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Chemical Composition and Antimicrobial Properties of *Teucrium polium* Essential Oil Collected from Dena Mountain in Yasuj, Iran

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ABSTRACT

Aims *Teucrium polium* is a medicinal plant that is used due to its antispasmodic and antimicrobial properties. This study aimed to evaluate the effect of the essential oil of *T. polium* against fungal, bacterial strains, and *Giardia lamblia*.

Materials & Methods In this clinical laboratory study, *Teucrium polium* was collected from the Dena mountain area in Yasuj, Iran. Essential oil was prepared from plant powder. Clinical Aspergillus species were isolated from otomycosis patients. For Candida species, the researchers used clinical isolates with otomycosis and vaginitis origin. 33 Aspergillus strains, 49 Candida species, and 33 non-albicans species were used as samples. The samples were collected and cultured on Sabouraud dextrose agar and identified based on PCR-sequencing analysis. Different concentrations of essential oil were assessed by broth microdilution method against clinical fungi and bacterial isolates. Also, anti-Giardia activity of this essential oil was investigated at different times and concentrations. Chemical analysis of the essential oil was done by Gas Chromatography–Mass Spectrometry.

Findings The inhibitory effect of essential oil of *T. polium* on Candida and Aspergillus strains was varied and dependent on species. Generally, the effect of essential oil on non-albicans species was better than *C. albicans* isolates. Also, essential oil had an inhibitory effect on *E. coli* and *Klebsiella* but did not affect methicillin-resistant *Staphylococcus* aureus. Its effect on Giardia isolates was dependent on time and concentration. Oxygenated monoterpenes are the major compound of *T. polium*.

Conclusion *Teucrium polium* essential oil has a considerable inhibitory effect on different strains of microorganisms.

Keywords *Teucrium polium*; Candida; Aspergillus; Bacteria; Giardia

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[1] Rare and emerging opportunistic fungal pathogens ... [2] A brief review of antifungal drugs ... [3] Nosocomial infections and their control ... [4] Recent advances in the genomic and molecular ... [5] Extra-intestinal and long term consequences of ... [6] Giardiasis: characteristics, pathogenesis and new insights ... [7] Giardia duodenalis in the UK: current knowledge of risk ... [8] Molecular genotyping of Giardia duodenalis ... [9] The effects of extracts from anti-diarrheic ... [10] Evaluation of Chenopodium ambrosioides ... [11] The anti-giardial effectiveness of fungal and commercial chitosan ... [12] Phytochemistry and medicinal properties of ... [13] The biological effects of Teucrium polium on ... [14] Comparative investigation of the sensitivity ... [15] Phytochemical screening, antibacterial, antifungal ... [16] Clinical and microbial epidemiology of otomycosis ... [17] Identification of Candida species isolated from ... [18] Antifungal susceptibility profile and molecular ... [19] Molecular identification and antifungal ... [20] Commentary: Impact of CLSI Breakpoint ... [21] A simple method for demonstrating ... [22] A comparative study of four methods for ... [23] Anti-giardial activity of Sambucus ... [24] Giardia and ... [25] Effect of Origanum vulgare hydroalcoholic ... [26] Chemical analysis and antimicrobial ... [27] Analysis of the essential oil of the ... [28] Comparison of chemical composition ... [29] Phytochemistry, antioxidant and antibacterial ... [30] Naturally occurring food ... [31] Mobile-based video learning outcomes in clinical nursing skill ... [32] Lead compounds from medicinal ... [33] Phytochemical analysis and study of ... [34] The Effect Of Aqueous And Ethanolic ... [35] Antimicrobial activity of some ... [36] Chemical analysis and biological ... [37] In vitro susceptibility of ... [38] Antimicrobial properties of Teucrium ... [39] Antiparasitic effects of medicinal plants ... [40] Anti-parasitic activity of some medicinal plants ... [41] In-vitro antigiardial activity and ... [42] Plants and chemical constituents ... [43] Anti-Giardia activity of phenolic-rich essential ... [44] Antiprotozoa activity of some essential ...

Introduction

Recently, due to the increase of predisposing factors, the rate of opportunistic infections with different microorganisms is raising. Major opportunistic fungal infections are associated with Candida and Aspergillus genera [1]. These agents can cause different forms of infection including superficial, cutaneous, systemic, and allergic diseases. Following the increasing rate of opportunistic infections, various classes of antifungal drugs are used [2]. However, several factors are involved in resistance to antifungal drugs; for instance, the increase in the use of these drugs and as a result drug resistance and inherent resistance in certain species of Candida and Aspergillus.

