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Antioxidant and Antibacterial Activity of *Tanacetum* spp. Essential Oil and Chemical Components

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ABSTRACT

Introduction: The members of the genus *Tanacetum* are important medicinal plants. This study investigated the chemical composition and antibacterial activity of *Tanacetum lingulatum* and *Tanacetum polycephalum* essential oils on human infectious bacteria. **Methods**: The aerial part of two plants were collected from Urmia Province, Iran. The essential oils were extracted using a Clevenger device. The antibacterial effect of essential oils was determined using the disc diffusion assay, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) by serial dilution method. Also, free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) was examined. Chemical composition was measured using the Gas Chromatography-Mass Spectrometry (GCMS). **Results:** The major constituents in *T. polycephalum* and *T. lingulatum* essential oils were 1,8- cineole and camphor, respectively. The highest sensitivity (MIC of 0.312 µg mL^{-}) was observed with *T. polycephalum* against *Bacillus subtilis*. The lowest IC50 (most potent radical scavenging activity) belonged to *T. lingulatum* essential oil. *Pseudomonas aeruginosa* and *Streptococcus pyogenes* showed resistance to *T. lingulatum* essential oil. **Conclusion:** The essential oil of *T. polycephalum* and *T. lingulatum* are potential natural antibacterials to treat human pathogenic bacteria and can be used as alternatives to produce antimicrobial agents.

INTRODUCTION

Some people use herbs with antimicrobial properties for treating infectious diseases [1]. The genus *Tanacetum* belonging to the Asteraceae family contains 200 species worldwide, of which 26 occur in Iran, comprising medicinal plants [2, 3]. In China, essential oils are used in traditional medicine and traditional ceremonies [4]. Due to bacterial resistance, the high treatment cost of synthetic medications, and the antibiotics side effects, attempts are made to develop new antibacterial agents [5]. Essential oils have a low molecular weight and evaporate rapidly at ambient temperatures. Herbal essential oils have antimicrobial properties, and their applications in pharmacy and medicine have been reported [6]. Essential oils are extracted by several methods, including water and steam distillation, vacuum distillation, gas chromatography, and high-performance liquid chromatography (HPLC) [6].

Due to the presence of flavonoids, sterols, and terpenoids, the *Tanacetum* species have anti-tumor, antifungal, antibacterial, and anti-migraine activities [7]. Terpenes and parathyroid compounds have anticancer and anti-migraine properties and powerful microbicidal effects against bacteria and fungi [8]. Plants contain high concentrations of carotenoids, tocopherols, tocotrienols, glutathione, ascorbic acid, and enzymes with antioxidant activity, which protect them from hazardous oxidative damage [9]. Phenolic compounds from medicinal herbs and dietary plants possess various bioactivities and play an essential role in preventing infectious diseases [10, 11]. Secondary metabolites reduce the risk of major chronic diseases (e.g., heart disease, cancer, and diabetes) due to potent antioxidant activities [12]. By inhibiting the polymerization of tubulin and preventing the destructive action of free radicals, flavonoids can play a crucial anticancer role [13]. Also, the anti-*Candida* activity of *T. polycephalum* has been reported [14].

Antiseptic, analgesic, anti-inflammatory, and antimicrobial properties of *T. vulgare* and *T. parthenium* are related to borneol, camphor, and terpenes compounds [15, 16]. Several factors, including harvest time, collection, drying, and storage methods, affect the quantity and quality of essential oils [12].

This study investigated the antibacterial and antioxidant activity of *T. lingulatum* and *T. polycephalum* essential oils on pathogenic bacteria *in vitro* and chemical compositions analysis.

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MATERIALS AND METHODS

The chemical materials, including 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Mueller-Hinton agar (MHA), Nutrient broth (NB), Nutrient agar (NA), and ascorbic acid, were purchased from a commercial company (Merck, Germany), and gentamicin and ciprofloxacin antibiotics were obtained from Padtanteb Company (Iran).

Preparation of essential oils. *Tanacetum lingulatum* and *T. polycephalum* were collected from Urmia Province, Iran, in 2015. The essential oil was extracted from aerial parts of collected samples using a Clevenger device at Bu-Ali Sina University (Biotechnology Department Laboratory). Amounts of 100 g of powders were heated in water for 3 h and then was dehydrated by anhydrous sodium sulfate [17].

