

IN VITRO COMPARISON OF ANTIFUNGAL ACTIVITY OF BIOPURE MTAD AND LAVANDULA ANGUSTIFOLIA EXTRACTS WITH NYSTATIN ON CANDIDA ALBICANS

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ABSTRACT:

Aim: One of the pathogens found in dental canals with necrotic pulp is *Candida albicans*. Teeth with unsuccessful root canal treatment sometime are found to have fungi. As a result, finding irrigation more effective on *Candida albicans* has a positive impact on treatment. The present study aimed to evaluate the antifungal activity of BioPure MTAD and lavender extract with nystatin on *Candida albicans* and to compare their effects.

Materials and methods: In this experimental study, after preparation of the materials, *Candida albicans* was cultured and transferred to liquid BHI medium and its turbidity was set to 0.5 McFarland. In this study, agar diffusion and cell counting methods were used. In agar diffusion method, seven wells were prepared after the culture of fungal suspension on solid BHI medium and the study solutions were added to each of them and after 48 hours, zone of inhibition was measured. In cell counting method in microplates fungal suspension and then drug were added. After 48 hours, the number of fungal cells was counted. The results were analyzed using ANOVA test and LSD. $P < 0.05$ was considered significant.

Results: The antifungal activity of BioPure MTAD, lavender extract and nystatin on *Candida albicans* in both methods was statistically ($P < 0.001$) significant, however BioPure MTAD was statistically more effective than lavender extract and nystatin showed the highest antifungal activity ($P < 0.001$).

Conclusion: All materials have antifungal activity against *Candida albicans*. The antifungal activity of BioPure MTAD is higher than that of lavender extracts against *Candida albicans*.

Keywords: BioPure MTAD, lavender extract, nystatin, *Candida albicans*, canal irrigant



INTRODUCTION:

Candida albicans: *Candida albicans* is the most common fungi that can be found in teeth with necrotic pulp. *Candida* is a yeast-like and gram-positive fungus. Various studies have isolated this

pathogen in the primary endodontic infections. ^[1] This pathogen can cause root canal treatment failure. ^[2]

C. albicans has a series of features that allow them to survive in the treated root canals. These include resistance to drugs

in the canal, ability to form biofilm, invasion to dentinal tubules and surviving long after deprived of food. [3]

C. albicans is part of the normal microflora of the mouth. They can be isolated from plaque, caries and sub-gingival microflora and also of the active periodontal pockets. [4]

This fungus enters into the root canal from the oral cavity in cases of opening of the pulp cavity due to pathological reasons, negligence in isolating the field of operation and also loss of pulp cavity's seal during several sessions in long-term treatments. [1]

Baumgarther [5] found *Candida albicans* by PCR method in 21% of samples taken from infected root canals. Fungi can also be found in teeth that have failed endodontic treatment. Siqueira et al found *Candida albicans* in 24 canals which failed endodontic treatment. Molander et al found *Candida albicans* in three of 68 teeth failed endodontic treatment with chronic apical periodontitis. These reports indicate that the fungi are the most resistant microorganism to medicinal treatment. The fact has also been confirmed by *Candida albicans* resistance to certain drugs with standard application in endodontic therapy such as calcium hydroxide mixed with a solvent. [1] Thus, cleaning the canal of the *C. albicans* during root canal therapy by using irrigation is of particular importance.

