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A comparative study of chemical composition and antibacterial properties of essential oils of *Tanacetum turcomanicum* (Krash.) and *Tanacetum walteri* (C.Winkl.) from northern Khorasan Province

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ABSTRACT

Background & Aim: *Tanacetum* is a member of Asteraceae family and its twelve species such as *Tanacetum walteri* are endemic to Iran. In this investigation, chemical compounds and antimicrobial activity of essential oils of *Tanacetum turcomanicum* (Krash.) and *Tanacetum walteri* (C.Winkl.) were studied.

Experimental: The plants were collected from north Khorasan province of Iran, in spring of 2016. The essential oils were extracted by the hydrodistillation method using clevenger. Identification of the essential oils chemical composition was conducted by GC/MS instrument. Also, Agar well diffusion method was used to investigate its antibacterial effects against *E. coli*, *S. aureus*, *B. atrophaeus* and *M. luteus*.

Results: Essential oil efficiency of *T. walteri* species was obtained 0.2% v/w. In total 92.84 percent of essence constituents of 43 components were recognized in which α-pinene (22.37%), 1,8-cineole (12.52%), camphor (11.91%) and ß-thujone (4.52%) were recognized as the main constituents. By isolating essence of *T. turcomanicum* species, 39 constituents of 89.28% of the whole essential oils were recognized with efficiency of 0.3% v/w. Davanone (20.79%), 1,8-cineole (15.63%), p-cymene (10.20%), camphor (10.14%) and borneol (4.95%) were the main components of the essence. The highest antimicrobial activity of the essential oils of *T. turcomanicum* and *T. walteri* were observed against *M. luteus*, *B. Atrophaeus* and *S. aureus*, respectively. The essential oil of *T.turcumanicum* did not show significant activity against *E. coli* bacterium.

Recommended applications/ industries: The results showed the environmental influences and the effect of plant species on the amount and type of volatile components of essential oils. Also, antibacterial efficacy shown by these oils provides a scientific basis and thus validates their use as medicinal remedies. Isolation and purification of different phytochemicals may yield to significant antibacterial agents.

1.Introduction

There is a scientific interest to screen essential oils and extracts of plants used medicinally across the globe (Heath,1981). The main volatile constituents of the essential oils have been used historically in the pharmaceutical, food and perfume industries owing to their antibacterial and antioxidant properties. Antimicrobial resistance, environmental problems,

cancer, side effects and high cost have limited the use of synthetic antibiotics (Gortzi *et al.*, 2006). Accordingly, the plant– products was suggested to replace conventional antibacterial. Extensive researches have been carried out to evaluate the antimicrobial effect of the essential oils and extracts (Aleksic andKnezevic, 2013; Liu *et al.*, 2012).

Asteraceae or Compositae is a famous family of medicinal and aromatic plant species. The family currently has 32,913 accepted species names, in 1,911 genera and 13 subfamilies. The genus Tanacetum belongs to the family Asteraceae or Compositae. This genus includes more than 200 species and is distributed throughout Western Asia, Europe and North America (Bremer, 1994). Tanacetums or Tansies are mainly perennial herbs, but some are annuals and subshrubs. In the flora ofIran, this genus has 26 species of herbaceous and perennial herbs, of which 12 species are exclusively Iranian (Rechinger, 1986; Mozaffarian, 1996). Tanacetum species are sources of natural products, essential oils, sesquiterpene lactones and bitter substances (Rezaee *et al.*, 2015). These species have a special taste or smell that is created by monoterpenes and sesquiterpenes and this is the reason for their use in native medicine. The species of the genus Tanacetum have been used in popular medicine as expectorants, antiseptic vermifuges, and spasmolytics (Oksuz, 1990).

According to studies, the essential oils and extracts of some species of the genus Tanacetum exhibit antibacterial (Akpulat *et al*., 2005; Salamci et al., 2007, Sonboli and Ghaderi, 2018;Habibi *et al.*, 2009), anti-inflammatory(Brown *et al.*, 1997), and antifungal effects (Hethelyi *et al.*, 1991; Neszmelyi *et al.*, 1992). Recently, many consumers prefer additive free foods or a safer approach, such as the utilization of more effective antioxidant and antibacterial agents from natural origins.

