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Investigating the Effects of *Thymus Vulgaris* Products and Clotrimazole Lotion to Prevent the Growth of CandidaAlbicans Fungus

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ABSTRACT

Since a long time, medicinal plants have been used for treatment of diseases, especially infectious diseases. Plant species diversity and the tendency of society to use the natural materials in the treatment of diseases have placed the screening of essences and herbal extracts in the core attention of researchers. The aim of this study was to evaluate the effect of *Thymus vulgaris* extract and essence on Candida albicans and compare its effects with clotrimazole lotion. Ethanolic, methanolic, and aqueous extracts of *Thymus vulgaris*prepared by *maceration* extraction method. *Thymus vulgaris* extract was prepared by water distillation. Then, they were used in research. *Thymus vulgaris* essence prepared fromBaryj Essence Company. Then paper discs which coated with herbal compounds placed in Candida albicans medium and theirzone diameters of growth inhibition compared with that of clotrimazole ointment. the essence and (ethanolic, methanolic) extracts and distilled extract of *Thymus vulgaris* haveantifungal effects and its essence zone diameter of growth inhibition was higher than its extracts zone diameter of growth inhibition. This study shows the good effects of *Thymus vulgaris*essence and (ethanolic, methanolic) extracts and distilled extract in preventing Candida albicans pathogenic fungi growth.It was also found that *Thymus vulgaris*is more effective in preventing Candida albicans pathogenic fungi than clotrimazole lotion .Therefore;Itcan be usedas a substitute forclotrimazole lotionin treatment ofcandidiasis.

Ke ywords: *Thymus vulgaris*,Clotrimazole, Candida albicans.

INTRODUCTION

Infectious diseases are the most common diseases in the world which have imposed a huge cost to the human society. In the past decades, although synthesis antibiotics played an important role in the treatment of infectious diseases, but the problems of bacterial resistance to antibiotics, hasled human society to more use of herbal remedies [1-3]. The use of herbal extracts and essences, as the alternatives of synthetic andprotective materials have been developed in food industry[4].Many researchers screen herbal essences and extracts to achieve their natural anti-fungal and anti-microbial ingredients[5, 6]. Mountain thyme with scientific name of *Thymus vulgaris* is from *Lamiaceae* species, its leaves and flowered branches strengthen the stomach; they also have anti-seizure and anti-menstrual effects and used in food industry as flavoring[7, 8]. Its essence has the same therapeutic uses and antiseptic effects. *Zataria multiflora* essence is obtained from plant

distillation and its two main components are thymol and carvacrol.Findingsshow antifungal effect of carvacrol[11, 10]. Candida albicans fungi are natural flora of the gastrointestinal tract, mouth and vagina.Ittransfers to human when passing through the vagina during birth. This fungus under specific conditions (including age, diabetes, use of antibiotics, and drugs containing steroids) can attack body mechanisms and cause disease.Sometimes, Candida albicans yeast in immunocompromised Patients (people with AIDS and various cancers) may be accompanied by fatal septicemia.In addition, itis one of the most important causes of fungal vaginitis in women which can be treated by available drugs[12]. Because of different clinical and immunological conditions of patients, patients with surface candidiasis should first be treated with topical anti-fungal drugs.Treatment of candidiasis diseases can cause side effects .For example , clotrimazole (topical ointment and vaginal tablets)may cause burning and itching, Itraconazole (capsules) causes fever, chills, itching, rash, and ketoconazole (tablets), can turn urine dark.Miconazole (ointment) causes blister, burning, redness of the skin, fluconazole (capsules) causes nausea, diarrhea and abdominal pain, nystatin (oral), stomach pain, nausea or vomiting, diarrhea, vaginal irritation of the vagina that did not exist before treatment[13].Hydro alcoholic extract of *Zataria multiflora*leaves has antibacterial effects against staphylococcus aureus standard strain[14]. *Zataria Multiflora* essence has antibacterial activity on Escherichia coli and Staphylococcus aureus which can be used as an alternative to ineffective antibiotics[15]. Considering the side effects of synthesis drugs and according to the previous studies[16], we decided to investigate the effect of ethanolic, methanolic, and aqueous) extract, essence and distilled extract of mountain thyme on Candida albicans yeast in vitro condition and compare the results with results related to the effect of clotrimazole lotion on this yeast .

MATERIALS AND METHODS

The preparation of fungal standard samples

Samples of the Candida albicans stellatoideafungus were provided from Imam Ali Hospital laboratory.To identify the Candida albicansstellatoideafungus, germ tube test was used as follow:1-We used human serum preparedfrom blood transfer organization. 2- By using a sterile swab, yeast was picked up from Pure culture and placed in serum .3-Suspension was heated for 2-3 hours at 37c .4-By using a sterile swab ,a drop of the suspension placed in a sterile slide and after covering it by a lamella, we Placed it under a microscope and observed the germ tubes.To obtain more complete observation, the suspension containing fungus heated for 8 hours at 37c.