Nosocomial bacterial pathogens, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), are the main cause of hospital infections and their prevalence in the world. Also, *Klebsiella pneumonia*, *Streptococcus* spp. , and *Escherichia coli* were introduced as the second agent of hospital-acquired infections [3].

Giardia lamblia is a protozoan parasite that is considered one of the main causes of diarrhea in all parts of the world with different levels of infection. This parasite infection is prevalent in tropical areas with low health facilities and places with high population density [4-7]. Generally, the prevalence of giardiasis has been reported 1.4% to 54.9% in different countries of the world [8]. It has been shown that there have always been failures in the treatment of human giardiasis, and in most cases the treatment has not been successful, and these cases are increasing [9]. As a result, despite these problems, the World Health Organization (WHO) has recommended the use of natural products, herbs, and related products in the treatment of parasitic diseases including *G. lamblia* [10, 11].

Medicinal plants are important sources with therapeutic effects. Currently, due to the increase in drug resistance, these herbal resources have been considered. Plants have a wide variety of secondary metabolites such as flavonoids, tannins and alkaloids with antimicrobial properties. Previous studies conducted on medicinal plants have shown low toxic and side effects compared to chemical substrates [12]. The genus Teucrium (Lamiaceae family) is characterized by more than 340 different species with a high geographical distribution. *Teucrium polium*, which is called "Halpeh" in the local language, is mainly found in the Mediterranean and western areas of Iran. This plant is covered with dense, long, and soft hairs with the height of 30-50 cm in its rather woody bushes and a dwarf, pubescent, aromatic shrub, posing oval leaves with enrolled margins and dense head of white flowers. In traditional medicine, this species is widely used as antidiabetic, anti-inflammatory, hypotensive, anorexic, antispasmodic, antiulcer, antipyretic, and antinociceptive [13]. Antimicrobial properties of *T. polium* have been reported in some studies [14]. For instance, the extract of this plant has high activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Paracoccus pantotrophus*, and some pathogenic fungi [15]. However, the use of various fungal species with different sources of isolation and the use of broth microdilution method are the differences between the present study and other similar studies.

The aim of this study was to categorize the chemical composition of *T. polium* essential oil and to evaluate its antifungal, antibacterial and anti-giardia properties, as well as to compare its antifungal activity with some routine antifungal drugs.

Materials and Methods

This study was designed based on the clinical laboratory research method.

Collecting plants: *Teucrium polium* was collected in 2020 from the highlands of Kohgiluyeh and Boyer Ahmad (Mount Dana), Iran. The collected plants were identified in the Medicinal Plants Research Center of Fars Agricultural and Natural Resources Research and Education center (Herbarium No: FANNREC14428). Other steps were performed at the Medicinal Plants Research Center of Yasuj University of Medical Sciences. The aerial parts of plants were dried and crushed aerobically at 25-28°C.

Preparation of essential oil: The essential oil was extracted from 200g of dried powder of *T. polium* using a Clevenger machine. The essential oils were stored in sterile bottles at 4°C and dark conditions for analysis.

Preparation of fungal strains: In the present study, the essential oil of *T. polium* was tested on clinical Candida and Aspergillus isolates. Thirty-three Aspergillus strains (11 *A. flavus*, 11 *A. niger*, 11 *A. tubengensis*) with otomycosis origin and 49 Candida species included 16 *C. albicans* (recurrent vulvuvaginit, vaginitis, blood culture), and 33 non-albicans species (otomycosis, recurrent vulvuvaginit, vaginitis) were used. These isolates were collected during previous studies and identified based on Polymerase Chain Reaction (PCR)-sequencing and PCR–Restriction Fragment Length Polymorphism (PCR-RFLP) [16-19].

MIC determination: The broth microdilution method was used to determine the antifungal activity according to CLSI M27-S4 and CLSI-M38 guidelines. For this purpose, serial dilutions of essential oil and antifungal drugs including Itraconazole (0.0019-4 µg/ml), fluconazole (0.125-16 µg/ml), and voriconazole (0.0019-4 µg/ml) were prepared with RPMI 1640 medium. Overnight cultures of Candida isolates and sporulated colonies of Aspergillus species were used to prepare a 0.5 McFarland suspension. Then, standard suspensions

were diluted 1:1000 and 1:50 in RPMI1640 for Candida and Aspergillus species, respectively. 100 µL of each serial dilution drug with 100 µL of fungal isolates were added to 96-well microtiter plates and incubated at 35°C for 24-48h. Finally, Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration that causes 50% inhibition of fungal growth, compared to the positive control.

