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The Effect of Aqueous and Ethanolic Extracts of Teucrium polium on Candida Albicans and Two Species of Malassezia

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Abstract

***Background*:** *Teucrium polium* L. is a medicinal plant, which due to its antimicrobial, antispasmodic and anti-tumor properties has been used in traditional medicine for over 2000 years. The aim of this research was to study the effect of aqueous and ethanolic extracts of *Teucrium polium* L. against three strains of *Candida albicans* (ATCC 62061, ATCC 1677, and NCPF 3153), *Malassezia furfur* and *Malassezia globosa* using pour plate method.

***Materials and Methods*:** *Teucrium polium* L. was collected from Broojen area during the spring. The plant was dried and powdered. The aqueous and ethanolic extracts were prepared from the fine powder. Different concentrations of extracts (1, 2, 4, and 8 mg/ml) were made in Sabouraud Dextrose Agar (SDA) and modified Leeming-Notman Agar (MLNA) medium for *Candida albicans*, *Malassezia furfur* and *Malassezia globosa*. 1.5×10^6 cfu/ml of yeasts, were cultured on media and incubated at 37ºC and 32ºC respectively. Pour plate method was used to assess the antifungal activity of these extracts.

***Results*:** The inhibitory effect of ethanolic extract of *Teucrium polium* L. on the three strains of *Candida albicans* was depended on concentration level of extracts in media. Aqueous extract had inhibitory effect on *Candida albicans* (NCPF 3153) only, and with increasing of the extract concentration, the number of colonies was decreased, so that in concentration of 8 mg/ml, no growth was seen. Aqueous and ethanolic extracts had no inhibitory effect on *Malassezia* species.

***Conclusion*:** *Teucrium polium* L. extracts have considerable inhibitory effect on different strains of *Candida albicans.* Further investigations are needed to detect the effectiveness of this plant in treatment of *Candida* infections.

Introduction

In recent years there has been a growing interest in using medicinal plants in prevention and treatment of illnesses throughout the world and particularly in Iran [1, 2]. Lack of satisfactory success in treatment of many chronic illnesses, undesirable side effects of chemical drugs and increasing resistance of microorganisms to many drugs and particularly to antibiotics have led to a growing interest in the use of herbal remedies [3, 4].

Teucrium polium L. which belongs to family of *Lamiaceae,* is a perennial plant, which is covered with dense long and soft hairs. Its rather woody bushes reach 30 -50 cm in height. This plant is a dwarf, pubescent, aromatic shrub, posing oval leaves with enrolled margins and dense head of white flowers. Flowers vary from white and off white to yellow and pink in color. The plant grows in arid and rocky areas of various parts of Europe, Mediterranean Basin, North Africa and South West Asia including Iran [5]. It is used to treat such illnesses as diabetes, rheumatoid and gastrointestinal tract diseases. It has antimicrobial, antispasmodic and anti-inflammatory effects. It is also known to reduce the glucose, triglyceride and cholesterol levels in blood. Research has also shown that it can improve appetite and increase the energy levels and fight fever [5-10]. It contains tannins, terpenoids, saponins, sterols and leucoanthocyanin. It also has antimicrobial activities, although no significant antifungal activities have been reported so far [11].

Since early 1990s an increase in the number of infections induced by pathogenic and opportunist fungi particularly among immunocompromised patients have been the main cause of fatalities among the hospitalized patients. Major opportunist pathogens such as *Candida albicans* and other species of *Candida* have had a significant role in contracting the infections [12]. *Candida* has been the most common cause of dermatological, oral and systemic diseases among immunocompromised patients [13].

Considering the increase in the application of antifungal medicines and the consequent resistance in certain types of *Candida* as well as the undesirable side effects of chemical drugs, it is important to explore new sources of remedies especially among herbal plants [14].

Malassezia is dimorphic yeast which is lipophilic and lives as a natural flora on the surface of skin. This fungus normally lives commensally along with other types of bacteria. It causes illnesses such as Pityriasis versicolor,

Seborrheic dermatitis, and Folliculitis [15].
Nature is a good source of medicinal plants and based on their traditional usage many modern drugs were developed [14, 16]. Research to date has not shown the antifungal activities of *Teucrium polium* against *Candida* and *Malassezia* [11]. The purpose of this study was to examine the activities of aqueous and ethanolic extract of *T. polium* on *C. albicans* (ATCC 62061, NCPF 3153, ATCC1677), *M. furfur* and *M. globosa* using the pour plate method.