Biopure MTAD: Biopure MTAD (Dentsply, Tulsa OK) is a mixture of tetracycline isomer (doxycycline), acid (citric acid) and detergent (Tween 80) has recently been

introduced to the market. Properties of the MTAD include smear layer removal and destruction of *E. faecalis*. [6]

Lavender: The science of identification and use of herbs is as old as human life. Until the 19th century, the use of natural resources, mainly plants was the main ways of treating diseases. But the rapid development of chemistry and the lack of natural resources caused the new chemical compositions to replace herbal medicines. However today, many scientists of various fields have again turned to herbal remedies. [7]

Lavender with the scientific name, *Lavandula Angustifolia*, is a plant of the Lamiaceae family. [8] the plant is mainly used in aroma therapy as a sedative, carminative and relaxant. In traditional medicine lavender oil is used as an antiseptic of wound, burns and insect bites as well as in veterinary to control lice and other parasites. [9]

Nystatin: nystatin is a Macrolide produced by *Streptomyces noursei*. It is a yellow powder that is amphoteric and dissolved in water to a very little extent (4mg / ml). Nystatin has the properties of fungal growth inhibition and at high concentrations and acidic PH shows strong fungicide activity. It has an excellent activity against yeasts of *Candida* genus especially *Candida albicans*. [10] Thus, in this study nystatin has been used as a positive control.

Considering the few studies on antifungal activity of lavender extracts and MTAD, therefore this study was conducted to

evaluate the antifungal activity of BioPure MTAD and lavender extracts with nystatin on *Candida albicans*.

MATERIALS AND METHODS:

In this study, in vitro antifungal activity of studied irrigation materials on standard *Candida albicans* with the code 5027 was evaluated. Regarding lavender extract, product of Kashan Barij Essence Company, supplied with concentration of 100% was used. The solution was diluted in dilutions of 1/2, 1/4, 1/8 and 1/16 with liquid BHI medium.

For preparation of fungal suspension; 2 to 3 colonies of fungi with the help of a inoculation loop were added to liquid BHI medium and turbidity was set to 0.5 McFarland (1x10⁶ CFU ml⁻¹) and checked under the microscope. Nystatin drops, 100,000 units per mL, was used as a positive control and physiologic serum as negative control.

Experiment with agar diffusion method:

For the evaluation and comparison of the antifungal effect of irrigations on *Candida albicans*, agar (solid BHI medium) was used. The fungal suspension was cultured with sterile swabs on agar plates, then seven wells with a diameter of about 6

mm and a depth of 3 mm were prepared using a Pasteur pipette on the agar medium and 50 µl of studied solutions were added to them using Sampler. Thus, 2 wells were allocated to negative and positive control, one well to BioPure MTAD and other wells to various dilutions (1/2 and 1/4 and 1/8 and 1/16) of lavender. The plates were incubated at 37 °c for 48 hours in incubator. After incubation, inhibition zone generated around the wells were measured by caliper.

Experiment with cell counting

(Microdilution): Alternatively, an experiment called cell counting was performed in the broth using hemocytometer slide (microdilution method), to determine the antifungal effect of MTAD and lavender extracts. Microdilution is a standard antifungal susceptibility test used as the preferred method for antifungal susceptibility in the laboratory. ^[11]

In order to culture fungi in broth, 7 sterile microplates were used so that in each microplate, 100 µl of the fungal suspension prepared was added with the help of Sampler, and then 100 µl of drug was added to fungal suspension to bring the total suspension amount to 200 µl.

Table 1: The type of material in each microplate

Microplate number	Type of material added
1	Physiologic serum (negative control)
2	Lavender extract with 1/2 dilution
3	Lavender extract with 1/4 dilution
4	Lavender extract with 1/8 dilution
5	Lavender extract with 1/16 dilution
6	BiopureMTAD
7	Nystatin drops (positive control)

Microplates were incubated at 37 °c for 48 hours followed by fungal cell counting with hemocytometer slide under microscope.

These experiments were repeated four times. The antifungal activity of studied irrigations against *Candida albicans* were recorded and entered into SPSS V.22 software and ANOVA (Tukey pair-wise comparisons) and LSD tests were used. P <0.05 was considered significant.

In experiment using agar diffusion, zone of inhibition was measured in millimeters after incubation in the incubator for 48 hours. In experiment using cell counting fungal cells were counted after 48 hours of incubation under a microscope by hemocytometer slide.

