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ANTIFUNGAL EFFECT OF THREE ESSENTIAL OILS AND NYSTATIN ON CANDIDA ALBICANS STRAINS: AN IN VITRO STUDY

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ABSTRACT

Background: Oral candidiasis is a common opportunistic infection that is presented in infants and young children. *Candida albicans* are the main causative species in oral candidiasis. Essential oils with their bioactive components were introduced to help in the treatment of oral candidiasis with minimal drawbacks.

Aim: To assess the antifungal effect of Moringa oleifera, Grape seeds, Tea tree, and Nystatin on Candida Albicans Strains in an in-vitro study.

Methods: Thirty-two samples were divided into four equal groups(n=8). Agar diffusion test was used in this study. Agar plates were inoculated with Candida Albicans suspensions, wells were made and filled with the Moringa, Grape seeds, tea tree oils, and nystatin. The agar plates were incubated at 37 °C and zones of inhibition around wells were measured after 24 hours, and 48 hours.

Results: There was a statistically significant difference between inhibition zone (mm) values of different oils ($p<0.001$) after 24 hours, and 48 hours. The highest value of inhibition zone was observed in the Tea tree oil group while the lowest value was reported in Grape seed oil group.

Conclusion: Tea tree and Moringa oils showed promising antifungal activity while Grape seed showed minimal antifungal activity against candida albicans. The herbal extracts can be used in the future treatment of fungal infections with minimal side effects.

KEYWORDS: Antifungal, Essential oils, Grape seeds, Moringa, Tea tree.

INTRODUCTION

Oral thrush (oral candidiasis) is a common opportunistic infection that may affect any child. It is caused by an overgrowth of the fungus Candida albicans (C. albicans) which is a normal commensal of the oral cavity and generally causes no problems in healthy children. Oral candidiasis is characterized by local discomfort, dysgeusia, and dysphagia. Its clinical presentation reveals a creamy white lesion, which adheres to whole parts of the oral mucosa like tongue, palate, gingiva, buccal mucosa, and oropharyngeal areas. These creamy white patches are easily scrapped and rubbed off resulting in bleeding and pain [1,2].

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Nystatin is rated as the primary line of treatment for uncomplicated oral candidiasis for several years [3]. The topical application of nystatin is the recommended route of administration in dentistry, as it is not absorbed from the gastrointestinal tract when the oral route is employed. In developing countries, nystatin has a crucial role within the prophylaxis of oral and systemic candidiasis in full-term and premature newborns and infants [4].

The worldwide increase of the incidence of microbial infections, and the increased nystatin resistance in some cases caused a rise within the importance to find other antifungal agents to exchange the existed drugs, widen the range of activity against C. albicans, and improve the effectiveness of treatment against oral candidiasis [5,6].

Herbal medicine has been used for several years in some countries of the globe. Natural products can replace synthetic chemical medicine. Plant essential oils provide anti-inflammatory and analgesic properties, have aromatic effects and antimicrobial, and anti-Candidal activities, including *Moringa oleifera*, grape seeds oil, and tea tree oil [5,7].

Moringa oleifera Lam. *(syn. Moringa pterygosperma Gaerthn, Moringa moringa Millsp.)*, is known as a miracle tree [8]. The Moringa plant plays an important role in economic, nutritional, and medicinal use. Furthermore, it contains good sources of vitamins, minerals, proteins, and carotenoids in all its parts. Many studies proved the antibacterial, anti-inflammatory, and anti-candidal properties of Moringa oleifera [8, 9-12].

Grape seeds belong to Vitis vinifera plant which is from Vitaceae family, the Mediterranean region, central Europe and South West Asia are the native origin [2]. Grape seeds have become popular in recent years as a medical and dental treatment [13]. Grape seed enhances skin wound healing, has antioxidant, antimicrobial, antiviral and anticancer effects [2]. It helps in increasing tooth re-mineralization. Moreover, grape seed oil has revealed antifungal activity against different Candida species [14,15].

Tea tree oil is that the oil obtained from the Australian native plant *Melaleuca alternifolia* [6]. It has powerful and broad-spectrum antimicrobial activity against a wide range of bacteria, viruses, and fungi, including yeasts. In the medical field, it is used as a topical antiseptic, especially when used in wound disinfection, inhalation therapy, aseptic surgery, dental medicine, and oral cavity rinsing [16].

Studies administrated on the anti-fungal activities of the essential oils are still emerging. Additionally, the clinical use of those plant extracts is not approved by regulatory agencies. Therefore, this study was performed to assess the antifungal effect of Moringa oleifera, Grape seeds, Tea tree, Nystatin on Candida Albicans Strains in an in-vitro study.

MATERIALS AND METHODS

Study design

The study design was in vitro.