Therefore, inthis study, the chemical composition of the essential oils of *T. turcomanicum* and *T. walteri* Iranian origin collected from North Khorasan was evaluated to compare the results with previous reports. Also, following previous studies on the antimicrobial activity of the aforementioned essential oils (Sonboli and Ghaderi, 2018; Habibi *et al.*, 2009),we evaluated the antimicrobial activity of prepared essential oils against a number of pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus atrophaeus* and *Micrococcus luteus*.

2. Materials and Methods

2.1. Plant material

The aerial parts of *T. turcomanicum* and *T. walteri* were collected from north Khorasan province of Iran, in May 2016, and identified by the Herbarium of Medicinal Plants Processing Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. Collection site information and voucher No. is summarized in (Table 1).

Table 1. Collection site information and voucher No.in the present work

Plant name	Latitude	Longitude	Altitude (m)	voucher No
Tanacetum turcomanicum (Krash.)	37° 23 ′N	56° 26 ′E	1550	43663
Tanacetum walteri (C.Winkl.)	37° 40 ′N	57° 57 ′E	2450	45836

2.2. Essential oil extraction

In this study, 250 g of aerial parts of *T. turcomanicum* and *T. walteri* were subjected to water-distillation for 3h using a Clevenger type apparatus, separately (Clevenger, 1928).The obtained essential oil was then dried over anhydrous sodium sulfate and stored at 4°C for further chemical and pharmacological analysis.

2.3. GC-MS Analysis

Analysis and identification of the chemical composition of essential oils of *T. turcomanicum* and *T. walteri* were performed on an Agilent 6890 series gas chromatograph interfaced to an Agilent 5973 N mass selective detector (Agilent Technologies, Little Falls, DE, USA). Helium was used as carrier gas at a flow rate of 1 mL/min. A TRB–5MS fused silica capillary column (30m×0.25 mm ID×0.25 µm film thicknesses) was used. Oven temperature was kept at 35°C for 5 min, and then gradually raised to 150°C at 25°C /min. It was kept constant at 150°C for 5 min, then planned at 20°C /min to final oven temperature of 280°C and kept constant for 10 min.

The mass range was 35–450 m/z and the identification of compounds was performed by comparing their mass spectra with the NIST 08 and Wiley 275 libraries. Also, relative indices were calculated using the retention times of C_3–C_{19} n–alkanes under the same conditions (Yang *et al.*, 2014).

2.4. Antimicrobial activity assay

The used protocols in this study were based on the guidelines of CLSI with little modification (Balouiri*et al.*, 2016). The antibacterial activities of the essential oils of *T. turcomanicum* and *T. walteri* were evaluated using Agar–well diffusion method on Mueller–Hinton agar (MHA). *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *B. atrophaeus* (DSM675) and *M. luteus* (PTCC 1170) were used as references for the antimicrobial assay of essential oils. At first, the concentrations of 1:2, 1:4, 1:8, 1:16 and 1:32 of essential oil were prepared. Then, using sterile tube (7mm diameters) wells were created in Muller Hinton agar (MHA). Thereafter, 200 µL of fresh bacterial suspension with a standard concentration (~10^8 CFU/mL) were added onto Muller Hinton agar (MHA). A positive control (containing Gentamycin 10 µg) and negative control (containing 10% DMSO) were selected. The wells were filled with 10 µL of the essential oils at different concentrations and then microplates were incubated at 37°C for 24 h. *B. atrophaeus* was incubated at 30°C. After 24–48 h of incubation, the antibacterial activity was evaluated by measuring the zone width of growth inhibition for the indicator organisms and compared with Gentamycin and 10% DMSO. The experiments were performed in triplicate.

3. Results and discussion

3.1. Composition of the essential oils

The chemical composition of *T .turcomanicum* and *T. walteri* essential oils is shown in (Table 2). By isolating the essential oil of *T. turcomanicum*, an efficiency of 0.3% v/w was observed in 39 constituents of 89.28% of the whole essential oil. Davanone 20.79%, 1,8-cineole 15.63%, p-cymene 10.20%, camphor 10.14% and borneol 4.95% were the main constituents. This essential oil consisted mainly of oxygenated monoterpenes (38.75%) and monoterpene hydrocarbons (25/85%), oxygenated sesquiterpenes (20.88%) and with a small amount of sesquiterpene hydrocarbons (1.02%).

The essential oil efficiency of *T. walteri* species was obtained as 0.2% v/w. As shown in (Table 2), analysis of the essential oil of *T. walteri* resulted in the identification of 43 compounds, representing 92.84% of the total oil. This essential oil was found to contain oxygenated monoterpenes (56.08%) and monoterpene hydrocarbons (30.40%), oxygenated sesquiterpenes (3.19%) and sesquiterpene hydrocarbons (0.47%). The major constituents of *T. walteri* oil were α-pinene (22.37%), 1,8-cineole (12.52%), camphor (11.91%) and ß – thujone (4.52%).