Antibacterial susceptibility: The essential oil was tested against different bacteria including Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC25923, *Escherichia coli* ATCC 25922, and *Klebseiella* (UTI isolates). These microorganisms were obtained from the stock collection of the Microbiology Laboratory of Yasuj. Susceptibility test was carried out by microdilution method according to CLSI- M 100 guideline [20].

Isolation of *G. lamblia* cysts: At first, stool samples infected with Giardia cyst were collected by referring to the laboratory of Shahid Ashrafi Clinic located in Yasuj, Iran. Cysts were isolated from patients' positive stool specimens by floating method. Detected cysts were identified with recognition keys and microscopic examination. They are typically 11-14 micrometers long and 7-10 microns wide and contain 4 nuclei and round or oval in shape, axons and middle bodies [21, 22]. Then 20 g of positive stool was broken down and filtered in 100 ml of tap water. 3 ml of 0.85 M sucrose was added to 3 ml of fecal suspension and centrifuged for 10 minutes at 2000-3000 rpm. Cysts were aspirated with a pipette on the sucrose-water interface and washed 3 times with water. The washed cysts were sensibly added to the upper of a discontinuous density gradient containing of 3 ml of 0.85 M sucrose sheets. After centrifugation at 2000-3000 rpm for 10 minutes, concentrated cysts on a 0.85 M sucrose interface were collected and washed again [23]. To maximize the number of cysts, 3 ml of normal saline was added to the washed cysts, then centrifuged for 5 minutes at 2000-3000 rpm, so that the cysts precipitated at the end of the tubes. Top-out and pure cysts were obtained.

Treatment of parasites with essential oils: At this stage, dilutions of 25, 50, 100, 200 µl/ml were used to prepare the essential oil. Towin 80 with a concentration of 3% was used to dissolve the essential oil in water. Then, in order to ensure the validity of the test, the positive control group with a concentration of 50 mg/ml was diluted with 5 oral metronidazole tablets 250 mg (Galenos Pharmaceutical Company; Iran) in 5 ml of normal silline [24]. The negative control group used normal silin without any additives. Then, to test the treatments, 1 ml of each group was transferred to the tubes separately and then approximately 5000 cysts (200 µl of cysts) were added to each group. These tests were performed for each group in 3 time. Tubes containing cysts were then incubated at 37°C for 30, 60, 120 and 180 minutes. At the end of the intended time, the vital staining method with 0.1% eosin dye was used to measure the lethal effect. Microscopic examinations showed dead cysts due to red eosin uptake and colorless living cysts [25].

Gas Chromatography–Mass Spectrometry (GC-MS) analysis: The components of essential oil were recognized by comparing their relative Retention Time (RT) with valid samples or comparing their Relative Retention Index (RRI) with a series of n-alkanes (Agilent Technologies-7890A). Computer matching with commercial (Wiley GC-MS Library, MassFinder 4 Library) and in-house "Başer Library of Essential Oil Constituents" libraries built up by genuine compounds and components of known oils as well as MS literature data were used.

Analysis method: Data were entered using Excel 2016 software. The chemical analysis of *T. polium* essential oil was done by GC-MS.

Findings

Chemical composition of essential oil: The yellowish-white essential oil was obtained by distillation of *T. polium*. The essential oil components and the percentage of these constituents are shown in Table 1. The analysis of essential oil of aerial part of *T. polium* by water distillation showed 1.3% of returns (w/w). 40 compounds were identified in the essential oil, which constitutes 99.99% of the total identified essential oil compounds. Oxygenated monoterpenes (71.87%) had the highest percentage of compounds. Other major compounds included Monoterpene hydrocarbons (14.04%), Oxygenated sesquiterpenes (4.76%), and Sesquiterpenes hydrocarbons (4.24%), espectively. Among all compounds, β-Thujone (37.57%) was identified as the highest compound of essential oil of *T. polium*. Followed by this composition, 1,8-cineole (8.13%), terpinene-4-ol (4.98%), Borneol (4.61%), cis-9-Octadecenal (3.92%), and trans-pinocarveol (3.20%) were detected as important compounds (Figure 1).

