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In Vitro Comparison of Inhibitory Activity of 10 Plant Extracts Against *Candida Albicans*

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Abstract: Aim: Because of scientific progression and paying more attention to health problem, using medical materials is increased. Because of the side effects, cost and difficulty of therapeutic-chemical materials production, using medical plants which have less side effects and are economically cost effective, have been recently taken into consideration. Researches on antimicrobial effects of plants assume certain importance. Materials and Methods: This study investigated the antimicrobial effect of 10 medical plants on Candida albicans. The following plants were selected: thyme, mint, garlic, cinnamon, chamomile, tea tree, clove, spearmint, sage and rosemary. These plants had been selected according to the medical traditional usage and previous researches. C. albicans was cultured in Sabouraud dextrose Agar containing Chloramphenicole. On each plate, one plant extract disc, one chlorhexidine disc and one nystatin disc; as positive controls; and one methanol and one blank disc; as negative controls; were placed. After 24 hours, the mean diameter of non-growth halo around every plant extract was compared with the mean diameter of non-growth halo of positive control disks by T test statistical analyze. Results: Non-growth halo in disks containing chamomile, garlic, clove, cinnamon, sage and thyme was observed. Cinnamon, garlic, chamomile, sage, clove and thyme had inhibitory effects on *C.albicans*. If similar results are confirmed in clinical trials, theses plant extracts can be used to produce new antifungal products.

Key words: Plant extract, *Candida albicans*, inhibitory effect, antifungal, nystatin, chlorhexidine.

INTRODUCTION

The experience of recent decades has shown that despite significant usefulness of artificial drugs, they have undesirable effects too.

Because resistance of the microbial species to the present artificial drugs has increased, nowadays researchers pay more attention to use medical plants.

Most of previous research has focused on special pathogens that affect skin, respiratory, digestive and urinary systems and there is a few studies about pathogens of oral mucosa (Ruddock *et al*, 2005). While in dentistry, systematic and local use of antimicrobial materials is more common and mouthwashes are usually used to decrease microorganisms of oral cavity. These chemical mouthwashes such as chlorhexidine have several side effects (Gürgan *et al*, 2006). Therefore, finding medical plants that have antimicrobial effects and using them as mouthwash has advantages as decreasing apparent side effects, decreasing poisonous effects on tissues, and also they are more economical. In this research, the antifungal effect of 10 plant extracts on *Candida albicans*, which is one current opportunist microorganisms in oral cavity, has been compared with chlorhexidine as antimicrobial mouthwash and nystatin as antifungal drug.

In previous research antimicrobial effects of some plant extracts have been studied with different methods. In one of them, in vitro antimicrobial activity of different natural agents against to a few mouth bacteria has

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been evaluated. Among them, Cinnamon, Clove oil and spices extract have inhibitory effects on mouth bacteria(Saeki *et al*, 1989).

Singh, *et al* identified "cinnamic aldehyde" in cinnamon as antifungal material (Singh ,1995).

A study done about effect of 45 Indian plants on human resistant pathogens to different drugs, 40 plants showed antimicrobial activity against one or more bacteria and 24 plants showed antifungal activity against Candida (Ahmad and Beg, 2001).

Reviewing the previous studies, it is clear that little research has focused on antimicrobial effect of plant extracts on oral pathogens. Because of fewer side effects of plant drugs, this study was initiated to evaluate the antimicrobial effects of 10 methanol plant extracts on one opportunistic pathogens of oral cavity: Candida *albicans*.

MATERIALS AND METHODS

Candida albicans was prepared from the Scientific and Industrial Center in Tabriz, Iran. *Candida albicans* was cultured in S.c medium (Sabouraud dextrose Agar containing Chloramphenicole).

After reviewing articles and review on traditional medicine, 10 species of plants that have shown more therapeutic properties on many pathogenic agents, have been selected.

