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RESEARCH ARTICLE

Chemical composition and antibacterial activity of essential oils of *Tripleurospermum disciforme* in three developmental stages

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Abstract

Context: *Tripleurospermum disciforme* (C.A. Mey) Schultz Bip. (Asteraceae) is a widespread biennial species which also has traditional medicinal uses. According to the few recent studies, essential oils of this species exhibit anti-inflammatory, antispasmodic, antiseptic, antifungal, antiulcer, and antioxidant activity.

Objective: The chemical compositions of the hydrodistilled oils of *T. disciforme* of Iranian origin are studied in the stages of prior to flowering, flowering, and post flowering, for the first time. Also, we investigated the antibacterial activities of the oils.

Materials and methods: The essential oils of air-dried *T. disciforme* were obtained by hydrodistillation in three different developmental stages and analyzed by gas chromatography-mass spectrometry (GC-MS). The antimicrobial activity of the isolated essential oil, in the three stages, was also investigated against four Gram-positive and four Gram-negative bacteria.

Results: Twenty-one components were identified in the essential oils of *T. disciforme,* and the highest amount of oil was extracted at the flowering stage. The main component of the species in the flowering stage was β-farnesene (22.46%) and the other major components were β-sesquiphellandrene (17.85%), *p*-methoxy-β-cyclopropylstyrene (16.64%), heptadecane (10.6%), *p*-methoxy-humulene oxide (6.88%) and benzene acetaldehyde (9.3%). The MIC of essential oil was evaluated from 4 μL ml^{-1} against *Staphylococcus subtilis* and *Bacillus cereus* to 22 μL ml^{-1} against *Citrobacter amalonaticus.*

Discussion and conclusion: This study demonstrates the occurrence of β-farnesene/β-sesquiphellandrene chemotype of *T. disciforme* in western regions of Iran that are different from previous reports. The findings also showed that the essential oils *T. disciforme* have excellent antibacterial activities and thus have great potentiality to be used as a resource for natural health products.

Keywords: *Antibacterial activity; Asteraceae; chemical composition; essential oils;* Tripleurospermum disciforme

Introduction

Tripleurospermum disciforme (C.A. Mey) Schultz Bip. (Asteraceae) is a widespread biennial species which can be found almost everywhere in farmland and gardens as a weed (Javidnia et al., 2008). According to recent studies, essential oils of this species exhibit anti-inflammatory (Hosseini et al., 2007), antispasmodic, and antiseptic (Grainger & Wichtl, 2004; Ghassemi-Dekhordi, et al., 2003), antifungal (Amin et al., 2004), antiulcer (Minaiyan et al., 2006) and antioxidant (Souri et al., 2005) activities.

So far, there has been very little research into the chemical composition of essential oils of *Tripleurospermum*

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disciforme (Jassbi & Rustaiyan, 1996; Javidnia, et al., 2008; Alipoor & Sefidkon, 2004). Fourteen compounds (representing 94.93% of the oil) were reported in the oil of *T. disciforme,* the main ones were *trans*-matricaria ester (39.93%) *cis*-calamenene (22.99%), (*Z*)-β-farnesene (12.54%), β-maaliene (7.98%) and β-sesquiphellandrene (2.22%) (Alipoor & Sefidkon, 2004). Javidnia et al. (2008) have showed that sesquiterpenes were the main components (57%) of the essential oil of *T. disciforme*, and among these, sesquiterpene hydrocarbons are the major compound (45.5%). The major compounds of this oil are *ρ*-methoxy-β-cyclopropylstyrene (18.8%), (*E*)-β-farnesene (15.6%), and β-sesquiphellandrene (15.4%) (Javidnia et al., 2008). *p*-Methoxy-β-cyclopropylstyrene and (*E*)-β-farnesene were reported in the extracts of *Tripleurospermum callosum* but in amounts less than 1% (Yasar et al., 2005).