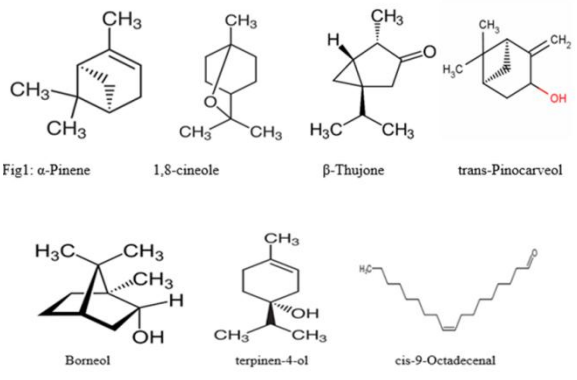

Figure 1) Chemical formula of important compounds of *T. polium* essential oil

Table 1) Chemical composition of *T. polium* essential oil

PK	Name	RT (min)	Standard Ritention Index	Conc. (%)	Formula
1	1,3-Cyclopentadiene,1,2,5,5-tetramethyl-	8.637	838	0.23	C_9H_{14}
2	(Z)-Salvene	9.048	863	0.47	C_9H_{16}
3	Santolina triene	10.597	908	0.88	$C_{10}H_{16}$
4	α-thujene	11.517	931	0.26	$C_{10}H_{16}$
5	α-Pinene	11.854	939	3.04	$C_{10}H_{16}$
6	Camphene	12.472	953	1.31	$C_{10}H_{16}$
7	Sabinene	13.220	976	2.26	$C_{10}H_{16}$
8	β-Pinene	13.466	980	1.64	$C_{10}H_{16}$
9	Myrcene	13.792	991	0.56	$C_{10}H_{16}$
10	α-Terpinene	14.815	1018	0.25	$C_{10}H_{16}$
11	O-Cymene	15.124	1020	2.79	$C_{10}H_{14}$
12	(+)-Santolina alcohol	15.289	1037	0.50	$C_{10}H_{18}O$
13	1,8-cineole	15.512	1039	8.13	$C_{10}H_{18}O$
14	γ-terpinene	16.329	1062	0.69	$C_{10}H_{16}$
15	Terpinolene	17.375	1088	0.36	$C_{10}H_{16}$
16	Linalool	18.050	1098	1.73	$C_{10}H_{18}O$
17	α-Thujone	18.256	1102	1.61	$C_{10}H_{16}O$
18	β-Thujone	18.941	1111	37.57	$C_{10}H_{16}O$
19	Cis-sabinol	19.536	1147	0.36	$C_{10}H_{16}O$
20	Pinocarvone	19.679	1162	0.96	$C_{10}H_{14}O$
21	Camphor	19.890	1143	1.23	$C_{10}H_{16}O$
22	Trans-Pinocarveol	20.37	1152	3.20	$C_{10}H_{16}O$
23	Borneol	20.856	1165	4.61	$C_{10}H_{18}O$
24	Terpinen-4-ol	21.090	1177	4.98	$C_{10}H_{18}O$
25	α-Terpineol	21.553	1189	2.20	$C_{10}H_{18}O$
26	Myrtenal	22.033	1195	0.53	$C_{10}H_{14}O$
27	Cuminaldehyde	23.228	1235	1.92	$C_{10}H_{12}O$
28	Trans-Chrysanthenyl acetate	23.634	1239	0.31	$C_{12}H_{18}O_2$
29	Carvone	24.165	1242	0.23	$C_{10}H_{14}O$
30	Bornyl acetate	24.657	1285	1.80	$C_{12}H_{20}O_2$
31	δ-elemene	24.976	1339	0.68	$C_{15}H_{24}$
32	Bicyclogermacrene	28.246	1494	2.24	$C_{15}H_{24}$
33	Germacrene B	31.898	1560	1.32	$C_{15}H_{24}$
34	Caryophyllene oxide	34.487	1581	0.29	$C_{15}H_{24}O$
35	γ-eudesmol	35.875	1630	0.85	$C_{15}H_{26}O$
36	β-Eudesmol	36.595	1649	1.75	$C_{15}H_{26}O$
37	14-hydroxy-a-Humulene	43.454	1716	0.87	$C_{15}H_{24}O$
38	palmitic acid	44.192	1984	0.62	$C_{16}H_{32}O_2$
39	Cis-9-Octadecenal	46.409	1989	3.92	$C_{18}H_{34}O$
40	Phytol	46.838	2114	0.84	$C_{20}H_{40}O$

