RESEARCH ARTICLE

Evaluation of the Effect of Some Plant Extracts as Antifungal Substances Isolated from Patients with Gingivitis and Endophthalmitis in Hilla City

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ABSTRACT

During this study, 8 isolates of *Candida* yeast were isolated and diagnosed on CHROMagar, and *Candida albicans* was the most prevalent species by 75%. The inhibitory activity of plant extracts of adrak, cloves, green tea, and cinnamon was tested by disc diffusion test against *C. albicans*, *C. krusei*, and the antibiotics gentian stain, fluconazole, kenazole, nystatin were used for comparison. As well as using oral and dental cleaning products for comparison as well. The study showed that clove extract had a weak inhibitory activity at a high concentration, while extracts of green tea, cinnamon and mint did not show any activity in inhibiting *C. albicans* and showed that toothpaste had high activity in inhibiting yeast better than the plant extracts that did not show any inhibitory effect despite their use in high concentrations. We conclude from the current study that it is better to use toothpaste instead of herbs to get rid of gingivitis because it plays a major role in inhibiting *Candida* yeast.

Keywords: *Candida*, Plant extract, Gingivitis, Antifungal.

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INTRODUCTION

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of *Candida* species. *Candida albicans* is one of the most common types in the oral cavity.[1] *Candida* infections of the mouth and throat are treated with well-known antifungals. The duration of treatment depends on the severity of the infection, the age, and the immune status of the person. Injuries can lead to dangerous forms resulting from the invasion of *Candida*.[2] The incidence varies with age and other factors such as smokers and people with diabetes, where the oral yeast infection is widespread. Oral candidiasis is one of the most common fungal infections in humans, especially in early life and in later life in extreme cases it can be fatal when it becomes widely disseminated.[3] It often occurs when the immune system is weakened as a result of disease or drugs, or when antibiotics disrupt the natural balance of microorganisms in the body and cause human diseases, as it affects the mouth, causing several diseases, the most important of which are gingivitis and oral whiteness. This infection is mainly caused by the yeast *Candida albicans*.[4] *Candida* infections of the mouth and throat are treated with well-known antifungals and continuous follow-up of the patient. Symptoms may include the presence of white, creamy soft spots, and lesions with an appearance similar to cheese in the mouth (on the tongue and inside the cheeks) or the throat, pain and difficulty swallowing, inflammation of the esophagus and other symptoms. Washing the mouth with chemical rinses improves oral hygiene and prevents tooth decay, while herbs are effective in preventing dental injuries and have fewer side effects and are more economical compared to chemical medicines and the use of chemical medicines has a dangerous effect in addition to being resistant to microorganisms. Some scientists chose a group of herbs based on their effectiveness in treating oral yeasts and their low cost.[5] Among the most important herbs that showed effectiveness on oral yeasts according to previous studies are: It is known the leaves of the cinnamon were used to prepare the

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extract, it was found that *Candida* is sensitive to the oils. The scientist[6] showed that about 85.5% of the *Candida* strains are sensitive to a concentration of 312.5 mcg/mL and gave areas of inhibition of 40 mm, while Hili showed that 72.2% of the *Candida albicans* strains were sensitive for a concentration of 500 mcg/mL.[7]

Cloves have a mechanism of action that involves direct damage to the cell membrane of the cell wall, leading to cell death. The effect of cloves on the cell membrane is more compared to the results obtained when using amphotericin B.[8] It is sensitive to a MIC of 0.64 µg/mL for cloves, while it is sensitive to a MIC of 1-µg/mL for fluconazole.[9]

Mint shows a significant decrease in the effectiveness of the biofilm composition and also leads to a change in shape, as it is in the form of bubbles sometimes with swelling in the cell and forms similar to clusters. *C. albicans* is sensitive to MIC of 0.025 µg/mL for peppermint while MIC is sensitive to 2 µg/mL MIC.[10] About 22.3 mm for the aqueous extract of mint and (9.2–17.7 mm) for the methanol extract of the same plant mentioned.[11]

The World Health Organization recommended the use of Arak in 1986 AD. The effect of Arak as an antibacterial and antiseptic may be due to the chemical content of the extract, which includes (sodium chloride, potassium chloride, tanning, silica, resin, alkaloid compounds, fatty acids, and vitamin C. About 12.5 mg/mL gave an area of inhibition of 9.1 mm in the case of the aqueous extract. The areas of inhibition ranged between (7.8–7.8).[12]

Synthesized antibiotics used for control gave MIC values of 64- (32 µg/mL) on 87.5 to 75% of *Candida* strains, respectively. These antibiotics are used for the treatment of superficial infections caused by *Candida*, nystatin is used to treat *Candida* at MIC of 0.5 to 8 µg/mL. Fluconazole is better than nystatin because it has fewer side effects, in addition to that nystatin stimulates the secretions of the salivary gland and thus leads to its dilution.[13]

MATERIALS AND METHODS

Sample Collection

Twenty samples of gum and oral lining swabs were collected from patients with gingivitis and/or thrush from several health centers in Hilla city using an Amis Transport medium. The samples were kept at 4ºC until the tests were conducted.