These plants were *Matricaria chamomillathyme* (chamomile), *Mentha arvensis* (mint), *Allium vineale* (garlic), *Cinnamomum zeylanicum* (cinnamon), *Melaleuca alternifolia* (tea tree), *Eugenia aromatica* (clove), *Mentha spicata* (spearmint), *Salvia officinalis* (sage) *Rosemaryinus officinalis* (rosemary) and *Thymus serpyllum* (thyme).

For Preparation of plant extracts, the method of Alade & Irobi (1993) was adapted with little modifications. Thirty grams of each powdered plant material were dissolved in 100- milliliters of pure methanol and placed on a shaker for 48 hours (Ahmad *et al.,* 1998; Ahmad *et al*., 2001).

Then, every solution was stirred every 24h using a sterile glass rod. Each extract was passed through Whatmann trez Filter No. 1(Whatmann, UK). Obtained refuses from filtrated specimens were weighted separately. The weight of filtrated specimens were in the range 29/985±0/010 grams.

In the next stage, filtered solutions were kept in an incubator 37°*C* for 48 hours to produce 0/5 to 2/5 mgr/ml concentration of solution.

In this experiment, standard paper disk diffusion method proposed by Baur *et al* (1966) was used to assess antimicrobial activity(Ahmad and Beg, 2001; Ahmad *et al*,1998).

Preparation of disks containing plant extract:

One hundred needle tips containing 10 disks were placed on a medical vial and were sterilized with autoclave. In sterile conditions, every needle consisting of 10 disks was placed into a glass containing one of the plant methanol extracts and covered.

After one hour, disks saturated, by pincer under the sterile conditions, the discs were removed and put in a 40 ℃ incubator for 20 minutes to be dried.

On each plate, one plant extract disc, one chlorhexidine disc and one nystatin disc which were used as positive controls and one methanol and one blank disc which were used as negative controls were placed. These disks were placed at a distance of 15 mm from the edge of the plate and 24 mm from the center of the next disk. There should be observed Candida non-growth halo around positive controls disks but there should not be observed non-growth halo around negative controls disks.

The plates were incubated at 37°C for 24 h.

For every plant extract, this test was repeated 10 times.

The zone of inhibition of each test plant against *Candida albicans* was compared with chlorhexidine and nystatin using T test analysis.

Results:

After 24 hours, the diameter of the zone of inhibition of each disc was measured using caliper.

There is a direct relation between the diameter of the zone of inhibition and antifungal ability of every plant extract.

As indicated in figures 1-5, blank disks and disks soaked in methanol, which were used as negative control, did not have any halo indicative of the lack of growth. Disks containing nystatin-as positive control disk- showed the greatest diameter of the zone of inhibition in all plates. The mean of inhibitory zone was 26.43 mm; S.D. = 2.14 mm.

The mean of inhibitory zone of chlorhexidine was 14.52mm; S.D. = 1.09 mm, less than the diameter of non-growth halo of nystatin.

Among 10 disks soaked in plant extracts at different plates, we observed non-growth halo in disks containing chamomile, garlic, clove, cinnamon, sage and thyme. Table 1 shows the mean diameter of halo indicative of the lack of growth around every plant at repeated tests. Among them, chamomile, garlic and clove had better effect than chlorhexidine and cinnamon and sage had similar effect with chlorhexidine according to statistical comparing. The inhibitory zone of thyme extract was significantly less than chlorhexidine. Also, all non-growth halos related to studied plants showed significantly difference to the mean diameter of halo indicative of the lack of growth related to nystatin disk. Nystatin had high antifungal effect than all studied plants. Figure 1, 2, 3, 4 and 5 show the mean of the inhibitory zone of these plants.