Sample size estimation

During the planning of this study, there was no relevant article in the published literature with the same sample grouping and studied outcome that could be used for sample size calculation. In order to judge the adequacy of the selected sample size, respective effect sizes were calculated and post hoc power analyses were conducted for the acquired data. The calculated effect sizes were all considered large based on Cohen specifications [17], and the calculated power for all intervals were well beyond the target power of 80%. So the chosen sample size of 32 samples was deemed sufficient.

Essential oils preparation

Three essential oils; Moringa (Moringa oleifera Lam.), Grape seeds (Vitis vinifera), and Tea tree (Malaleuca alternifolia) were used in this study.

Moringa oil: cold maceration technique [18,19] was used to derive the extract of moringa leaves, and obtained from the Agriculture Department, Moringa Unit, National Research Centre, Egypt.

Grape seeds oil (International Standard ISO 9235): prepared by the cold-pressing method using hydraulic press machine [20] was used in this study.

Tea tree oil (International Standard ISO 4730): prepared by steam distillation process [21] from using Australian tea tree ("Oil of Melaleuca, terpinen-4-ol type").

The three prepared essential oils used in this study were 100% natural products, free from preservatives, free from coloring agents, free from artificial fragrances, and environmentally friendly. The concentration of these oils was expected to be around 99% (one single source and not blended with other natural ingredients or diluents) were used in this study due to unavoidable contamination of environmental humidity.

Test organism

The study was carried out on reference strain the main causative microorganisms in oral candidiasis; Candida albicans (ATCC 1023) obtained from the Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Microorganisms were stored in 10% glycerol brain-heart infusion broth at -80°C until used.

Sample grouping

A total of 32 samples were divided into four equals groups(n=8). Group 1: Moringa oil (leave extract), Group 2: Grape seeds oil, Group 3: Tea tree oil, and Group 4: Nystatin (Control). The antifungal effect of each group was assessed after two-time intervals: 24 hours, and 48 hours.

Antimicrobial assay

The antifungal activity was assessed using agar diffusion test as suggested by Kumar et al. [22]. Fresh subculture of microorganism from the stocks was made on Sabouraud's dextrose agar for Candida albicans and incubated at 37°C for 18-24 hrs. Microbial suspension was prepared from individual colonies on the fresh subculture agar plate and adjusted to 0.5 MacFarland standard to be used for inoculation of the test agar [23,24]. From microbial suspension (nine hundred microliters) was mixed with 60 ml of the molten soft agar (to ensure even distribution of the microorganism) to which Tween 20 at a level of 0.5% was added to reinforce the oil solubility and so the mixture was poured immediately onto Petri dish. The plates were left for ten minutes to solidify. A sterilized 5 mm cork borer was accustomed make holes. Two hundred microliters of every one of the three essential oils along with nystatin as control were pipetted into the holes. After 24, and 48 hours of incubation at 37°C, the diameter of the expansion inhibition zones (in mm) was measured by a digital caliper at the two outermost points at two intervals time of this in-vitro study. The experiment was repeated eight times in each group [25].

Statistical analysis

Numerical data was represented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Data showed non-parametric distribution, so they were analyzed using Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction for intergroup comparisons and Wilcoxon signed rank test for intragroup comparisons. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows [26].

RESULTS

This study was conducted to assess the antifungal effect of three essential oils proposed for the treatment of oral thrush. The antifungal effect of these oils was assessed after 24, and 48 hours.

Inter-group comparison of the examined essential oils

The results revealed that there was a statistically significant difference between inhibition zone (mm) values of different oils ($p<0.001$) after both time intervals. The highest value of inhibition zone was

observed in the Tea tree oil group, followed by Moringa oil then Nystatin (control), while the lowest value was found in Grape seed oil group. Post hoc pairwise comparisons between the different groups, showed that the value of Tea tree oil inhibition zone was significantly higher than that of the other groups except for Moringa oil. There was no a statistically significant difference between the inhibition zone values of Moringa oil and Nystatin (control) ($p>0.05$). Moreover, the value of inhibition zone of Grape seed oil group was found to be significantly lower than those of other groups except for Nystatin (control) ($p<0.001$), (Table 1 & Figure 1).