Table 2. Chemical composition of essential oils of two Tanacetum species cultivated from Iran.

No	Compound	KI[a]	*T. walteri*	*T. turcomanicum*
1	*santolinatriene*	908	0.36	-
2	*2,5-diethenyl-2-methyltetrahydro furan*	916	-	0.67
3	*tricyclene*	926	0.1	0.1
4	*artemisia triene*	929	-	0.63
5	*α-thujene*	931	0.25	0.09
6	*α-pinene*	939	22.37	2.89
7	*camphene*	953	1.48	1.49
8	*verbenene*	967	0.17	0.83
9	*sabinene*	976	1.36	0.11
10	*-pineneβ*	980	1.02	0.59
11	*6-methyl-5-hepten-2-one*	985	0.13	-
12	*1,5,8-p-menthatriene*	998	-	2.42
13	*α-phellandrene*	1005	0.14	2.33
14	*α-terpinene*	1018	0.64	2.58
15	*P – cymene*	1026	0.83	10.22
16	*1,8-cineole*	1033	12.52	15.63
17	*γ-terpinene*	1062	1.02	1.56
18	*cis-sabinenehydrat*	1068	0.10	-
19	*α-terpinolene*	1088	0.56	1.78
20	*filifolone*	1095	-	0.91
21	*linalool*	1098	2.00	0.33
22	*-thujoneα*	1102	2.97	-
23	*isopentylIsovalerate*	1103	-	0.15
24	*-thujoneβ*	1114	4.52	-
25	*α-campholenal*	1125	2.51	0.34
26	*4-acetyl-1-methylcyclohexene*	1131	2.67	0.37
27	*camphor*	1143	11.91	10.14
28	*pinocarvon*	1162	2.36	0.15

29	*borneol*	1165	3.27	4.95
30	*terpin–4-ol*	1177	3.68	0.87
31	*P- cymen-8-ol*	1183	0.21	-
32	*α-terpineol*	1189	1.28	0.9
33	*myrtenal*	1193	0.33	-
34	*myrtenol*	1194	0.44	-
35	*verbenone*	1204	0.43	0.43
36	*trans-carveol*	1217	0.67	-
37	*nordavanone*	1229	-	0.44
38	*chrysanthenyl acetate*	1235	3.58	0.58
39	*geraniol*	1255	2.47	-
40	*isobronyl acetate*	1285	-	0.26
41	*sabinyl acetate*	1291	0.37	-
42	*eugenol*	1356	-	0.14
43	*isoledene*	1373	-	0.18
44	*geranyl acetate*	1383	0.19	1.91
45	*cis-jasmone*	1388	-	0.62
46	*-elemeneβ*	1391	0.09	-
47	*cyperene*	1398	0.30	-
48	*davana furan*	1414	-	0.09
49	*trans-caryophyllene*	1418	0.08	-
50	*davana ether*	1474	-	0.20
51	*viridiflorene*	1493	-	0.47
52	*epizonaren*	1497	-	0.16
53	*delta-cadinene*	1524	-	0.37
54	*spathulenol*	1576	0.73	-
55	*caryophyllene oxide*	1581	0.48	-
56	*davanone*	1586	0.50	20.79
57	*-bisabololα*	1683	0.57	-
58	*frnesyl acetate*	1817	0.91	-
	oxygenated monoterpenes		56.08	38.75
	monoterpene hydrocarbons		30.4	25.85
	oxygenated sesquiterpenes		3.19	20.88
	sesquiterpenes		0.47	1.02
	total		92.84	89.27

[a]Kovats index calculated on the TRB-5MS column relative to C3–C19 n–alkanes.

3.2. Antimicrobial activity

Numerous microorganisms cause damage to human health due to inappropriate use of antibiotics. Thus, there is a need to discover new substances from natural sources. In the present study, the antibacterial activity of the essential oils obtained from *T. turcumanicum and T. walteri* were assessed using the agar well diffusion method by measuring the zone width of growth inhibition. The results are shown in Table 3.

