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. 1.5×10^5 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 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,
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 activities 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, ATCC1677), *M. furfur* and *M. globosa* using the pour plate method.

Effect of *Teucrium polium* on *C. albicans* (ATCC 62061, NCPF 3153, ATCC1677), *M. furfur* and *M. globosa* using the pour plate method.

**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.
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 alkald 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 antifungal 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.

**References**


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**Conflict of Interest**

The authors declare no conflict of interest.

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