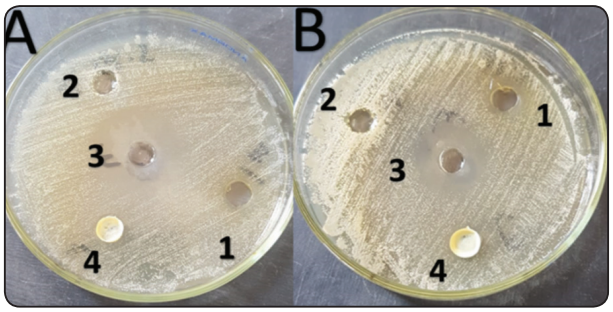

Fig. (1) Agar plates with Candida albicans showing the zones of fungal growth inhibition among the tested groups. (A: after 24 hours, B: after 48 hours, 1: Moringa, 2: Grape seed, 3: Tea tree, 4: Nystatin)

Intra-group comparison of the examined essential oils:

The findings of the present study demonstrated that there was no significant difference between the inhibition zone values measured at both time intervals for all tested materials ($p>0.05$). The recorded inhibition zone values for Moringa and Tea tree oils revealed that the highest values for both oils were observed after 48 hours, followed by 24 hours. While the inhibition zone values for Nystatin (control) were the same after 24 and 48 hours, (Table 1).

TABLE (1): Inter and intragroup comparison of Candida albicans inhibition zone (mm) among the tested groups.

Time	Inhibition zone (mm) (Mean±SD)				p-value	Eta squared (95%CI)	Power
	Group 1 Moringa oil	*Group 2 Grape seed oil*	*Group 3 Tea tree oil*	*Group 4 Nystatin (control)*			
24 hours	11.75 ± 2.19^{ABa}	0.00 ± 0.00^{Ca}	27.50 ± 2.67^{Aa}	10.00 ± 0.00^{BCa}	**<0.001***	**0.928 (0.840-0.980)**	**1.000**
48 hours	12.00 ± 2.62^{ABa}	0.00 ± 0.00^{Ca}	28.00 ± 3.30^{Aa}	10.00 ± 0.00^{BCa}	**<0.001***	**0.927 (0.850-0.970)**	**1.000**
p-value	**0.346ns**	**1ns**	**0.346ns**	**1ns**			

Different upper and lowercase superscript letters indicate a statistically significant difference within the same horizontal row and vertical column respectively; *significant ($p<0.05$)

Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction for intergroup comparisons

Wilcoxon signed rank test for intragroup comparisons

DISCUSSION

Herbal medicine has been introduced as a possible alternative to traditional drugs because of its competitive price, availability, long-lasting therapeutic effects, and potential effectiveness [27].

Thus, this study was carried out to assess the antifungal effect of Moringa oleifera, Grape seeds, Tea tree, and Nystatin in an in-vitro study. The antifungal effect was evaluated after 24, and 48 hours, following the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for antifungal susceptibility testing of Yeast and Filamentous Fungi [28,29].

In the present study, an agar diffusion test was utilized to evaluate the antifungal effect of the three oils as it is rated as the most frequently used method to assess the antimicrobial potential of essential oils and their components [30].

C.albicans. is normal inhabitant of the oral cavity and is commonly found on mucosal surfaces, when immune system impairments occur the colonization of this organism will arise. Therefore, it is important for the clinicians to be aware of the risk factors, early diagnosis, and proper treatment of oral candidiasis, especially in children [31].

The results of this study revealed that, there was a statistically significant difference between inhibition zone (mm) values of different oils ($p<0.001$) after 24-, and 48-hours. The highest value was found in the Tea tree oil group. The previously reported superior results of Nystatin over Tea tree oil reported by *Lydiawati el al*.[6] were due to the great dilution of Tea tree oil (5%).

Tea tree oil antimicrobial activity is attributed mainly to terpinen-4-ol, the main bioactive component present in Tea tree oil [16]. Tea tree oil and its components increase the permeability of the cell membrane. This could inhibit the growth of Candida species. In addition to this mechanism, tea tree oil could also produce membrane lipid bilayers like structure that change the component to make the membrane intact. These changes lead to inhibition of the growth of Candida [6].

The results of the present study reported that, there was no statistically significant difference between Moringa oil and Nystatin (control) groups ($p>0.05$) at the 2 intervals investigations. This comes in agreement with *Isitua, et al*., [11] who showed comparable zones of inhibition. The antifungal activity of the bioactive compounds in Moringa leaves, against C. albicans was proved by previous studies [10,11,32].

Despite the popularity of grape seed oil as an antifungal agent [14], the results of this study demonstrated that the value of the inhibition zone of Grape seed oil group was lower than Nystatin (control) after 24- and 48-hours assessment with no statistically significant difference ($p>0.05$). The inefficacy of the grape seed oil in this study may be attributed to the different preparation method of the oil, the method used to investigate the antimicrobial property, or the difference in the reference strain of the studied microorganism. These findings are in accordance with *Sherestha et al*.,[33], *Eslami et al*.,[2] and *Volety et al*.,[15].

CONCLUSION

Teatree and Moringa oils showed promising antifungal activity while Grape seed showed minimal antifungal activity against candida albicans. The herbal extracts can be used in the future treatment of fungal infections with minimal side effects.

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