The preparation of plants distilled extracts

To produce intended distilled extracts, the thyme is washed and then dried in dark place.Since cinnamon is not cultivated in our country, its dried bark can be used.To obtain ethanolic extracts of Cinnamon and Thyme, their dried bark is powdered. Then 100g dried powder placed in distillation flask and 1lit water added to the compound and heated on distiller.The resulting distilled extract was used for testing.In this study, cinnamon distilled extract was produced through water distillation.To prepare the alcoholic extracts of cinnamon and thyme, first, their powder was prepared (the initial tests showed that they limit fungal growth).10 g cinnamon powder added to ethanol 30 and poured in one flask and 5g thyme (thyme has higher absorption power than cinnamon) added to ethanol 30 and poured in another flask and stored for 48 hours at room temperature.The derived extracts called ethanoic extracts.To prepare methanolic extracts, the same method was exactly used.to prepare aqueous extracts of cinnamon and thyme through ethanolic extraction process, the distilled extraction method was used.In this approach distilled water was used instead of ethanol and the compound was sterilized in autoclave.

Themethod of using distilled extracts within the medium

The controlled medium which indicated fungal growth contained distilled water and dextrose agar powder.The plant pure or diluted distilled extracts were used in samples instead of distilled water.After sterilization, the medium was placed in sterile petri dishes as control sample and marked with labels.After a few minutes, the jelly-like medium was ready for fungal culture.then by using a sterilized swap, a sample of fungi pick up

from gelatin medium and cultured in intended medium and stored in Incubator for 7 days at 37c and results were investigated. Each test was triplicated.The results showed that, the cinnamon was more effective in limiting fungal growth than thyme.

The Preparation of sabouraud dextrose agar and potito dextrose agar medium and the use of disc blank

Sabouraud dextrose agar and Potito dextrose agar mediums were prepared and candida albicans stellatoidea fungus was cultured on their all surface.Thenthe discs blanks were separately coated by ethanolic, methanolic, aqueous and distilled extracts of cinnamon and thyme and cultured on the mentioned medium. Then the alcoholic extracts of discs as control sample coated by ethanol or methanol and stored in incubated for 24 hours. The zone diameter of growth inhibition was measured by ruler.Tests were conducted by using clotrimazole ointment (one of the most common anti-fungal medications).In this regard, sabouraud dextrose agar was prepared and fungal suspension cultured on all over the medium.A drop of ointment of the approximate size of disk was placed on medium after homogenization. The zone diameter data of growth inhibition analyzed with Minitab software in all samples and control group.

Findings

The results of using thyme extracts in sabouraud dextrose agar medium.

According to conducted tests, the mean of zone diameter around blank discs coated with thyme methanol extracts was 9.5mm. Zone around blank disks containing methanol (control) was not found. The mean zone diameter of thyme ethanolic extracts was 10.33. Zone around blank discs containing ethanol (control) was not found. The mean of zone diameter of thyme distilled extract was 12.8 mm. The mean of zone diameter of thyme aqueous extract was equal to thediameter ofthe disc itself. In other word, thyme aqueous extract has no antifungal effect. The results of using thyme extracts in Potito dextrose agar medium indicated that the zone diameter mean around blank disks coated with thyme methanolic extracts was 9.83 mm. Zone around blank discs containing methanol(control) was not found. The zone diametermeanof thyme ethanolic extracts was 10 mm. zones around blank discs containing ethanol (control) were not found. Thezone diametermean of thyme distilled extract was 11.28. The zone diameter meanof thyme aqueous extract was equal to thediameter ofthe disc itself.In other word, thyme aqueous extract has no antifungal effect. The mean zone diameter of thyme essential oil was 40mm.Zone diameter of growth inhibition was investigated by Minitab software and results indicated that , considering zone diameter of growth inhibition, there was a significant difference between thyme ethanolic , methanolic and distilled extracts. Thecomparison of zone diameter of growth inhibition between thyme methanolic extracts and control sample indicates that there is a significantdifference at the significant level of á=0.01. The comparison of zone diameter of growth inhibition between thyme ethanolic extracts and control sample indicates that atthe significant level of á=0.01and á=0.05 there is no significant difference but at the significant level of á=0.1 there

Table 1:The results of assessing zone diameter of growth inhibition of disc blank containing essence and extracts in sabouraud dextrose agar medium

Plants	Herbal compounds	Medium of zone diameter of growth inhibition	Control sample disk(ethanol, methanol)	Medium of zone diameter of growth inhibition
Thyme	essence TH(101.IX)	40	thyme	38
	methanolic extract	9.5	methanol	7.5
	ethanolic extract	10.33	ethanol	8
	aqueous extract	-	-	-
	extract	12.28		-

is a significant difference. The comparison of zone diameter of growth inhibition between thyme distilled extracts and control sample indicates that at the significant level of á=0.01 there is no significant difference but, there is a significant difference at the significant level of á=0.05.