The few studies mentioned above display the different oil chemotypes, which strongly correlate with a different geographical origin, the plant material, the vegetative period, and method used for isolating the essential oils. In this research work, we propose to study the chemical composition of the hydrodistilled oils of *T. disciforme* of Iranian origin that were collected in three stages, before flowering, at flowering and after flowering stages, and then compare the results with the previous reports in area. The behavior of antibacterial activities of essential oils prepared from the species is studied against some bacterial strains. According to our bibliographical studies, there is no research on antibacterial effects of essential oils of *T. disciforme.*

Material and methods

Plant materials

The aerial parts of *Tripleurospermum disciforme* were collected from Alvand Mountain in Hamedan (west of Iran) in July 2007 at three stages including prior to flowering, flowering stage, and after flowering, and shade-dried. A voucher specimen of the species is deposited in the local herbarium of the Department of Biology, Faculty of Science, Bu-Ali Sina University, Iran. The location information is recorded as: *Tripleurospermum disciforme* (C.A. Mey) Schultz Bip. Iran, Hamedan, Road of Razan to Avaj, Amirabad village, 1700 m, *Chehregani & Hajisadeghian*, 24.06.2007 (BASUH, 17673), n = x= 9, $2n = 2x = 18$.

Isolation procedure

The dried plant samples (500 g) were subjected to direct hydrodistillation (plant material in boiling water) using a Clevenger apparatus (Faraz Teb Tajhiz, tehran, Iran) for 4 h. The oils were dried over anhydrous Na_2SO_4. Hydrodistillation of *T. disciforme* yielded 0.74, 0.92, and 0.86% (v/w) of essential oils in the above mentioned stages, respectively. The yields were based on dry mass of the plant samples.

Identification of components

Essential oil components were identified by GC-MS via peak matching and by utilizing their retention indices on an Innowax FSC column (Shimadzu, Kyotone, Japan). *n*-Alkanes (C9–C20) were used as reference points in the calculation of retention indices (RRI) (Curves et al., 1985). Computer matching against commercial libraries (Wiley and Mass Finder Version 2.1) (Adams, 1995; McLafferty & Stauffer, 1989; Joulain et al., 2001), Baser Library of Essential Oil Constituents, which was built from genuine compounds and components of known oils, and the reported MS literature library data (Joulain & Koenig, 1998; Jennings & Shibamoto, 1980) were utilized in the final characterization of oil components.

Test organisms

The standard strains of the following microorganism were used as test organisms: *Enterobacter aerugenes* (PTCC 10009), *Serratia marcescens* (PTCC 1330), *Proteus vulgaris (*Lio), *Citrobacter amalonaticus* (Lio), *Bacillus cereus* (ATCC 7064), *Bacillus megaterium* (PTCC 1672), *Staphylococcus subrogation* (Lio), and *Staphylococcus aureus* (ATCC 6633). We provided some of these microorganisms from the Persian Type Culture Collection (PTCC), Tehran, Iran and the others are locally isolated (Lio). To be used in experiments, the organisms were sub-cultured in nutrient broth and nutrient agar (Oxoid, Cambridge, UK) while diagnostic sensitivity test agar (DST) (Oxoid) was used for antibiotic sensitivity testing.

Sensitivity testing

For bioassays, suspension of approximately 1.5×10^8 cells per mL in sterile normal saline was prepared as described by Forbes et al. (1990). The sensitivity testing was determined using the agar-well diffusion method (Russel & Furr, 1977; Irobi et al., 1996). In each disk of 30 µL, essential oil were loaded. The bacterial isolates were first grown in nutrient broth for 18 h before use. The inoculum suspensions were standardized and then tested against the effect of the essential oils at amounts of 30 µL for each disk in DST medium. The plates were later incubated at 37°C ± 0.5°C for 24 h after which they were observed for zones of inhibition. The effects were compared with that of the standard antibiotic chloramphenicol at a concentration of 1 mg/mL (Khan & Omosto, 2003). The minimum inhibitory concentration (MIC) of essential oils was also determined by tube dilution techniques in Mueller-Hinton broth (Merck) according to NCCLS (2008).

Results

The essential oil yielded (v/w, on dried mass basis) from *T. disciforme* were 0.74, 0.92 and 0.86% in the stages of pre-flowering, flowering, and post-flowering, respectively. The results obtained by the analysis of essential oils are present in Table 1. Results showed that in the oil of *T. disciforme,* 21 compounds were identified, at all the above mentioned stages. But results indicated that there are some differences between the stages. In the stage of flowering, β-farnesene appears at the highest percentage (22.46%) and together with β-sesquiphellandrene (17.85%). The presence of *p*-methoxy-β-cyclopropylstyrene (16.64%), heptadecane (10.60%), *p*-methoxy-humulene oxide (6.88%) and benzene acetaldehyde (9.30%) is also important for the oil profile in the flowering stage.