Bacterial strains. Human pathogenic bacteria including *Streptococcus pyogenes* (PTCC-1447), *Bacillus subtilis* (PTCC-1156), *Bacillus cereus* (PTCC-1247), *Micrococcus luteus* (ATCC 10987), *Enterococcus faecalis* (PTCC-1195), *Staphylococcus aureus* (PTCC-1189), *Escherichia coli* (ATCC-25922), *Shigella boydii* (PTCC1744), *Salmonella typhi* (PTCC-1609), *Pseudomonas aeruginosa* (PTCC-1181), *Enterobacter aerogenes* (PTCC-1221), *Proteus mirabilis* (PTCC-1287), *Neisseria meningitides* (PTCC-4578), *Acinetobacter baumannii* (PTCC-4413) and *Klebsiella pneumoniae* (PTCC-1129) were obtained from the Tehran University, Iran. The bacteria suspension concentration was prepared equal to 0.5 McFarland standard (1.5×10^8 CFU) [18].

Disc diffusion assay. The antibacterial activity of essential oil was performed by disc diffusion assay [6]. Various essential oil concentrations 5, 10, and 20 µg mL^- were prepared in dimethyl sulfoxide (DMSO). A volume of 20 µl of the bacterial suspension (1.5×10^8 CFU) was cultured on an MHA medium. Then, the 20 µl essential oil was spread on discs and incubated at 37 ^{0}C for 24 h [19]. Gentamicin (10 µg) and ciprofloxacin (5 µg) discs were used as positive controls [20] and DMSO as a negative control. The inhibitory zone (mm) formed around each disc was measured, and the data were analyzed by SPSS software.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The MIC and MBC for *T. lingulatum* and *T. polycephalum* essential oils were determined using the micro-dilution broth method in 96-well plates [21]. Various dilutions of essential oils, i.e., 10, 5, 2.5, 1.25, 0.625, and 0.312 µg mL^- were prepared. The wells in a 96-well plate were filled with 185 µl of NB medium, and 200 µl of 20 µg mL^- essential oil was added to the first well plate. Then, 200 µl from the first well plate was added to the second well plate, and the process continued. Finally, 15 µl of bacterial suspension was added to each well plate, followed by incubation at 37 ^{0}C for 24 h. The lowest dilution with no bacteria growth was defined as MIC. To calculate MBC, 5 µl from the plates with no bacterial growth was cultured on an MHA medium and incubated at 37 ^{0}C for 24 h. The minimum concentration with no bacterial growth was considered as MBC.

Free radical scavenging activity. The free radical scavenging activity was assayed as described by others [22]. Various dilutions of essential oils were prepared, i.e., 2, 4, 6, 8, and 10 µg mL. The ascorbic acid and 2, 2- DPPH were used as the standard and reagent. The sample absorption was calculated using a spectrophotometer at 517 nm after 30 min [23]. The IC50 for the essential oil and ascorbic acid were measured. The experiments were tested in triple.

Gas Chromatography-Mass Spectrometry (GCMS). Chemical compositions of essential oils were analyzed by GCMS (Tehran University, Iran). The GCMS analysis was carried out using an Agilent 6890N coupled with an Agilent S973 mass detector, using an HP-5, 30 m column. The instrument was programmed with initial heating at 275 ^{0}C for 2 min. The temperature dropped to 120 ^{0}C, at an increasing rate of 8 ^{0}C /min; then to 285^{0}C, an increasing rate of 3.5^{0}C /min. Injection port temperature was ensured as 350 ^{0}C and the helium flow rate at 0.9 ml/min. The samples were injected in split/splitless mode. Solvent delay adjusted for 5 min and 1 µl volume injected [5].

Statistical analysis. Data as mean ± SD and mean comparison by Duncan test at (P<0.05) in a triple with SPSS software were expressed.

RESULTS

Antibacterial activity. Generally, the sensitivity of tested bacteria against essential oils increased by concentration except for *E. coli*. Moreover, the *T. lingulatum* essential oil did not affect *S. pyogenes*, *P. aeruginosa*, and *E. faecalis* growth. The *T. polycephalum* essential oil showed a more inhibitory effect than *T. lingulatum* essential oil. Furthermore, the highest sensitivity was exhibited with *T. polycephalum* essential oil (20 µg mL^-) against *B. subtilis*. Finally, the Gram-positive bacteria demonstrated more sensitivity than the Gram-negative bacteria. The antibacterial activity of *T. lingulatum* and *T. lingulatum* essential oil are represented in Table 1.