Table 1: Comparison of the mean inhibitory zone of the plant extracts with chlorhexidine

Herbal extracts	Diameters of no-growth halo (mm)	Comparison to chlorhexidine (PV)
Chamomile	18.98	0.003
Garlic	18.64	0.001
Clove	15.26	0.003
Cinnamon	14.92	0.751
Sage	14.80	0.561
thyme	8.81	0.009

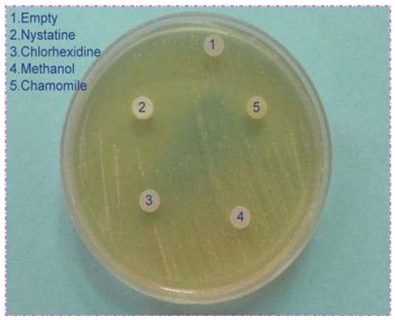

Fig. 1: The inhibitory zone related to chamomile

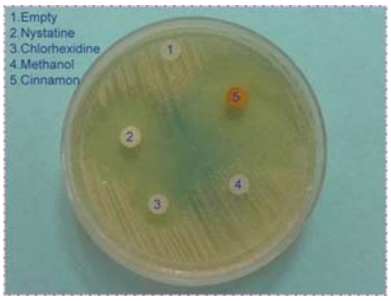

Fig. 2: The inhibitory zone related to cinnamon

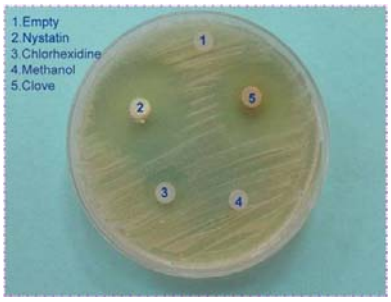

Fig. 3: The inhibitory zone related to clove

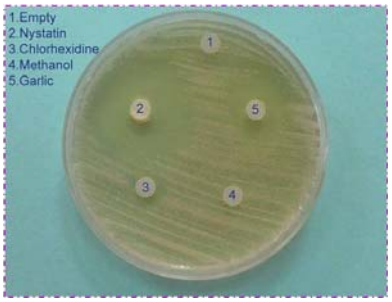

Fig. 4: The inhibitory zone related to garlic

Fig. 5: The inhibitory zone related to sage

Discussion:

This study showed the antifungal effects of 10 plant extract against *Candida albicans* using standard disk diffusion method. In comparison with effects of chlorhexidine and nystatin mouthwash, methanol extract of chamomile, garlic, clove, cinnamon, and sage had inhibitory activity against *Candida albicans*. Similar to results of the research done by Xia, *et al* (Xia and Ng, 2005) and Ghannoum (Ghannoum,1988), in this study, antifungal effect of garlic against *C.albicans* was observed. Ghannoum defined that water extract of garlic shows antifungal effect by oxidation of thiol groups of proteins. Thiol oxidation causes inactivating of enzymes and inhibition of microbial growth(Ghannoum,1988). In other study, the effect of the garlic on *Candida albicans* was evaluated and observed that alliumin of this plant has inhibitory effect on mycosphaerella arachidicola (Xia and Ng, 2005).

In other study, Ankri *et al* studied activity of allicin of freshly crushed garlic and showed that garlic has a broad spectrum inhibitory activity against Gram-positive and Gram-negative bacteria and *Candida albicans (*Ankri and Mirelman,1999).

In a research done in 2001, the biological property of steroid saponins present in garlic was investigated. That study cleared that this material has antifungal, antitumor preventing firming clot and cytotoxicity effects(Matsuura, 2001).

The study done on the allium plants detected that extract of garlic, onion and shallot scallion, chiness leck, chiness chive, bakeri garlic had the antifungal effect. Garlic had higher antifungal activity than other plants(Yin and Tsao, 1999).

In another study, Yoshida, *et al* assessed six fractions derived from garlic. Ajoene had inhibitory effect on *Candida albicans* and *Aspergillus niger* (Yoshida *et al*,1987).

Wang, *et al* isolated allivin and lillin, which are special proteins with antifungal effects, from garlic (Wang and Ng , 2000; Wang and Ng 2001).

Mau, *et al*., (2001), De, *et al* (1999), Saeki, *et al*., (1989), Quale, *et al*., (1996) studies, confirmed the inhibitory effect of cinnamon on different microorganisms, such as E.coli, Sacharomyces cerevisiae, Bacillus subtilis, and oral bacteria, such as Streptococcus sp, Actinomyces sp, Actinobacillus sp, Bacteroides sp, Capnocytophaga sp, Eikenella sp, Fusobacterium sp, propionibacterium sp and a few species of Candida.