Table 2: The results of assessing zone diameter of growth inhibition of disc blank containing essence and extracts in potito dextrose agar medium

Plant	Herbal compounds	Medium of zone diameter of growth inhibition	Control sample disk(ethanol, methanol)	Medium of zone diameter of growth inhibition
Thyme	methanolic extract	7.25	methanol	9.83
	ethanolic extract	7.0	ethanol	10.0
	aqueous extract	-	-	-
	extract	-	-	11.25

Table 3: The results of using antifungal medicines (clotrimazole lotion) on fungal medium

Plant	Frequency	The zone diameter of growth inhibition(mm)
Clotrimazole lotion 0.01 on disc blank in medium	3	14.5

DISCUSSION

Microorganisms are the main cause of illness in human.As the chemical materials extracted from plants havefewer side effects, they have been used as alternatives to synthetic medicines. In the study entitled "The Effect of Growth Inhibition of Common Mallow on Microorganisms", the results indicated that aqueous extracts exhibit no antimicrobial effect. Gram-positive bacteria staphylococcus and candida albicans fungus were sensitive to common mallow black ethanolic extract. Common mallow flowers had the greatest inhibitory effect.

Mallow extracts had no effect on bacteria, but its root extract prevented the growth of candida albicans fungus [21]. In recent years, there has been a growing interest in herbal medicine especially in treatment of infectious diseases. For example pityriasis versicolor extract has antifungals effects which has medical use and can be used against cassia alata fungus[22]. Inula viscose aqueous extract has inhibitory effect against dermatophytes such as trichophyton rubrum and Microsporum canis[23].A plant of the family of Polyporaceae has inhibitory effect against dermatophytes such as Trichophyton mentagrophytestrichophyton rubrum, microsporum canis, and microsporum gypseum as well as candida albicans[24]. Agastache extract is effective in treatment of genital candidiasis and tineaversicolor[25]. Agastache rugosa extract increased the effect of clotrimazole ointment in treatment of Blastoschizomyces capitatus (a deadly fungus in people with immunosuppressive). Agastache rugosa extract increases the Therapeutic effects of clotrimazole[26]. Some extracts increase the antifungal activity of macrophages[27]. Some plants extracts can be used in treatment of subcutaneous fungal infections[28]. Treatment with chemical drugs may be difficult and requires long term treatment period.It may also lead to complications. For example the more common antifungal drugs such as clotrimazole and nystatin can lead to complications such as burning, itching, blister, rash, redness of the skin and skin desquamation. In a study entitled The Study of the Antimicrobial Effect of *Zataria Multiflora* Extract on Staphylococcus Aureus ,it was reported that the extract of *Zataria Multiflora*hydroalcoholic leaves

has antibacterial effects against standard strains of Staphylococcus aureus[14]. In another study entitled, TheAntibacterial Activity of *Zataria Multiflora*Extract on the Growth of Staphylococcus Aureus and Escherichia Coli, it was reported that *Zataria Multiflora*extract has antibacterial activity against E. coli and staphylococcus aureus which can be used as an alternative to ineffective antibiotics[15].

CONCLUSION

The anti-fungal effect of thyme essence to thyme distilled , ethanolic and methanolic extracts, is indicative of the fact thatthe effectiveness of different types of essences and ethanolic and methanolic extracts is different in a plant and can be affected by factors such as plant type, plant growth condition, plant environmental characteristics, or extract type, and the type of solvent.The zone diameter of growth inhibition of thyme essence was 40 mm and the zone diameter of growth inhibition of clotrimazole ointment was 14.5 mm. Therefore, it was concluded that thyme essence is more effective against antimicrobial activities and can be used for manufacturing pharmaceutical products.