The MIC and MBC for *T. polycephalum* and *T. lingulatum* essential oil against human pathogenic bacteria are represented in Table 2. The MIC for *T. polycephalum* essential oil on *B. subtillis* was 0.312 µg mL^-. In addition, the MIC for *T. polycephalum* essential oil on *S. aureus* and *K. pneumoniae* and the MIC for *T. lingulatum* on *B. cereus* was 1.25 µg mL^-. However, *P. aeruginosa* and *S. pyogenes* exhibited resistance against various dilutions of *T. polycephalum* and *T. lingulatum* essential oils, i.e., 10, 5, 2.5, 1.25, 0.625, and 0.312 µg mL^-. Furthermore, *T. polycephalum* essential oil showed

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more inhibitory activity than *T. lingulatum*. The *T. lingulatum* essential oil had no MIC and MBC with *K. pneumoniae* and *E. faecalis*. The MIC and MBC of gentamicin on *E. faecalis* was 0.156 µg mL^{-} and MIC and MBC of ciprofloxacin on *E. faecalis* and *E. coli* was 0.07 µg mL^{-}.

Table1. Antibacterial activity (mm) of *T. polycephalum* and *T. lingulatum* essential oils against human pathogenic bacteria

Bacteria	*T. polycephalum* (µg mL^{-})			*T. lingulatum* (µg mL^{-})			Gentamicin (µg mL-)	Ciprofloxacin (µg mL^{-})
	20	10	5	20	10	5	10	5
S.boydi	12±0.88 mm	11±0.33 mm	9±0.33 mm	11±0.88 mm	10.5±1.2 mm	8±0.57 mm	29±0.57 mm	29.5±0.33 mm
N.meningitides	14±0.88 mm	12±0.57 mm	-	9±0.22 mm	8±0.66 mm	-	19±0.33 mm	28.5±0.66 mm
A. baumannii	15±0.33 mm	13±0.66 mm	7.5±1.2 mm	10±0.33 mm	9±00 mm	-	20±1 mm	28.5±0.66 mm
K.pneumoniae	17±0.88 mm	14±0.88 mm	9±00 mm	12±0.33 mm	-	-	20±0.57 mm	31.5±0.33 mm
S.typhi	12.5±0.33 mm	11±0.22	8±0.66 mm	15±0.88 mm	14±0.88 mm	12±1.2mm	22±0.33 mm	30±1 mm
P.aeruginosa	8.5±0.33 mm	7±0.66 mm	-	-	-	-	16±0.33 mm	17.5±0.57 mm
E.coli	10.5±0.33 mm	12±0.88 mm	14±0.33 mm	11±0.33 mm	9±0.66 mm	7±0.22 mm	19±0.57 mm	37.5±0.66 mm
E.aerogenes	13±0.88 mm	13±0.88 mm	8±0.66 mm	9±0.33 mm	-	-	20±0.33 mm	24.5±0.66 mm
P. mirabilis	12±0.33 mm	11±0.66 mm	10±0.33 mm	14±0.66 mm	13±0.33 mm	10±0.33mm	19.5±1 mm	24.5±0.57 mm
S.pyogenes	13.5±0.22 mm	12±0.33 mm	10±0.88 mm	-	-	-	11±0.33 mm	28±0.33 mm
M.luteus	18±0.33 mm	15±0.88 mm	13±0.55 mm	15±0.33 mm	12±0.66 mm	10±0.66 mm	15±0.33 mm	17±0.57 mm
S.aureus	18.5±0.33 mm	16±0.66 mm	10±0.33 mm	13±0.88 mm	10±0.33 mm	-	16±0.88 mm	17.5±0.88 mm
B.subtillis	23±0.88 mm	20±0.33 mm	15±0.66 mm	16±0.33 mm	14±00 mm	10±1 mm	15±0.88 mm	16±0.33 mm
B.cereus	20±0.33 mm	19±0.66 mm	16±0.22 mm	14±0.33 mm	10±0.66 mm	9±0.33 mm	15±0.57 mm	16.5±0.57 mm
E. faecalis	16±0.33 mm	12±00 mm	8±0.22 mm	-	-	-	29.5±1 mm	33±0.57 mm