Singh, *et al* attributed identified cinnamon aldehyde as the active fungitoxic constituent of cinnamon (Singh *et al*, 2005).

Also, in a study on 12 plant species with therapeutic effect, cinnamon, spearment, and thyme had more antifungal activity (Soliman and Badeaa, 2002).

Ahmad N, *et al* showed strong antifungal activity of the clove oil against different fungi such as *Candida albicans*. The achieved result was similar to the result which was observed in the present research (Ahmad *et al*, 2005).

In another study, the inhibitory effect of cinnamon, thyme and clove against a few different fungi such as *Candida albicans* was cleared (Suhr and Nielsen ,2003). Similar to this study, antifungal effect of chamomile was observed in another research, too (McKay and Blumberg, 2006).

As mentioned in this paper, sage, chamomile and rosemary antimicrobial effects. Before, in one research, the inhibitory effects of these plants on other microorganisms such as the arcobacter strains, was determined (Cervenka *et al* ,2006).

The zone of inhibitory was observed around 2 of 10 disks containing thyme. In another research, thyme showed moderate antifungal effect(Suhr and Nielsen,2003). But, Hammer *et al* investigated antimicrobial activity of 52 plant extracts. This study cleared the lowest minimum inhibitory concentration is related to thyme (Hammer *et al*,1999).

According to these results, it is needed to more studies done about the antifungal effect of thyme.

Thyme and sage have different strains in different countries. Therefore, this controversy could be related to various species of these plants. Other research done in 2008 showed the essential oils of rosemary and sage did not have the antifungal activity against Candida isolates (Pozzatti*et al*,2008). But, the research done by (Hammer *et al*,1999), (Bozin *et al*, 2007), and (Ahmad and Beg, 2001), showed that rosemary and sage have antifungal effect.

Also, the difference between our achieved results in the present study and some of the previous research can be due to different Candida strains. These plants have inhibitory effect on one strain of Candida and these plants do not have any inhibitory effect on other strains such as *Candida albicans*.

In addition, differences in extract type and concentration could lead to differences in results. For example, Mahasneh *et al* evaluated the antimicrobial activity of 9 medical plants against 4 bacteria and 3 fungi and observed that methanol and hexan extracts of these plants did not have any antimicrobial effects. However, the botanol extract of Ononis spinosa and Brynia syriaca had high moderate antifungal activity against 3 fungi including *Candida albicans* (Mahasneh and El-Oqlah, 1999).

Ahmad *et al* studied effect of 82 Indian plants against pathogenic and opportunist microorganisms. Among them, 56 plants had the antimicrobial activity against one or more pathogen. Five plants showed strong antibacterial. Also, they discovered that alcoholic extract of the plants had higher activity than water and Hexan extracts (Ahmad *et al*,1998).

Besides, another research done by Cervenka *et al* on methanol and chloroform extracts of 17 plants such as rosemary, sage, chamomile and cinnamon, showed that methanol extract of these plants had higher antimicrobial effects than their chloroform extracts (Cervenka *et al*,2006). The difference in obtained results is due to using different kinds and different concentrations of extracts in our study and previous researches.

Briefly, about some plants that showed antimicrobial activity, clinical trial is necessary to determine the usefulness of these plant extracts for the treatment of oral candidiasis.

If similar results are confirmed in clinical trials, theses plant extracts can be used to produce new, useful and economic antifungal products.

Conclusion:

Among plants being studied, methanol extract of garlic, cinnamon, chamomile, clove, and sage had greater inhibitory activity against *Candida albicans*. Garlic, chamomile, and clove showed better inhibitory effect than cholorhexidin.

Because of the antimicrobial effects of some medical plants, which have minimal side effects in comparison with chemical drugs, more in vivo and in vitro investigations about oral cavity flora and antibacterial and antifungal effects of different plants on oral bacteria and fungi should be recommend.

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