RESULTS:

Table 2: Comparison of mean and standard deviation of the studied variables in 7 discussed groups

Groups variables	Physiologic serum (1)	1/2 lavender (2)	1/4 lavender (3)	1/8 lavender (4)	1/16 lavender (5)	MTAD (6)	Nystatin (7)	P-Value
Zone of inhibition in mm	0 a	1.75±0.96 b	0 a	0 a	0 a	20.25±2 c	47.5±3.7 d	<0.001
Fungi cell counted in CCx10 ⁶	36.44±7.33 a	2.81±0.66 b	4.34±13.1 a	19.38±13.1 a	58.75±19.73 a	0.75±0.45 c	0.24±0.025 d	<0.001

* The numbers in the table are the values of mean ± standard deviation.
 ** Similar letters in each row represent no significant difference between each pair of groups at α=0.05 level.

According to the results of tests, BioPure MTAD, lavender extract and nystatin, all showed significant antifungal activity (P <0.001). Antifungal activity of BioPure MTAD was significantly more than that of lavender extract and less than that of nystatin. Antifungal activity of lavender

extract on *Candida albicans* was significant only in a dilution of 1/2 (P <0.001) and was not statistically significant in other dilutions. The highest rate of antifungal activity was statistically for nystatin and the extracts of lavender showed the lowest antifungal activity (P <0.001).

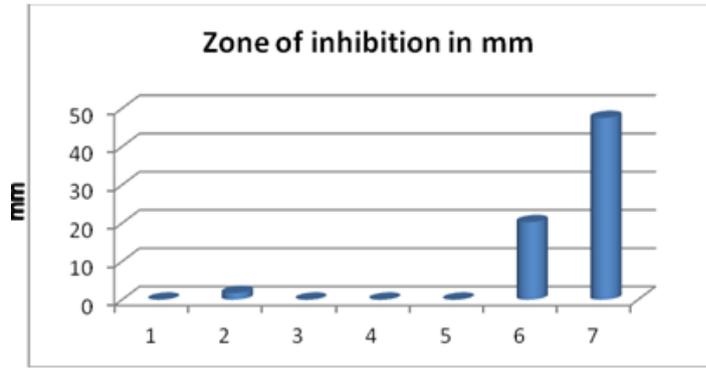


Figure 1: diagram of the mean inhibition zone diameter of each plate separated by irrigation used in millimeters

* The numbers in the diagram indicate the type of material taken from Table 2.

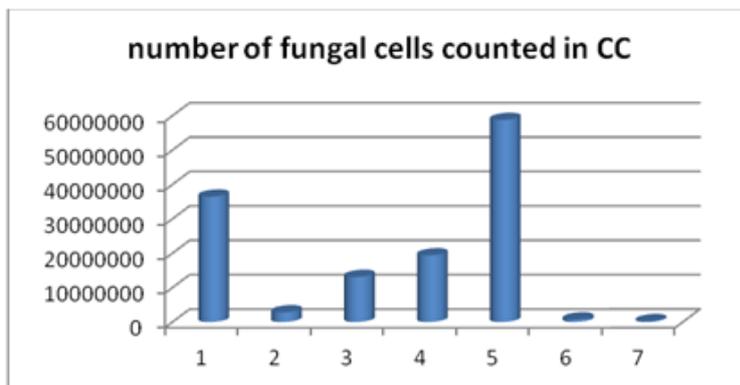


Figure 2: diagram of the mean number of fungal cells counted separated by irrigation used in cc

* The numbers in the diagram indicate the type of material taken from Table 2.