Table 2. MIC and MBC of *T. polycephalum* and *T. lingulatum* essential oil against human pathogenic bacteria

Bacteria	*T. polycephalum* (µg mL^{-})		*T. lingulatum* (µg mL^{-})		Gentamicin (µg mL^{-})		Ciprofloxacin (µg mL^{-})	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. boydii	5	10	5	10	1.25	2.5	1.25	2.5
N.meningitides	2.5	5	10	10	2.5	5	0.625	1.25
A. baumannii	5	5	5	-	0.625	1.25	0.312	0.625
K. pneumoniae	1.25	5	-	-	0.625	1.25	0.312	0.312
S.typhi	10	-	2.5	5	0.312	0.625	0.156	0.312
P. aeruginosa	-	-	-	-	5	5	2.5	2.5
E. coli	5	10	10	10	2.5	2.5	0.07	0.07
E. aerogenes	2.5	5	10	-	1.25	2.5	0.625	1.25
P. mirabilis	5	5	5	10	5	5	1.25	1.25
S.pyogenes	-	-	-	-	5	5	2.5	2.5
M.luteus	2.5	5	2.5	5	5	5	2.5	5
S. aureus	1.25	10	2.5	5	5	5	2.5	2.5
B. subtillis	0.312	1.25	1.25	5	5	5	2.5	2.5
B. cereus	2.5	5	1.25	5	2.5	5	1.25	2.5
E. faecalis	5	10	-	-	0.156	0.156	0.07	0.07

Anti-radical activity by DPPH. The amount of DPPH free radical inhibition increased by essential oil concentrations. A significant difference was observed between *T. polycephalum* essential oil IC50 and ascorbic acid as the control. The lowest IC50 (most potent radical scavenging activity) was obtained with *T. lingulatum* essential oil (Table 3).

Table3. The DPPH inhibition percentage and IC50 of *T. polycephalum* and *T.lingulatum* essential oil

Organ	Inhibition percentage of DPPH in different concentrations (µg mL^{-})					IC50
	2	4	6	8	10	
T. polycephalum	12.53	16.11	19.3	25.74	31.62	0.94^{a}
T.lingulatum	24.85	45.31	52.47	70.64	82.88	0.42^{b}
Ascorbic acid	25.58	49.02	56.51	79.85	92.04	0.39^{b}

The same letters are not significantly different at $P<0.05$

GCMS analysis. GCMS analysis identified 23 (91%) and 20 (86%) chemicals in *T. polycephalum* and *T. polycephalum*, respectively (Table 4). The main constituents of *T. polycephalum* essential oil were 1,8 cineole (15.7%), camphor (10.94%), alpha-pinene (5.4%), and trans-sabinene (5.13%), and of *T. lingulatum* essential oil was 1,8 cineole (18.83%), camphor (14.95%), linalyl propionate (8.5%), and alpha-terpineol (8.02%). The chemical structures of some essential compounds of *Tanacetum* spp. are reflected in Figure 1.

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Table4. The chemical compositions of *T. polycephalum* and *T.lingulatum* essential oil

T. polycephalum	Compound content (%)	*T.lingulatum*	Compound content (%)
1,8- Cineole	15.7	**1-8,cineole**	18.83
Camphor	10.94	**Camphor**	14.95
α –pinene	5.4	**Linalyl propionate**	8.5
Trans- sabinene	5.136	**α –terpineol**	8.02
α-Terpineol	4.75	**Sabinene**	5.81
Berbenol	4.66	**Terpinen-4-ol**	5.76
Piperitol isomer	4.35	**Isobutylbenzene**	5.56
2- hexene,4,4,5-trimethyl	3.09	**α –pinene**	4.17
Chrysanthenyl acetate	3.02	**Chrysanthenone**	3.87
Borneol	2.37	**γ-Eudesmole**	3.28
Linalool	2.29	**Gamma-terpinene**	1.87
Isoborneol	2.13	**Eucarvone**	1.64
Pinocarvone	2.05	**α - terpinene**	1.48
Trans-caryophyllene	1.96	**1-(hydroxymethyl) methylamino adamantine**	1.47
Tetrahydropyranyl ether	1.44	**Isocaryophillene**	1.37
Bornyl acetate	1.68	**Endobornyl-acetate**	1.34
Beta-pinene	1.67	**α -terpineol(p-menth-1-en-8-ol)**	1.16
Cyclohept-4-enone	1.62	**Globulol**	1.03
4-terpineol	1.62	**Camphene**	1.04
Nerolidol	1.46	**Dichloromethane**	0.99
Terpinen-4-ol	1.43		
Camphene	1.4		
Sabinene	1.28		