Materials and Methods

Collection of plants: *T. polium* was collected from the highlands of Broojen in Isfahan Province during April 2011. The samples were identified in Isfahan Agricultural and Natural Research Centre (Herbarium No. 15124). Extraction and laboratory examinations were carried out in Falavarjan Branch, Islamic Azad University.

The aerial parts of plants were aired indoors in room temperature and then finely powdered using an electric grinder. It took two days to extract 20 grams of plant materials in a soxhlet with 120 ml ethanol 96%. The extracts were then aired in a rotary evaporator at 60ºC and were preserved in sterilized airtight bottles at 4ºC.

In order to prepare the aqueous extracts the plants were brewed using the local traditional method. 250 ml of distilled water was added to 25 grams of the plant material and heated for 15 minutes not allowing it to boil. The mixture was then cooled in a closed container at room temperature. It was then filtered using the whatman filter No.1. In order to prepare the dried extracts, the solution was placed in a Bain Marie at 40ºC for 24 hours prior to use.

Preparation of Organisms: Standard strains of *C.albicans* (ATCC 62061, NCPF 3153, ATCC 1677) were supplied by department of Mycology, Faculty of Veterinary Medicine, Tehran University. They were cultured in Sabouraud Dextrose Agar (SDA) medium and were refrigerated when ready.

M. furfur and *M. globosa* were taken from patients' samples and were cultured in modified Leeming and Notman Agar medium and were identified using biochemical and physiological methods. To carry out the tests the 24-hour yeast cultures were used. Yeast suspensions were prepared with concentration adjusted at 1.5×10^6 cfu/ml in sterile distilled water as described by Forbes et al [17].

Determination of the activity of extracts using the pour plate method: Various values of 1, 2, 4, and 8 mg of the aqueous and ethanolic extracts were individually incorporated into 1 ml of dimethyl sulfoxide (DMSO). These were then mixed in ratios of 1 to 10 in erlenmeyer containing SDA medium for *Candida* yeast and MLNA medium for *Malassezia* yeasts. The settled mixture was then poured into sterile Petri dishes [18]. 20 microliter of yeast suspension was incubated to the surface of discs containing culture mediums with various extract concentrations. The media containing the *Candida* were maintained at 37ºC and *Malassezia* at 32ºC for 48 hrs. The growth rate and the colonies of each Petri dish were then counted and recorded [18].

Minimum Inhibitory Concentration (MIC): The macrodilution broth method was used to determine the minimum inhibitory concentration of the aqueous and ethanolic extracts.

To this end 1 ml of Sabouraud Dextrose Broth medium was added to C. *albicans* and same amount of Modified Leeming and Notman Broth medium was added to *M. furfur* and *M. globosa*. They were then poured into 11 tubes numbered from 1 to 11. Then 1 ml of dried extract was dissolved into dimethyl sulfoxide and was added into the first tube. Then 1 ml of the solution in tube 1 was poured into tube 2.

This procedure was repeated serially all the way to tube 10. 1ml of the solution in tube 10 was discarded and tube 11 was kept as blank. This way extract dilutions of 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 were prepared. 20 microliter of each fungal suspension was added to the tubes. Tubes containing *C. albicans* were kept at 37ºC and the ones containing *M. furfur* and *M. globosa* were kept at 32ºC for 48 hours. The tubes were then observed for turbidity. The tests were carried out in triplicate.

Determination of Minimum Fatality Concentration (MFC): 20 microliters of solution from the tubes with no turbidity were inoculated in Sabouraud Dextrose Agar medium for *C. albicans* and into MLNA medium for *M. furfur* and *M. globosa* and were preserved in required temperature for 48 hours. No growth in fungi was regarded as an indication of MFC.

Results

To determine the effects of extracts using pour plate method: The inhibitory effect of ethanolic extracts of *T. polium* was different in various strains of *C. albicans*. *C. albicans* ATCC 62061 showed less sensitivity compared to the other two strains. The minimum inhibitory concentration for *C. albicans* ATCC 62061, ATCC 1677 and NCPF 3153 was 8, 4 and 4 mg/ml respectively.