Based on our results the amounts of β-farnesene, β-sesquiphellandrene, *p*-methoxy-β-cyclopropylstyrene, heptadecane and benzene acetaldehyde were the highest during flowering time and they were reduced after flowering and during the on-coming seed maturation (Table 1). In conclusion, this study demonstrates the occurrence of β-farnesene/β-sesquiphellandrene of *T. disciforme* in western region of Iran.

Antibacterial activities of the oils were studied against eight bacterial strains (Table 2). The oils inhibited the growth of bacterial strains producing a zone diameter of inhibition from 12 to 28 mm, depending on susceptibility of the tested bacteria. In the flowering stage, inhibition zones against some bacterial strains (*Citrobacter amalonaticus, Staphylococcus aureus, Bacillus megaterium* and *Bacillus cereus*) were greater even than those of chloramphenicol, and showed wide inhibition zones at a very low concentration.

The minimum inhibitory concentration (MIC) of the oils was also determined using the method of NCCLS (2008). Results showed that the minimum inhibitory concentration (MIC) of plant oils against the tested organism was varied between 4 and 22 μL mL^{-1}. The standard chloramphenicol had MIC values varying between 1 μg mL^{-1} and 8 μg mL^{-1}. The results indicated that standard antibiotic chloramphenicol had the nearest activity to plant oil against some bacterial strains. The lowest MIC (4 μL mL^{-1}) was detected for *T. disciforme* against *Staphylococcus aureus* and *Bacillus cereus.*

Discussion

The result of this research work showed that the essential oils yielded (v/w, on dried mass basis) of *T. disciforme* were 0.74, 0.92 and 0.86% in the pre-flowering, flowering and post-flowering stages, respectively; similar to that published in Javidnia et al. (2008), who reported 0.81% yield, and very different from a report of Alipoor and Sefidkon (2004), who reported 0.45% yield. The results of the analysis of essential oils showed the presence of 21 compounds in the essential oil of *T. disciforme*. The amounts of most compounds were evaluated at the highest in the flowering stage. For example, β-farnesene

Table 1. Chemical composition of aerial parts of *Tripleurospermum disciforme* prepared in three developmental stages. Amounts were expressed as percentage of dry weight.

	Compound	Prior to flowering	During flowering	After flowering
1	Alloaromadendrene	0.43	0.6	0.75
2	Pentadecane	0.75	0.45	0.5
3	Cycloisolongifolene	0.5	0.62	0.4
4	β-Ylangene	0.72	0.92	0.56
5	δ-Cadinene-δ-guaiene	2.2	1.74	1.5
6	β-Farnesene	19.24	**22.46**	18.3
7	Dodecane	0.2	1.1	1.96
9	Unknown	1.35	0.68	0.56
10	β-Caryophyllene	1.65	0.5	0.38
11	Unknown	2.13	1.94	1.65
12	Benzene acetaldehyde	6.5	9.3	7.7
13	Spathulenol	0.93	1.35	1.48
14	Unknown	1.45	Unknown	1.26
15	Caryophyllene oxide	0.92	1.36	1.14
16	p-methoxy-Humulene oxide	8.5	6.88	4.75
17	Unknown	0.49	0.1	0.4
18	β-Eudesmol	0.81	1.25	2.86
19	Heptadecane	9.8	10.6	9.65
20	β-Sesquiphellandrene	15.4	**17.85**	12.78
21	p-methoxy-β-Cyclopropylstyrene	16.94	16.64	13.85
	Total	90.91	97.74	82.43

Table 2. Antimicrobial activity of the essential oil of *T. disciforme* that was expressed as diameter of inhibition zone (mm).