Antifungal activity of essential oil: Table 2 shows the *in vitro* results of the essential oil against Candida species compared to fluconazole. Generally, the range of MIC was varied from 0.009 to 0.312 µl/ml among different Candida species. *C. albicans* had the highest MIC_{GM} (0.41 µl/ml), which was approximately equal to the MIC_{GM} of fluconazole (0.46 µl/ml). However, *T. polium* had interesting activities against non-albicans species. Among these species, *C. krusei* was markedly inhibited at low concentrations of essential oil (0.009-0.019 µl/ml).

For other saprophytic fungi, Aspergillus species had high MIC ranges (0.39-50 µl/ml) compared to voriconazole (0.156-1 µg/ml) and itraconazole (0.0625-2 µg/ml). Among all tested isolates, *A. flavus* was slightly inhibited from concentrations 12.5 to 50µl/ml. Whereas, *T. polium* had great antifungal activity against A. *niger* species complex. For instance, the growth of *A. tubingensis* was inhibited at concentrations of 0.39-12.5 µl/ml (Table 3).

Table 2) The MIC Range, MIC50, and MIC90 (µl/ml) of essential oil of T. polium compared to fluconazole against Candida species

Organisms	*T. Polium*	Fluconazole
Candida albicans **(n=16)**		
MIC range	0.087-0.312	0.125-0.25
MIC_{50}	0.156	0.25
MIC_{90}	0.312	0.25
MIC GEOmean	0.41	0.46
Candida parapsilosis **(n=12)**		
MIC range	0.019-0.078	0.125-0.25
MIC_{50}	0.039	0.125
MIC_{90}	0.078	0.237
MIC GEOmean	0.041	0.14
Candida glabrata **(n=11)**		
MIC range	0.019-0.039	0.125-0.25
MIC_{50}	0.039	0.125
MIC_{90}	0.039	0.237
MIC GEOmean	0.028	0.14
Candida kruzei **(n=10)**		
MIC range	0.009-0.019	0.125-0.25
MIC_{50}	0.019	0.125
MIC_{90}	0.019	0.125
MIC GEOmean	0.016	0.125

Table 3) The MIC range, MIC50, and MIC90 (µl/ml) of essential oil of T. polium compared to voriconazole and itraconazole against Aspergillus species

Organisms	*T. Polium*	Voriconazole	Itraconazole
Aspergillus flavus **(n=11)**			
MIC range	12.5-50	0.0156-0.5	0.0625-1
MIC_{50}	12.5	0.25	0.5
MIC_{90}	27.5	0.5	0.55
MIC GEOmean	12.5	03.5	0.35
Aspergillus niger **(n=11)**			
MIC range	1.56-3.125	0.25-0.5	0.5-2
MIC_{50}	3.125	0.25	1
MIC_{90}	3.125	0.5	2
MIC GEOmean	3.125	0.5	0.7
Aspergillus tubingensis **(n=11)**			
MIC range	0.39-12.5	0.25-1	1-2
MIC_{50}	1.56	0.5	2
MIC_{90}	12.5	0.55	2
MIC GEOmean	1.1	0.5	2

Antibacterial activity: Essential oil of *T. polium* showed no inhibitory effect on MRSA. However, the results of MIC and Minimum Bactericidal Concentration (MBC) showed that the MIC value was lower for *E.coli* (3.75 µl/ml) compared to *Klebsiella* (7.5 µl/ml).

Efficacy of *T. polium* essential oil for *Giardia lambelia*: According to the comparison of the mean concentration and time of treatments, the effectiveness of different concentrations of The essential oil of *T. polium* in 30 min was less than other treatments. The best activity was observed in 30 min for a concentration of 200 µl/ml. At this time the activity of positive control (metronidazole 50 mg/ml) was not completed and its best activity was observed in 120 min. The increase in efficacy for each concentration occurred gradually and for all

groups, the most activity was observed in 180 minutes (Table 4).