Various concentrations of *T. polium* aqueous extracts had no effect on *C. albicans* ATCC 1677 and ATCC 62061. However in regards to *C. albicans* NCPF 3153 the number of colonies present in the culture media decreased with an increase in the concentration level of the extracts, so that in the concentration value of 8 mg/ml the fungi stopped growing altogether. Neither the ethanolic nor aqueous extracts exhibited any effects on *M. furfur* and *M. globosa* (Table 1).

Results of MIC test on *C. albicans*: The examination of the tubes after 48 hours showed that *C. albicans* ATCC 62061 exhibited no turbidity up to tube number 4. Therefore, the MIC for this fungus was determined to be at 8 mg/ml.

Table 1. The inhibitory effect of ethanolic and aqueous extracts of *Teucrium polium* on *Candida albicans* strains and two species of *Malassezia*

Extract concentration	Number of colonies on each plate				
	Candida albicans	*Candida albicans*	*Candida albicans*	*Malassezia furfur*	*Malassezia globosa*
mg /ml	(NCPF 3153)	(ATCC 1677)	(ATCC 62061)		
Ethanolic 1	30	50	100	Uncountable	Uncountable
Ethanolic 2	15	25	30	Uncountable	Uncountable
Ethanolic 4	0	0	17	Uncountable	Uncountable
Ethanolic 8	0	0	0	Uncountable	Uncountable
Aqueous 1	300	Uncountable	Uncountable	Uncountable	Uncountable
Aqueous 2	200	Uncountable	Uncountable	Uncountable	Uncountable
Aqueous 4	120	Uncountable	Uncountable	Uncountable	Uncountable
Aqueous 8	0	Uncountable	Uncountable	Uncountable	Uncountable

Table 2. Results of MIC and MFC of ethanolic extract of Teucrium polium on Candida albicans strains (mg/ml)

Candida albicans	MIC	MFC
NCPF 3153	4	8
ATCC 1677	4	8
ATCC 62061	8	16

In regards to the other two strains i.e., *C. albicans* ATCC 1677 and NCPF 3153 up to tube number 5 no growth was observed at all. Therefore their MIC was determined to be at 4 mg/ml (Table 2).

Aqueous extract: Examination of *C. albicans* NCPF 3153 revealed that tube number 3 exhibited no growth at all. Therefore the MIC for this fungus was determined to be at 16 mg/ml. As for *C. albicans* ATCC 1677 and ATCC 62061 growth was evident up onto the last tube (Table 3).

Table 3. Results of MIC and MFC of aqueous extract of *Teucrium polium* on *Candida* albicans strains (mg/ml)

Candida Albicans	MIC	MFC
NCPF 3153	16	32
ATCC 1677	—	—
ATCC 62061	—	—

Results of MIC test on *Malassezia*: In regards to *M. furfur* and *M. globosa* the turbidity that indicated the growth of the fungi was observed in all tubes.

Results of the MFC test on *C. albicans*: In regards to ethanolic extracts, examination of the culture media after 48 hours showed that the MFC for *C. albicans* ATCC 62061, ATCC 1677 and NCPF 3153 were 16, 8 and 8 mg/ml respectively (Table 2). As for the aqueous extract, the MFC for *C. albicans* NCPF 3153 was determined to be at 32 mg/ml (Table 3).

Discussion

Based on the findings of the present study, ethanolic extract of *T. polium* had a significant inhibitory effect on the growth of various strains of *C. albicans*. Yeasts are widespread fungi that despite all the developments in medical treatments continue to cause rather common infections.

Candida and *Malassezia* are the most common and most important yeast genus that are spread around the world and are found in various parts of Iran [19]. Research findings show that 61% of all hospital acquired fungal infections are caused by *C. albicans* and the rest are caused by other strains of *Candida* [20, 21].

Considering the ever-increasing intake of antifungal drugs and the consequent resistance of some genus of fungi to them, it's becoming necessary to explore new sources of remedies that have fewer side effects and as well as being cost effective. Research supports the anti-*Candida* activities of herbal medicines, which can be considered as a good source of anti-fungal elements [19, 22].