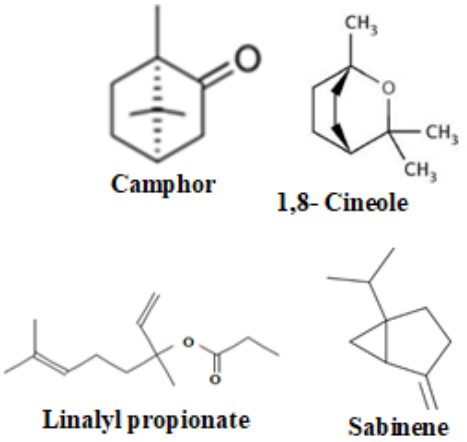

Fig. 1. The chemical structures of important compounds

DISCUSSION

The chemical compositions, including cineole and camphor with antimicrobial properties, are used to control human pathogenic agents, especially bacteria [24]. The 1,8-cineole, a natural monoterpene, also known as eucalyptol, has anti-inflammatory activity [25].

In this study, the important chemical compositions of *T. polycephalum* essential oil comprising 1,8 cineole (15.7%), camphor (10.94%), alpha-pinene (5.4%), trans-sabinene (5.13%), α-terpineol (4.75%), berbenol (4.66%), piperitol isomer (4.35%), linalool (2.29%), pinocarvone (2.05%), bornyl acetate (1.68%), and camphene (1.4%) were measured. The dominant compounds, included camphor (18.5%), pinocarvone (31.4%), α-pinene (9.5%), and bornyl acetate (5.9%), from the aerial part essential oil [26], camphor (59.1%), camphene (14.9%) and 1,8-cineole (10.1%) from flower essential oil [27], linalool (9.72%) from flower essential oil [28] and borneol (28.3%), β-pinene (10.1%), α-pinene (6.5%), camphene (6%), α-terpineol (5.16%) and 1,8- cineole (5.1%) [6], from essential oil of *T. polycephalum* were identified. The result of these groups showed a close similarity with the present study. The factors including area, harvest season, climate, and physiological conditions affect types and chemical compositions [29].

Moreover, the chemical compounds including 1,8 cineole (18.83%), camphor (14.95%), linalyl propionate

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(8.5%), alpha-terpineol (8.02%), sabinene (5.81%), and α–pinene (4.17%) in *T. lingulatum* essential oil were obtained in the present study. The chemical compositions including 1,8- cineole (18.6%) and camphor (13.9%) [28], and α-pinene (22.876%), 1,8-cineole (21.472%), sabinene (17.902%), 2-pyrrolidinone (7.196%), and camphor (6.794%) [30] have been reported from the aerial part and flower essential oils of *T. lingulatum*, similar to the present study.

The essential oils catalytic action increases permeability in the cell wall and membrane, destroying the pathogens [31]. According to this research, some bacteria, including *S. typhi*, *P. aeruginosa*, and *S. aureus* showed sensitivity against *T. polycephalum* essential oil, especially in high concentrations. There are reports of antibacterial activity of *T. polycephalum* essential oil on *S. typhi, S. epidermidi, S. saprophiticus, P. aeroginosa,* and *S. aureus* [6], similar to the present research.

Based on this research, the amount of DPPH free radical inhibition increased by essential oil concentrations, and the lowest IC50 belonged to *T. lingulatum* essential oil. The free radicals play a pivotal role in some medical conditions, including cancer and cardiovascular diseases, neural disorder, and diabetes [32].

Some Gram-positive bacteria, including *M. luteus, S. aureus,* and *B. subtillis* due to compounds like camphor, sabinene, 1,8 cineole, and linalyl propionate in *T. polycephalum* and *T. lingulatum*, showed more sensitivity to essential oils than tested antibiotics. Finally, antibacterial constituents of *T. polycephalum* and *T. lingulatum* essential oils can be used to develop antimicrobials against pathogenic bacteria.

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CONFLICT OF INTREREST

The authors declare no conflicts of interest associated with this manuscript.

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