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

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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. 1.5×10^3 cfu/ml of yeasts, were cultured on media and incubated at 37°C and 32°C 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.

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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 opportunistic fungi particularly among immunocompromised patients have been the main cause of fatalities among the hospitalized patients. Major opportunistic 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 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
 turbdidity 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.
The growth of various strains 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 alkaid 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 fungal infections are caused by *C. albicans* and the rest are caused by other strains of *Candida* [20, 21].

### Discussion

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>
<thead>
<tr>
<th>Extract concentration</th>
<th>Candida albicans NCPF 3153</th>
<th>Candida albicans ATCC 1677</th>
<th>Candida albicans ATCC 62061</th>
<th>Malassezia furfur ATCC 1677</th>
<th>Malassezia furfur ATCC 62061</th>
<th>Malassezia globosa ATCC 1677</th>
<th>Malassezia globosa ATCC 62061</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC</td>
<td>30</td>
<td>25</td>
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<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
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<tr>
<td>MIC</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>8</td>
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<td>16</td>
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<tr>
<td>MFC</td>
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<td>15</td>
<td>25</td>
<td>0</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
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<tr>
<td>MIC</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
</tr>
<tr>
<td>MFC</td>
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<td>50</td>
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<tr>
<td>MIC</td>
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<td>0</td>
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<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
</tr>
<tr>
<td>MFC</td>
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<td>0</td>
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<td>Uncountable</td>
<td>Uncountable</td>
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</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Candida albicans</th>
<th>MIC</th>
<th>MFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCPF 3153</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>ATCC 1677</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>ATCC 62061</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Candida Albicans</th>
<th>MIC</th>
<th>MFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCPF 3153</td>
<td>16</td>
<td>52</td>
</tr>
<tr>
<td>ATCC 1677</td>
<td>16</td>
<td>52</td>
</tr>
<tr>
<td>ATCC 62061</td>
<td>16</td>
<td>52</td>
</tr>
</tbody>
</table>

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).

Based on the findings of the present study, the aqueous and alcoholic extracts of *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.

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

<table>
<thead>
<tr>
<th>Extract concentration</th>
<th>Candida albicans NCPF 3153</th>
<th>Candida albicans ATCC 1677</th>
<th>Candida albicans ATCC 62061</th>
<th>Malassezia furfur ATCC 1677</th>
<th>Malassezia furfur ATCC 62061</th>
<th>Malassezia globosa ATCC 1677</th>
<th>Malassezia globosa ATCC 62061</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC</td>
<td>30</td>
<td>25</td>
<td>0</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
</tr>
<tr>
<td>MIC</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>MFC</td>
<td>2</td>
<td>15</td>
<td>25</td>
<td>0</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
</tr>
<tr>
<td>MIC</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
</tr>
<tr>
<td>MFC</td>
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<td>120</td>
<td>50</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
</tr>
<tr>
<td>MIC</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
</tr>
<tr>
<td>MFC</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
<td>Uncountable</td>
</tr>
</tbody>
</table>

### Table 4. Results of MIC and MFC of ethanolic extract of *Teucrium polium* on Candida albicans strains (mg/ml)

<table>
<thead>
<tr>
<th>Candida albicans</th>
<th>MIC</th>
<th>MFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCPF 3153</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>ATCC 1677</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>ATCC 62061</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

### Table 5. Results of MIC and MFC of aqueous extract of *Teucrium polium* on Candida albicans strains (mg/ml)

<table>
<thead>
<tr>
<th>Candida Albicans</th>
<th>MIC</th>
<th>MFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCPF 3153</td>
<td>16</td>
<td>52</td>
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<tr>
<td>ATCC 1677</td>
<td>16</td>
<td>52</td>
</tr>
<tr>
<td>ATCC 62061</td>
<td>16</td>
<td>52</td>
</tr>
</tbody>
</table>

**Discussion**

Based on the findings of the present study, the aqueous and ethanolic extracts 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].
infections related to these fungi. However, in regards to \textit{C. albicans} it was proved that various strains of this fungus show different reactions to the same degrees of concentration in ethanolic extracts of \textit{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 \textit{T. polium} have various degrees of antimalarial 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 \textit{T. polium} are a potential source of antibacterial agents, which can be used to prevent various illnesses.

\textbf{References}


\textbf{Acknowledgements}

This article is based on post graduation thesis (code 17230507892034 approved by Falavarjan Islamic Azad University Research Committee) submitted in partial fulfillment of the requirements for the Degree of M.S in Microbiology at Falavarjan Branch, Islamic Azad University. Special thanks go to all the people involved in carrying out the research.

\textbf{Authors’ Contributions}

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

\textbf{Conflict of Interest}

The authors declare no conflict of interest.

\textbf{Funding/Support}

Falavarjan Branch, Islamic Azad University, Isfahan.