Kalpooreh is an herbal medicine, which is used in traditional medication for its antibacterial effects. *Teucrium polium* is a well-known species of Kalpooreh, which is used for its anti-diarrhea and anti-inflammatory effects [23]. This plant grows in the rocky fields and mountainous areas [20]. Contrary to the findings of this study, some previous research indicated that *T. polium* has in fact no inhibitory effect on the growth in fungi. The difference in findings are probably due to such factors as weather, soil contents, plant parts utilized, age and development stage of the plants and the harvest time [19, 20, 24-27].

Further research is required to examine the antibacterial and antioxidant activities of pure active components in natural settings [28].

Findings of research on the antimicrobial effects of the essence derived from *T. polium* revealed that the essence of this plant has a considerable inhibitory effect on majority of both gram positive and gram negative bacteria and in fact it is more effective than Gentamicin antibiotics. Therefore, it seems beneficial to use this plant to treat gram negative bacterial infections in the digestive system and urinary tract [29]. According to research, the aqueous and alcoholic extracts are commonly used for commercial and treatment purposes rather than the chloroform or alkaloid based extracts [20].

Research to date has indicated that *T. polium* has no inhibitory effect on fungi growth [11]. However, considering the traditional use of this plant in the treatment of *Candida* infections in females in some parts of Isfahan and Chahar Mahal Bakhtiary province encouraged the idea of a scientific investigation of any effects this plant might have on these two yeast species.

Based on the findings of this study the aqueous and ethanolic extracts of *T. polium* had no effect on *M. furfur* and *M. globosa*. Therefore, it cannot be used to treat

infections related to these fungi. However, in regards to *C. albicans* it was proved that various strains of this fungus show different reactions to the same degrees of concentration in ethanolic extracts of *T. polium*. Therefore, more research is needed to determine and identify the exact species and strains of the fungus in order to yield more thorough results.

This study also proved that ethanolic extracts of *T. polium* have various degrees of anticandidial activities against the examined fungi. The antifungal activities increased with the density of the extracts.