Cultivation and Isolation of Oral Fungi

Swabs were cultured on PDA medium to obtain single colonies. The dishes were incubated at 37ºC for 24 hours.

Isolate Diagnosis

A sample of pure colonies was stained on a glass slide using a Lactophenol-cotton blue stain, and then the samples were examined under the compound microscope.

The positive samples in the previous paragraph were plotted on CHROM agar medium to ensure the purity of the isolates and to diagnose the type of yeast isolated according to the color of the colonies.

Preparation of Plant Extracts

Aqueous extracts of several common plants clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), arak (*Salvadora persica*), green tea (*Camellia sinensis*), and mint (*Mentha cervina*), were prepared as additives for oral and dental cleaning products, where spices and plants were obtained from local markets, either in their dry form (cloves, sage and green tea) or fresh (Arak and mint) where they were dried in the oven. at 60ºC. All materials were ground using an electric mill and kept in glass bottles. The aqueous extract was prepared as follows:

A total of 100 g of the dried plant was weighed in a glass beaker and then 1 liter of distilled water was added to it. The mixture was boiled with constant stirring for one minute, then left to cool then filtered using Whatman no.1 filter paper and Buechner funnel, then the filtrate was transferred to sterile containers of known weight. The extract was dried using a Rotary evaporator at 100ºC, and then the dry weight of the extract was calculated.

The extract was dissolved in 50 mL of distilled water and the final concentration of the concentrated solution was calculated (Table 1). Several double dilutions (1:1, 1:2, 1:4, 1:8, 1:16) were made in addition to the concentrated solution. The plant extracts were sterilized using fine filters (0.45 µm) and kept at 4ºC until use.

Sensitivity Test for Fungal Isolates

The sensitivity of the fungal isolates was tested using the method used by CLSI M44A, using different concentrations of plant extracts, in addition to different types of commonly used toothpaste and mouthwashes which were purchased from the local market, and the results were compared with some common antifungal agents: Gentian stain, fluconazole, kenazole, nystatin and as follows:

Table 1: Dry weight and the final concentration of the aqueous extract of the plants' understudy

Code	*Common name*	*Extract dry weight (g)*	*Extract final conc. (mg/mL)*
1	Clove	10	200
2	Cinnamon	3	60
3	Arak	13	260
4	Green Tea	15	300
5	Mint	13.5	270

Table 2: Identified yeast species

Code	*Species*	*Color on CHROM agar*
C1	*C. albicans*	Green
C6	*C. krusei*	Pale pink
C7	*C. albicans*	Green
C8	*C. albicans*	Green
C9	*C. albicans*	Green
C12	C. albicans	Green
C13	C. albicans	Green
C18	*C. krusei*	Pale pink

A 1-mL of yeast suspension (0.5 McFarland) was spread on Molar Hinton agar plates and left for 1-minute. Discs of filter paper with a diameter of 5 mm were made for the sensitivity test by diffusion method where each disc was dipped in the materials under test to be immersed, i.e., up to 20 µL of the substance and then distributed on the surface of the dish in triplicates (CLSI M44A). The dishes were incubated at 35ºC for 48 hours. The inhibition diameter was measured for each tablet of diluted extracts and compared to the rest of the tablets representing oral and dental hygiene preparations and antibiotics.

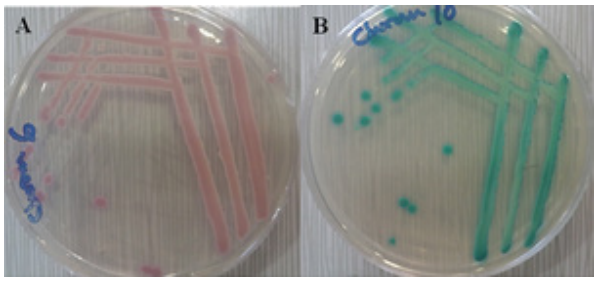

Figure 1: Yeast isolates, A: *C. Krusei* and B: *C. albicans* as shown on CHROM agar medium

RESULTS AND DISCUSSION

Eight isolates of yeast belonging to the genus *Candida* were isolated and purified, i.e. 40% of the total samples under study. The diagnostic results appeared as in Table 2 and Figure 1. *Candida albicans* is the most prevalent among the fungal isolates, i.e. 75%. This agrees with (1).