The results of this study showed that the essential oils of *T. turcumanicum* and *T. walteri* had antimicrobial activity against the selected bacteria and the activity order was obtained as: *E. coli*< *S. aureus*,< *B. atrophaeus*< *M. luteus*. No inhibitory activity was observed against *E. coli* for essential oil of *T. turcomanicum*. This essential oil showed better activity against Gram-positive than Gram-negative bacteria. The lipopolysaccharide outer membrane of Gram-negative bacteria may be responsible for resistance to antibacterial materials (Amarowicz *et al.*, 2003).

The results of the present study differ from previous reports. In previous studies, the leaves of *T. turcomanicum* were examined. In this study, trans-chrystanthenyl acetate (19.2%), trans-thujone (13.5%), chrysanthenone (11.2%), and camphor (7.3%) were observed as major constituents (Habibi *et al.*, 2009). Ghaderi and Sonboli (2018) analyzed the essential oil of the aerial parts of *T. walteri* and 43 compounds were identified, of which the main oil constituents were thymol (22.50%), 1,8- cineole (8.20%), umbelloulone (6.9%), α-bisabolol (6.3%) and camphor (5.3%). In the present study, the highest inhibition zone was observed against *S. aureus*. This difference in the amount of chemical compounds may be attributed to the genotype and various environmental conditions. It is well known that the biological activity of essential oils depends on their chemical composition, and the yield components of plants are determined by a series of factors, including plant genetic, climate, edaphic, elevation, and topography and also, an interaction of various factors (Golparvar and Hadipanah, 2016; Ardalani *et al.*, 2017).

It has shown that 1,8- cineol and camphor are the most important antimicrobial compounds separated from various plant species (Kazemi *et al.*,2011). Also, α-pinene is one of the main components of essential oil of *T. walteri* with antimicrobial properties (Burt, 2004). Davanone, camphor and thujone have been reported for their antibacterial activity against several bacterial strains(Juteau *et al.*, 2002; Delamare *et al.*, 2007; Lopes *et al.*, 2008). Moreover, the essential oils of *T. turcumanicum and T. walteri* are composed of various components with small amount which each of the components have different antimicrobial effects. For example, oxygenated monoterpenes such as,

camphene and borneol, which were detected in the essential oils of plants, have been demonstrated to exhibit antibacterial activity (Amiri, 2007).

Antibacterial effect of essential oils containing terpinene-4-ol has been reported previously (kordali *et al.*, 2005). Considering the fact that camphor and 1, 8-cineol majorly contribute to the essential oils, it seems that the inhibitory and lethal effect of the essential oil of this herb on tested bacteria is mostly due to the existence of these components. However, synergistic effects and negative interactions of other components of the essential oil in the incidence of antimicrobial properties should not be ignored because the essential oil is a mixture of different chemical components. For example, p-cymene has weak antimicrobial properties, but based on literature reports, it can be said that p-cymene enhances the activity of other antimicrobial agents through synergism, antagonism and additive effects (Marchese, 2017).

Table 3. The average diameter of the halo against the various concentrations of the essential oil (mm)

Type of bacteria	Different concentrations of essential oil										Gentamicyn (10µg)	DMSO
	T.turcomanicum					*T. walteri*						
	1:2	1:4	1:8	1:16	1:32	1:2	1:4	1:8	1:16	1:32		
M. luteus	12.5	14	16.2	17	18	14	15	17.6	19	21	26	-
B. atrophaeus	11	13.2	14	16	17	12	14	16.8	19.3	21	25	-
S. aureus	-	12	12.5	14	16	-	-	13	15	17	18	-
E.coli	-	-	-	-	-	-	-	-	10	11	20	-

4. Conclusion

The results of this study showed that environmental influences have effect on the amount and type of volatile components of essential oil of *T. turcumanicum and T. walteri.* α-pinene, 1,8-cineole, camphor and ß – thujone were obtained as the major constituents of essential oil of *T.walteri,* and davanone, 1,8-cineole, p-cymene, camphor and borneol for *T. turcumanicum.* Also, the results of the antimicrobial tests showed that both essential oils showed good activity against *M. luteus, B. atrophaeus* and *S. aureus.* This inhibitory power was observed more in essential oil of *T. walteri*. This essential oil showed weak activity against *E. coli*, while the essential oil of *T. turcumanicum* did not show significant activity against *E. coli*. The results of this study showed that the gram-positive bacteria were more sensitive than gram- negative. Due to the lipopolysaccharide outer membrane of gram-negative bacterium, it can be concluded that these bacteria are less sensitive to the antibacterial effects of essential oils.

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