DISCUSSION:

This study aimed to determine the antifungal activity of BioPure MTAD, lavender extract and nystatin on *Candida albicans*. According to the results, all materials have antifungal activity against *Candida albicans* among which nystatin has the highest activity followed by BioPure MTAD. Lavender extracts showed the lowest antifungal activity. ($P < 0.001$)

When irrigations or intracanal medicament are used for cleaning canals especially infected canals, one point must be considered that there are microorganisms that are resistant to these materials and mechanical cleaning and cause the endodontic treatment to fail, *Candida albicans* is one of these microorganisms. [4]

Root canal infection is an infection with a variety of microorganisms and available medicines have different antimicrobial effects and this has led researchers to continue searching for an irrigation which meets all the requirements. [12] *Candida*

albicans like many microorganisms adapt to changes in different conditions to survive, thus this study was conducted on the standard type of this fungus.

Sequera et al in a study showed that candida albicans can make colonies in the dentin of the root canals and penetrate into the dentinal tubules; it shows the dentinophilic nature of this fungus. [13] C.albicans also can survive for a long time even in the event of deprivation of food. [3]

BioPure MTAD as having the highest penetration into dentin among irrigating solutions and the ability to remove the smear layer, preserving its effectiveness for a long time [6] and also due to few studies that have been conducted on this material, was selected in the present study. However, the antifungal activity of this irrigation solution is not as ideal, but its properties can complement the impact of this material on Candida albicans.

Ruff ML et al in a study in 2006 evaluated the antifungal activity of four irrigations as final irrigation.[14] The aim of their study was to compare the in vitro antifungal efficacy of sodium hypochlorite, CHX, EDTA and BioPure MTAD. The antifungal activity of BioPure MTAD was shown in this study that the results are consistent with the results of present study.

Arslan et al in an in-vitro study evaluate the antimicrobial activity of Propolis, BioPure MTAD, NaOCL and CHX on Candida albicans and E.faecalis [15]. The results of the study showed little antifungal activity for BioPure MTAD that

is similar with the results of present study. Also in another study, Mohammadi z et al evaluated the antifungal activity of four materials in the laboratory [16] that BioPure MTAD was one of them and showed its antifungal effect that is consistent with the results of present study.

Everyone knows the medicinal properties of plants, medicinal plants have helped human against various diseases for a long time. However, because of their frequency and also replacing them with chemical drugs, few studies have been conducted on these drugs and their properties; few side effects as well as low cost of medicinal plants compared to chemical types could be the main reason for the new approach to these medicines, hence lavender extract was selected in this study. Although lavender extract showed antifungal activity in the present study, the activity has been low and at only 1/2 dilution.

Studies on the effects of antifungal activity of lavender are different, but most of them have reported a positive antifungal effect. [17]

Konstantia Adam et al in a study evaluated the antifungal activities of marjoram, mint, lavender and sage extracts against human pathogenic fungi. [18] lavender extract showed antifungal activity of low to moderate. In a similar study conducted by D'Auria [9] et al it was shown that lavender oil and its major components, linalool and linalyl acetate have inhibitory and fungicide properties that are similar to the results of this study.

F Haghigati et al ^[19] in a study compared antimicrobial effects of extracts of ten plants with chlorhexidine mouthwash on three types of microorganisms causing mouth pathology (including *Candida albicans*) in vitro. In that study, *Candida albicans* was cultured in Mueller Hinton Agar. Lavender was one of the plants studied and did not show any antimicrobial effect that is contrary to the results of present study. It may be due to differences in culture.

Behmanesh et al in a study investigated the effects of lavender and clotrimazole on growth of standard species of *Candida albicans* in vitro. ^[20] In this study, cell counting method was used and it was shown that the number of fungal cells has decreased significantly compared to clotrimazole in Lavender extract, which is similar with the results of present study

except that it was compared with clotrimazole.

Because of differences in the results of studies carried out, prior to generalizing the results of this study, further laboratory studies with larger sample size are recommended.

CONCLUSION:

1. BioPure MTAD, lavender extract and nystatin have antifungal activity against *Candida albicans*.
2. The highest antifungal activity is for nystatin, followed by BioPure MTAD and lavender extracts have the lowest antifungal activity against *Candida albicans*.

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