Table 4) Efficacy or percentage of killed/inactive cysts for different concentrations of *T. polium* essential oil in different times (mean±SEM)

Concentrations	30 min	60 min	120 min	180 min
25 µl/ml	$48±4.35^{Dc}$	$60±3^{Db}$	$74±3.60^{Ca}$	$82.33±2.51^{Ba}$
50 µl/ml	$59±4.35^{Dc}$	$91.33±3.05^{Cb}$	$95.33±4.16^{Ba}$	$99.33±1.15^{Aa}$
100 µl/ml	$76±4.35^{Bd}$	$97.67±2.08^{ABc}$	$100±0^{Ab}$	$100±0^{Aa}$
200 µl/ml	$86.33±3.21^{Ac}$	$100±0^{Ab}$	$100±0^{Aab}$	$100±0^{Aa}$
Negative control	$2±0^{Eb}$	$4±0^{Ea}$	$5±0^{Da}$	$5±0^{Ca}$
Positive control	$92±0^{Ab}$	$95±0^{Ba}$	$100±0^{Aa}$	$100±0^{Aa}$

Uppercase letters show significant differences in concentration treatments at each time and non-common lowercase letters show significant differences in times per concentration using LSD test at 0.05 level.

Two factors, concentration and time, are important in parasitic studies. The best activity (i.e., low duration and minimum concentration) can be reported at a concentration of 200 µl/ml in 60 min or 100 µl/ml in 120 min. 100% fatality was observed at this time (Figure 2).

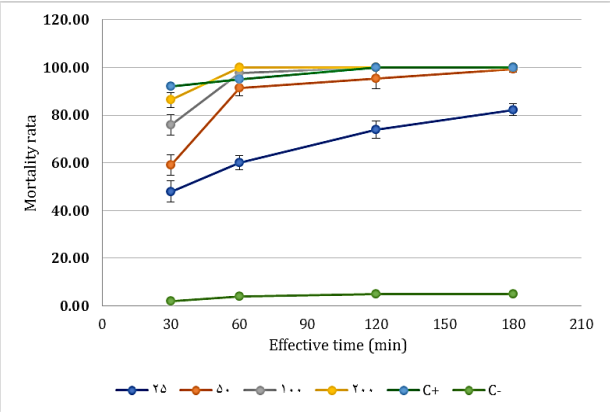

Figure 2) Mortality percentage of Giardia lamblia cysts by essential oil according to concentration and duration of exposure

Discussion

Numerous factors such as the increasing use of synthetic drugs, immunocompromised patients, and the emergence of drug-resistant microorganisms are causing global health concerns. These factors make researchers to look for a new antimicrobial agent with low cost and resistance. Herbal medicines are a good resource with therapeutic effects with low costs and side effects. *T. polium* is one of the traditional herbal plants that has been used as a herbal medicine in Iran for a long time.

In the present study, the returns of essential oil was 1.3% (w/w), which is higher than the value reported in a study in Algeria (0.27%) and lower than the value reported in a study in Saudi Arabia (1.65%) [26, 27].

Chemical analysis of the essential oil of this plant showed that Oxygenated monoterpenes (71.87%) had the highest percentage of compounds. According to a previous study, the number of *T. polium* compounds was 25 compounds and the total amount detected was 92.9%, of which 21% were Monoterpene hydrocarbons, and 53.4% were Sesquiterpenes hydrocarbons [28]. In another study, there were 31 compounds and the percentage of total compounds of *T. polium* essential oil was 94.49% [29]. In this group, the most compounds were Oxygenated monoterpenes (3.82%), Sesquiterpenes hydrocarbons (64.11%), and Oxygenated sesquiterpenes (12.3%), respectively.

The results of these two studies are in conflict with the present study. Because in our study, the highest percentage of compounds was related to oxygenated monoterpenes. This difference may be due to the distribution of various plant subspecies in different climatic regions, different geographical locations in terms of physicochemical factors of soil, as well as plant physiological conditions and environmental conditions [12].

Besides, β-thujone was identified as the highest compound of essential oil of *T. polium*. This compound is one of the main compounds and biologically active components of essential oils derived from a variety of plants of the families Laminaceae, Asteraceae, and Cupressaceae [30-32]. Previous studies on the chemical components of *T. polium* essential oil have shown that in some studies similar compounds were found with different percentages. For example, α-Pinene, which is a hydrocarbon monoterpene, was an important compound in the study of Asgharipour and Shabankare, whose percentage was reported as 3.92% [28]. In another study, this value was 6.76% for α-Pinene [33]. Generally, the difference between our results and the results of other studies regarding the quantity and quality of essential oil compounds and the type of compounds is due to physiological changes in different vegetation stages, different environmental conditions (e.g., habitat soil conditions, availability of factors such as moisture, temperature, evaporation, and transpiration), geographical changes and genetic factors that directly affect the biosynthesis of essential oils.