					MIC	
Bacterial strains	Prior to flowering	Flowering	After flowering	Chloramphenicol	EO[a] (μL/mL)	STD[b] (μg/mL)
Citrobacteramalonaticus	12±4	16.5±4	13±4	13±4	22	8
Proteusvulgaris	14±3	18±3	15±3	35±8	20	4
Serratiamarcescens	14±6	17±3	16±4	22±5	20	4
Enterobacteraerogenes	18±4	22±4	18±6	22±3	8	4
Staphylococcusaureus	22±5	**28±6**	25±7	25±3.5	4	4
Staphylococcussubtilis	12±2	15±4	14±2	25±4	20	4
Bacillusmegaterium	18±4	22±6	19±3	18±2.5	8	1
Bacillus cereus	21±4	25±2	25±5	17±3	4	4

[a]Essential oil.
[b]Chloramphenicol standard.
Each datum represents the means ± SE of 4-5 samples.

(22.46%) and β-sesquiphellandrene (17.85%) were determined to be present at the highest percentage. The presence values of *p*-methoxy-β-cyclopropylstyrene (16.64%), heptadecane (10.6%), *p*-methoxy-humulene oxide (6.88%) and benzene acetaldehyde (9.3%) is also important for the oil profile in the flowering stage.

Our results are different from prior reports. Data reported by Alipoor and Sefidkon (2004) demonstrated that the main compounds of essential oils are *trans*-matricaria ester (39.93%) *cis*-calamenene (22.99%), (*Z*)-β-farnesene (12.54%), β-maaliene (7.98%) and β-sesquiphellandrene (2.22%). In an another study, the major compounds of the oil were also reported as *p*-methoxy-β-cyclopropylstyrene (18.8%), (*E*)-β-farnesene (15.6%), and β-sesquiphellandrene (15.4%) (Javidnia et al., 2008). *p*-Methoxy-β-cyclopropylstyrene and (*E*)-β-farnesene were also reported in the extracts of *Tripleurospermum callosum* Boiss. & Herldr., but in amounts less than 1% (Yasar et al., 2005).

Our results showed that the amounts of most compounds were the highest in the flowering stage. The amounts of a few chemicals were decreased in the flowering stage in comparison to pre-flowering stage. They include pentadecane, *p*-methoxy-humulene oxide and *p*-methoxy-β-cyclopropylstyrene (Table 1). In the post-flowering stage the amounts of most compounds were decreased in the essential oils. However, β-farnesene (18.30%) and *p*-methoxy-β-cyclopropylstyrene (13.85%) were the prominent chemicals in this stage.

In conclusion, this study demonstrates the occurrence of β-farnesene/β-sesquiphellandrene chemotype of *T. disciforme* in the western region of Iran and also this is the first report about the chemical composition of essential oils of *T. disciforme* in three different developmental stages. Since *T. disciforme* is a medicinal plant (Grainger & Wichtl, 2004; Ghassemi-Dekhordi, et al., 2003; Amin et al., 2006; Minaiyan et al., 2006; Souri et al., 2005), this research suggests harvesting in this stage because the essential oil yield and the amounts of chemicals in the flowering stage are greater than the two other stages.

Antibacterial tests showed good antibacterial activities of the oils (Table 2). In the flowering stage, inhibition zones were greater against some bacterial strains (*Citrobacter amalonaticus, Staphylococcus aureus, Bacillus megaterium* and *Bacillus cereus*) even than those of chloramphenicol, and showed wide inhibition zones at a very low concentration. On the other hand, it can be seen from Table 2 that the oil of flowering stage was more effective against all the studied bacterial strains than oils prepared from the two other stages. This means that the reason for higher antibacterial effect is the presence of β-farnesene, β-sesquiphellandrene, benzene acetaldehyde and heptadecane in the flowering stage with a higher percentage than at the two other stages. Although there are some reports about anti-inflammatory, antispasmodic, antiseptic (Grainger & Wichtl, 2004; Ghassemi-Dekhordi, et al., 2003), antifungal (Amin et al., 2002), antiulcer (Minaiyan et al., 2006) and antioxidant activity (Souri et al., 2005) of essential oils of *T. disciforme*, our bibliographical studies indicated that our work is the first research on its antibacterial activity. In conclusion, the findings demonstrated that the essential oils *T. disciforme* have excellent antibacterial activities and thus have great potentiality to be used as a resource for natural health products. The results also indicated that the oil of flowering stage has more prominent effects than the oil of other developmental stages.

Declaration of interest

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