REFERENCES

1. Cowan MM. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12**(4): 564-582 (1999).
2. Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review. *Int. J. Food. Microbiol.;* **94**: 223-253 (2004).
3. Ayfer D, Turgay O. Antimicrobil activities of various medicinal and commercial plant extracts. *Turk. J. Biol.;* **27**: 157-162 (2003).
4. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J.Appl. Microbiol.;* **86**: 985-990 (1996).
5. Sonboli A, Azizian D, Yousefzadi M, Kanani, Mehrabian AR. Volatile constituents and antimicrobial activity of Tetrataenium lasiopetalum (Apiaceae) from Iran. *Flavour. Frag. J.* **22**: 119-122 (2007).
6. Abdelwahed A, Hayder N, Kilani S, Mahmoud A, Chibani J, Hammami M, et al. Chemical composition and antimicrobial activity of essential oils from Tunisian Pituranthos toruosus (Coss). Maire. *Flavour. Frag. J.* **21**: 120-133 (2006).
7. Erdurak CS, Coskun M, Demirci B, Baser KHC. Composition of the essential oil of fruits and roots of Ferulago isaurica Pesmen and F. syriaca Boiss. (Umbelliferae) from Turkey. *Flavour. Frag. J.* **21**: 118-121 (2006).
8. Kurkcuoglu M, Husnu KCB, Iscan G, Malyer H, Kaynak G. Composition and anticandidal activity of essential oil of Chaerophyllum byzantinum Boiss. *Flavour. Frag. J.*: **21**: 115-117 (2006).
9. Omidbaygi R. *Medicinal Plants,* **1 & 2**: Publication Designers; 1379.
10. Akhundzade S, Encyclopedia of Medicinal Plants; 1379: 152.
11. Zargari A, medicinal plants, Publishing TehranUniversity, **4**; 1369: 59.
12. KhosraviAR, specialized curriculum booklet candidiasis, Faculty of Veterinary Medicine Research Publications, TehranUniversity, 1378.
13. Zayni F, Emami M, Mahbod A, Comprehensive Medical Mycology - The Tehran University, 1382.
14. Parent P, et al. the study of anti-microbial effect of Shiraz thyme extract a Staphylococcus aureus - the seventh congress of Microbiology Iran (Semnan) 1383.
15. Sattari M, et al. reviews Shiraz thyme essential oil antibacterial activity on Staphylococcus aureus growth and general Eshrishia - the Seventh Congress of Microbiology (Semnan) 1383.
16. Bayat M, Kousha A, Azizi Saraji A.R, Rohani R, Nissiani M. Study Effects of Some Kinds of Standard Essences over Two Microorganisms (Candida albicans and Gardnerella vaginalis) Related to Leucorrhoea Disease as in vitro.; *World Appl. Sci. J.* **5** (4): 418-421 (2008).
17. Momeni C.M, plant extracts - Publishing

martyr Farhad Reza, 1379.
18. Mirza M, White F, Ahmad L, natural oils - Publishing the Institute Vmrat forests, 1375.
19. Momeni C.L, plant oils and their therapeutic effects, PublishingTehranUniversity, 1377: 9-13.
20. Mousavi K.M.R, Pharmacology and treatment, Third Printing. Publishing Sepehr Tehran,1360: 8.
21. Asgharlo S, Majd A, Mehrabian S, extract growth deterrent effect of different organs of hollyhock hock and some micro-organisms - Seventh Congress of Microbiology (Semnan) 1383.
22. Damodaran S, Venkataraman S. A study on the therapeutic efficacy of Cassia alata, Linn. Leaf extract against Pityriasis versicolor. *J Ethnopharmacol.;* **42**(1):19-23 (1994).
23. Maoz M, Neeman I. Antimicrobial effects of aqueous plant extracts on the fungi: Microsporum canis and Trichophyton rubrum and on three bacterial species. *Lett Appl Microbiol*.; **26**(1):61-3 (1998).
24. Steinmetz MD, Rascol JP, Regli P, Gargadennec A and Andary C. Invitro antifungal activity of Polyporaceae against yeasts and dermatophytes. *Mycoses;* **38**(7-8):305-9 (1995).
25. Na M, Li Ly, Yang YD. Anti-fungal test of composite agastache lotion on seven pathogenic fungi and its clinical application. Zhongguo Zhong Xi Yi Jie He Za Zhi.; **23**(6): 414-6 (2003).
26. Shin, S, Kang, CA. Antifungal activity of the essential oil of Agastache rugosa Kuntze and its synergism with ketoconazole. *Lett Appl Microbiol.;* **36**(2):111-5 (2003).
27. Akagawa G, Abe S, Tansho S, Uchida K, Yamaguchi H. Protection of C3H/HE J mice from development of Candida albicans infection by oral administration of Juzen-taiho-to and its component, Ginseng radix: possible roles of macrophages in the host defense mechanisms. *Immunopharmacol Immunotoxicol.;* **18**(1):73-89 (1996).
28. NwosuMO, Okafor JI. Preliminary studies of the antifungal activities of some medicinal plants against Basidiobolus and some other pathogenic fungi. *Mycoses.;* **38**(5-6):191-5 (1995).