According to the findings of this research it seems that the aerial parts of the *T. polium* are a potential source of antibacterial agents, which can be used to prevent various illnesses.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Niazmand S, Erfanian Ahmadpour M, et al. [The inotropic and chronotropic effects of aqueous ethanolic extract of Teucrium polium L. on guinea pig isolated heart] Persian. J Babol Univ Med Sci 2008; 10(1): 7-13.
2. Mohajeri D, Doostar Y. [Antioxidant effect of extract of the grape seed in streptozotocin induced diabetic rats] Persian. Zahedan J Res Med Sci 2010; 12(1): 9-14.
3. Mirzaei A, Jaberi-Hafshejani H. [Effects of hydroalcoholic extract of Teucrium polium on biochemical and hematological parameters of hepatotoxic rats] Persian. J Armaghane-danesh 2010; 15(1): 67-75.
4. Mirjana S, Nada B, Valerija D. Variability of Satureja cuneifolia ten. Essential oils and their antimicrobial activity depending on the stage of development. Eur Food Res Technol 2004; 218 (4): 367-371.
5. Esmaeili A, Amiri H. [Effects of antimicrobial and identification of composition of essential oil of Teucrium polium L.] Persian. J Esfahan Univ 2008; 31(2): 15-22.
6. Shakhanbeh J, Atrouse O. Teucrium polium inhibits nerve conduction and carrageenan induced inflammation in the rat sikn. Turk J Med Sci 2001; 31: 15-21.
7. Seyyednejad SM, Motamedi H. A review on native medicinal plants in khuzestan, iran with antibacterial properties. Int J Pharmacol 2010; 6 (5): 551-560.
8. Gharib-Naseri Mk, Omidi-birgani F, Vakilzadeh G. [Effect of antispasmodic of plant Teucrium polium on the virgin rat uterus] Persian. Iran J Basic Med Sci 2005; 8(1): 31-37.
9. Akin M, Oguz D, Saracoglu HT. Antibacterial activity of essential oil from Thymbra spicata var. spicata L. and Teucrium polium. Int J Pharm Appl Sci 2010; 1(1): 55-58.
10. Karimi F, Abbasi S, Bateni A. [Effect of medicinal extracts of plant Teucrium polium on glycemic control compared with Glibenclamide in type 2 diabetic patients] Persian. Iran South Med J 2001; 4(2): 96-103.
11. Mosaddegh M, Dehmobad-Sharifabadi A, Nasiri P, et al. [Survey phytochemical two plants Astraceae and Teucrium polium and evaluate their antimicrobial and antifungal effects] Persian. Sci J Kordestan Univ Med Sci 2002; 7(1): 23-28.
12. Razaghparast A, Shams-ghahfarrokhi M, Yadegari MH and Razzaghi-Abianeh M. [Effect of antifungal of onion and some azoles individual and in combination with to gather on pathogenic yeasts] Persian. Kowsar Med J 2008; 13(2): 103-113.
13. Anaissie EJ, McGinnis MR, Pfaller MA. Clinical Mycology. Philadelphia: Churchil Livingstone; 2003: 195-6.
14. Madani M, Khosravi A, Shirani M. [Effect of plant Allium jesdianum extracts on different Candida strains in vitro] Persian. J Biol Sci 2009; 3(1): 63-71.
15. Falahati M, Pakshir K, Alinejad-Talesh K. [Separation and identification of different species of Malassezia in patients referred to medical centers in Shiraz] Persian. Iran J Med Sci 2005; 12(45): 130-140.
16. Bentley R, Trimen H. Medicinal plants. India: International Book Distributors; 1981: 172-177.
17. Forbes BA, Sahm DF, Weissfeld AS, editors. Methods for testing antimicrobial effectiveness. In: Baron EJ, Peterson LR, Finegold SM. Bailey and Scotts diagnostic microbiology. Philadelphia: Mosby Press; 1990: 171-194.
18. Niazamand S, Nemati-Karimuee H, Esparham M. [The effect of aqueous- alcoholic extracts of Teucrium polium on blood pressure heart rate and intra- abdominal pressure in rabbits] Persian. J Med Plants 2009; 9(1): 90-97.
19. Aiyegoro OA, Okoh AI. Phytochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of Helichrysum pedunculatum. Int J Mol Sci 2009; 10(11): 4990-5001.
20. Cichewicz RH, Thorpe PA. The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine. J Ethnopharmacol 1996; 52(2): 61-70.
21. Darwish-Rula M, Aburjai-Tala A. Antimicrobial activity of some medicinal plants against different Candida species. Jordan J Pharm Sci 2011; 4 (1): 70-80.
22. Bokaeian M, Nakhaee A, Moodi B. [Effect of Eucalyptus Globulus upon Candida colonization in normal and diabetic rats] Persian. Zahedan J Res Med Sci (ZJRMS) 2012; 13(9): 21-26.
23. Shahraki M, Mirshekari H, Palen M. [Comparison of analgesic effect of aqueous extract of Kalporeh and morphine in the rat] Persian. Ofogh-e-Danesh 2006; 12(1): 10-14.
24. Shahraki M, Mirshekari H, Shahraki A, et al. [Toxic Effect of aqueous extract of kalpore on liver and blood lipoproteins in male rats with normal glucose] Persian. Zahedan J Res Med Sci (ZJRMS) 2006; 8(3): 9-15.
25. Kirby GC. Medicinal plants and the control of protozoal disease, with particular reference to malaria. Trans R Soc Trop Med Hyg 1996; 90(6): 605-609.

26. Romero CD, Chopin SF, Buck G. Antibacterial properties of common herbal remedies of the southweat. J Ethnopharmacol 2005; 99(2): 253-257.
27. Scalbert A. Antimicrobial properties of tannins. Phytochem 1991; 30(12): 3875-3883.
28. Tarawneh-Khaled A, Irshaid F, Jaran-Adnan S, et al. Evaluation of antibacterial and antioxidant activities of methanolic extracts of some medicinal plants in northern part of Jordan. J Biol Sci 2010; 10(4): 325-332.
29. Ljubuncic P, Dakwar S, Portnaya I, et al. Aqueous extracts of Teucrium polium possess remarkable antioxidant activity in vitro. Evid Based Complement Alternat Med 2006; 3(3): 329-338.

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