In the present study, the essential oil of *T. polium* aerial parts was effective against opportunistic fungi and bacteria. The inhibitory effect of essential oil on the growth of *C. albicans* isolates was approximately equal compared to fluconazole (MIC_{GM}= 0.46 µg/ml). However, in the present study MIC ranges of *T. polium* against *C.albicans* isolates (0.087-0.312 µl/ml) were less than the value reported by Nadimi *et al.* (4-16mg/ml) [34]. There are differences in our study method with Nadimi *et al.*'s study, which used alcoholic and aqueous extracts with the macro dilution method. Besides, Darwish *et al.* indicated that methanolic extract of *T. polium* had no anti-Candida activity, which is completely inconsistent with the result of our study [35]. The moderate activity of essential oil of *T. polium* on *C. albicans* was shown in the study of El-Shazly and Hussein [36]. It seems that this disagreement in the results of

studies is related to the origin of Candida isolates and the type of method. It should be noted that fluconazole is a choice of treatment in various forms of candidiasis. Better susceptibility to essential oil against non-albicans species compared to fluconazole is important. Our results showed that the MIC_{GM} value was lower than fluconazole for non-albicans species. Bonyadpour *et al*. reported that all clinical isolates and standard non- Candida species were sensitive to *T. polium* smoke product [37]. Also, their study showed that the concentration of 180 µl of the plant on the disk has an antifungal effect.

In our study, *T. polium* had high MIC a value range against Aspergillus species (0.39-50 µl/ml). Also, the essential oil was showed great antifungal activity against *A. niger* species complex. By comparing the results with itraconazole and voriconazole, we observed that *T. polium* has lower activity against Aspergillus species. Alreshidi *et al*. indicated that methanolic extract of *T. polium* has low activity against filamentous fungi, especially in *A. fumigatus* and *A. niger* [15]. Since no other study has been done on the effect of this essential oil on Aspergillus isolates, further studies seem necessary.

Overall, based on the minimum inhibitory concentration, the antibacterial compounds present in *T. polium* essential oil act as an antibacterial agent. In our study, *T. polium* essential oil did not inhibit the growth of MRSA. Darabpour *et al*. reported that combination therapy of antibacterial drug with *T. polium* extracts could be important in the management of MRSA infection [38].

Giardia lamblia is a protozoan parasite that is considered one of the main causes of different levels of diarrhea infection in all parts of the world. The antiparasitic activity of some medicinal plants from different families on several parasites has been previously studied. For instance, some herbal compounds including *A. sativum* (Acrolein ,Allicin), *A. sieberi* (1,8-cineole, camphor, α-thujon), *Z. multiflora* (thymol, carvacrol, linalool, p-cymene, borneol, beta-pinene), *C. botrys* (Ascaridole), *E. globulus* (eucalyptol, butyric aldehyde, valeric aldehyde, pinocarveol) have been identified as antiparasitic agents [39]. A study conducted by Azadbakht *et al*. on the effect of essential oils of some medicinal plants on *G. lamblia* cysts concluded that the essential oil of *Eucalyptus globulus* can kill 79.7% of cysts in 30 min [40].

In another study, Dehghani-Samani *et al*. reported that the highest anti-Giardia activity (73.55%) for Eucalyptus essential oil at 480 min after exposure [41]. Amaral *et al*. reported that 153 plant species (from 69 families) have anti-Giardia activity [42]. Also, the anti-Giardia activity of essential oils rich in phenols, polyphenols derived from some aromatic herbs has been reported by Machado *et al*. [43]. The activity of 42 essential oils from different plant families on protozoa was investigated. The results showed that plants belonging to the Lamiaceae family had the best yield and the highest lethality [44]. The results of the present study with previous studies show the high activity of essential oil against Giardia cysts at different concentrations and at different times.

In the end, it can be said that due to the high susceptibility of fungal isolates to this plant and the presence of recurrent fungal infections, the results can be tested in clinical trial studies. If *T. polium* essential oil has a positive effect on fungal, bacterial strains and *Giardia lamblia* in clinical trial studies, it can be recommended as a complementary medicine for patients with these problems.

Conclusion

The essential oil of *T. polium* has antifungal, antibacterial, and anti-Giardia properties and can be used in drug and food industries to prevent various infections and contamination.

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