Sensitivity test

Table 3 and Figure 2 show the results of the sensitivity assay for the yeast isolates under study. The results showed that the

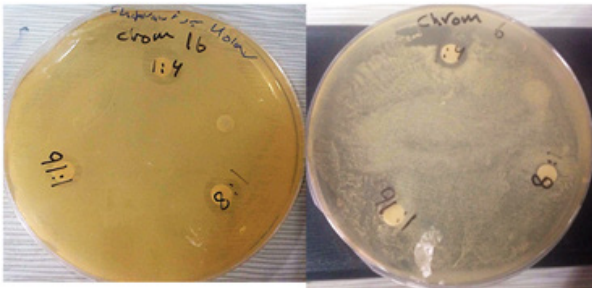

Figure 2: Sensitivity test of some plant extracts

Table 3: Sensitivity examination of the isolated yeast

Material and concentration								*Isolates and Inhibition zone diameter (mm)*
	C1	C6	C7	C8	C9	C10	C13	C18
Clove								
*concentrated	-	15	-	-	-	-	-	-
*1:1	-	10	-	-	-	-	-	-
*1:2	-	-	-	-	-	-	-	-
*1:4	-	-	-	-	-	-	-	-
*1:8	-	-	-	-	-	-	-	-
*1:16	-	-	-	-	-	-	-	-
Cinnamon 1:1*	-	-	-	-	-	-	-	-
Arak *1:1	-	-	-	-	-	-	-	-
Green tea 1:1*	-	-	-	-	-	-	-	-
Mint 1:1*	-	-	-	-	-	-	-	-
Toothpaste 1 *1:1	14	12	23.	14	15	-	-	13.5
Toothpaste 2 1:1*	10	12	13	13	-	10	-	13.5
Toothpaste 3 *1:1	13	12	10	11	11	-	-	12
Mouth wash 1								
*concentrated	12.0	15.0	15.0	14.0	26.0	13.0	20.0	16.0
*1:1	10.0	11.0	13.5	13.5	14.5	13.5	-	15.0
*1:2	9.5	12.0	10.0	13.5	-	9.0	-	15.0
*1:4	9.75	9.5	15.0	13.0	-	9.0	11.5	13.5
*1:8	7.75	10.0	9.0	13.0	-	8.0	17.7	14.2
*1:16	6.00	6.0	-	7.5	-	4.5	9.0	11.2
Mouth wash 2 *1:1	-	-	-	-	-	-	-	-
Nystatin	20	20	-	22	25	23	21	19
Gentian stain	13	13	22	19	25	19	20	25
Fluconozol	-	25	-	-	-	-	20	25
Kenazole	-	26	9	-	25	23	23	23

aqueous extracts of the selected plants had no significant effect on the inhibition of yeast growth, except for the concentrated extract of clove and the 1:1 dilution, which showed a somewhat acceptable inhibition ratio on isolate C6 (*C. kruzei*).

Dental cleaning products showed inhibitory activity on most of the isolates, except mouthwash 2, which had no inhibitory activity at all.

Gentian stain has a high inhibitory effect on all isolates under study compared to the rest of the antifungal agents. Whereas the antibiotics nystatin and kenazole showed varying effectiveness on most of the isolates. As for the antifungal fluconazole, it had no qualitative effect as an antifungal, as most of the *C. albicans* isolates showed resistance to this antifungal.[14,15] Whereas, both C6 and C18 isolates of *C. kruzei* showed high sensitivity.

In general, *C. albicans* showed remarkable resistance to all extracts used in addition to some oral hygiene products and antibiotics in comparison with *C. kruzei.*

CONCLUSION

Most of the fungal isolates belonged to *C. albicans.* Plant extracts that are used as additives to dental cleaning products do not have any role as anti-fungal substances in the mouth, but only add flavor.

Dental cleaning products have an effective role as an anti-fungal in the mouth without the need for any other additives, especially toothpaste.

Antibiotics have varying efficacy against oral fungi.

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