack of inhibition can only be traced to a combination of the two medications. On the

contrary, the combination of origanum oil and tissue conditioner produced good results, which is consistent with Srivastava's findings that adding origanum oil reduced the adhesion of *C. albicans* to the surface of the tissue conditioner without affecting its physical qualities.⁽²⁶⁾ In addition, the inclusion of garlic oil with tissue conditioner demonstrated the predicted efficacy because garlic oil is renowned for its fungicidal properties.

GODIL et al. (2021), using scanning electron microscopy (SEM) studies, also proved the effectiveness of incorporated antifungal agents on the cell morphology of *C. albicans* at their respective MIC values. This approach permits prolonged drug release in the oral cavity, treating both the injured denture-bearing tissues and the infection biofilms of *Candida* without compromising their physical properties.⁽²⁷⁾ This study concurred with the findings of OAamir et al.'s study (2021), which showed that their method permits sustained medication release in the oral cavity without impairing the physical qualities of the denture-bearing tissues and *candida* biofilms. These studies are important and have enormous medical and therapeutic significance.⁽²⁷⁾

In conclusion, the antifungal activity of garlic oil and origanum oil combined with Acrosoft tissue conditioner might be utilized as an alternative treatment for denture stomatitis.⁽⁵⁾

CONCLUSION

The addition of garlic oil and origanum oil to tissue conditioner reduced the growth of *C. albicans*, suggesting a new intra-oral effective antifungal management for denture stomatitis.

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دراسة الفعالية المضادة للكانديدا للزيوت العشبية المختلفة الداخل فيها مكيف للأنسجة (دراسة مقارنة)

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ملخص

تم إجراء هذه الدراسة في المختبر بهدف اختبار فعالية مكيف الأنسجة الممزوج بأربعة زيوت عشبية مختلفة متوفرة تجاريًا وهي (زيت الزعتر البري، زيت الحبة السوداء، زيت الثوم وزيت الزنجبيل) ضد الكانديدا. كانت مجموعات المراقبة التي تم اختبارها باستخدام أقراص اختبار مضادات الفطريات هي الفلوكونازول، تم إجراء اختبار الحساسية للمستخلصات النباتية وحدها (أقراص فلوسيتوزين ومكيف الأنسجة (أكروسوفت)). كانت المجموعات المركبة التي تم اختبارها عبارة عن أكروسوفت ممزوجًا بزيت الزنجبيل، وأكروسوفت ممزوجًا بزيت الزعتر البري، وأكروسوفت ممزوجًا بزيت الحبة السوداء، وأكروسوفت مع زيت الثوم. وكانت أقراص الاختبار مدمجة تمامًا في منعم الأنسجة الممزوج بالمستخلصات النباتية، وتوضع بلطف على أطباق الأجار. تم تحضين الأطباق عند 35 درجة مئوية لمدة 72 ساعة. بعد الحضانة، لوحظ تثبيط أقطار المجموعات المختلفة. وكانت هناك مقاومة كاملة للكانديدا لكل من فلوكونازول وفلوسيتوزين، ولم تكن هناك منطقة تثبيط فيما يتعلق حساسية زيت الزنجبيل، وزيت الزعتر البري، وزيت الحبة السوداء، وزيوت الثوم. وبالمثل، لم يكن هناك أي تثبيط لوحظ للكانديدا في مادة أكروسوفت. لمزيج منعم الأنسجة مع مستخلص النبات أظهرت النتائج عدم وجود تثبيط في زيت الحبة السوداء وزيت الزنجبيل مع مكيف الأنسجة. على النقيض من ذلك، أظهر مزيج زيت الثوم وزيت الزعتر البري مع مكيف الأنسجة منطقة تثبيط، وتراوح أقطار التثبيط من 5-9 ملم.

الكلمات الدالة: زيوت عشبية، مكيف أنسجة، مضادات الفطريات.

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