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Chemical Composition and Antimicrobial Activity of Feverfew (*Tanacetum parthenium*) Essential Oil

ZAHRA IZADI, MAHMOOD ESNA-ASHARI[1]†, KHOSRO PIRI‡ AND POORANDOKHT DAVOODI¶
Department of Agronomy, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran
†Department of Horticultural Sciences, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran
‡Department of Biotechnology, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran
¶Department of Oral medicine, Dental Faculty, Medical University, Hamedan, Iran
[1]Corresponding author's e-mail**:** m.esnaashari@basu.ac.ir

ABSTRACT

Essential oils from the whole aerial parts as well as stem/leaf, inflorescence and unripe and ripe seeds were isolated through hydro-distillation from aerial parts of feverfew (*Tanacetum parthenium* L.) plants were collected from Hamedan and Tehran regions at the vegetative, flowering and seeding stages. The amount of essential oil obtained from the above parts of the Hamedan plant samples were 3.80%, 6.01%, 3.61%, 0.49% and 0.31% (w/w) and from those collected from Tehran were 4.96%, 6.94%, 3.39%, 0.96% and 0.87% (w/w), respectively. Analysis of extracted oil by GC and GC/MS showed that camphor was the major constituent in total oils (10.3%–53.3%) followed by chrysanthenyl acetate (4.3%–22.5%) and camphene (4.1%–10.4%). However, bornyl acetate, α-pinene and *p*-cymene were found in the plant samples from Hamedan only. The antimicrobial activity of the oils was determined using the disk diffusion method against Gram positive bacteria (*Bacillus subtilis*, *B. cereus*, *Micrococcus luteus* & *Staphylococcus aureus*), Gram negative bacteria (*Yersinia enterocolitica*, *Klebsiella oxytoca*, *Serratia marcescens*, *Escherichia coli* & *Pseudomonas aeruginosa*) and yeast (*Candida albicans*). Results showed a significant difference between Gram positive and Gram negative bacteria in their susceptibility to the oil, so that Gram positive bacteria were more susceptible to the antimicrobial activity of feverfew oil. In addition, the oil extracted from Hamedan samples showed more antimicrobial activity compared to those from Tehran.

Key Words: Feverfew; Antimicrobial activity; Essential oils; GC-MS

INTRODUCTION

Increasing the number of antibiotic resistant bacteria has led to a demand for new agents that could be used to decrease the prevalence of bacterial diseases (Lis-Balchin & Deans, 1997). Available evidences show that essential oils extracted from plants could be employed as antimicrobial agents in food systems (Sefidkon & Ahmadi, 2000). Recently, screening for new plants with antibacterial activity has been the subject of many investigations since their essential oils with antibacterial activity could be the promising agents for this purpose (Dorman & Deans, 2000; Imelouane *et al*., 2009). Feverfew (*Tanacetum parthenium* L.) is a perennial herbaceous essential oil bearing plant belongs to Asteraceae family. The species of genus *Tanacetum* have been used as medicinal plants for over 2000 years (Omidbeigi, 2007). Interest in the genus has been stimulated by its biological activities, particularly as insect antifeedants, antitumor and antimicrobial activities due to its sesquiterpenoid constituents (Burt, 2004). This genus has been found in different regions of many countries including Iran, Anatolia, Jordan, Iraq, Turkey, Afghanistan and Pakistan (Awang, 2000).

Feverfew, is an aromatic plant with about 65 cm height, white inflorescence and achene fruit that grows in stony slopes and river beds (Mozaffarian, 1996; Rechinger, 2002). *Tanacetum* species contain sesquiterpenoids and flavonoids mainly, whereas the other terpenoids and phenolic compounds are rarely found in these plants (Bernath, 2000). Sesquiterpenoids as the main constituents of the genus, supposed to be the bioactive principles of plants. Flavonoids and essential oils are also pointed out as active substances in some species. Oil composition of *T. parthenium*, *T. argyrophyllum*, *T. aucheranum* and *T. chiliophyllum* has previously been reported (Gören *et al*., 2002; Akpulat *et al*., 2005; Omidbaigi, 2007; Salamci *et al*., 2007; Tabanca *et al*., 2007; Askari, 2008). However, to the best of our knowledge no biological assays of feverfew have so far been performed.

Composition of the oils extracted from the aerial parts of *T. argenteum* has also been reported by Kalodera *et al.* (2000) from two regions near Wurzburg in Germany and Riva Del Garda in Italy. The main component of plant oils collected from both regions has been *cis*-thujone with the

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amounts of 69.9% and 37.3%, respectively. The German plant samples have also shown to have *trans*-thujone (5.6%) camphor (6.5%) and 1, 8-cineole (3.2%); while those from Italy contained α-Pinene (29%), (*E*)-sesquilavandulol (16%) and camphor (14%).

The aim of this study was to determine the chemical compositions of essential oil extracted from different parts of feverfew plant and to investigate antimicrobial activities of the oil against some bacteria and fungi.

MATERIALS AND METHODS

Plant materials: Plant materials were collected from Hamedan in the west and Tehran in the center of Iran at the vegetative (begining of June), flowering (middle of June) and seeding stages (middle of July to late September 2009). Two kinds of seeds including unripe and ripe seeds were considered. The unripe seeds were collected when the plant inflorescence was formed and the ripe seeds were harvested when their color changed into light brown. All samples were dried in room temperature.

Oil extraction: Essential oils of different plant samples and seeds were separately extracted through hydro-distillation method. To do that, dried samples were crushed to small particles using an electric blender. They were then hydrodistilled for 2 to 2.5 h in a Clevenger type apparatus to obtain their oils. Three distillations were performed and the oils were dehydrated over anhydrous sodium sulfate and stored in sealed vials at 4°C before analysis.

GC analysis: The oils were analyzed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m×0.25 mm with 0.25 m film thickness), J and W Scientific Corporation. Oven temperature was 40°C for 5 min and then set to 260°C for a rate of 4°C min^{-1}. The injector and detector (FID) temperature were 270°C and helium was used as carrier gas with a linear velocity of 32 cm s^{-1}. The percentages were calculated using the area normalization method without the use of response factor correction. The retention indices were worked out for all compounds using a homologous series of n-alkanes.

GC/MAS analysis: GC/MS analyses were carried out by the use of a Varian 3400 GC/MS system equipped with the same fused silica column as above. Oven temperature was 50°-260°C at a rate of 4°C min^{-1} and the transfer line temperature set to 270°C. The other components of GC/MS system included helium as carrier gas with a linear velocity of 31.5 cm s^{-1}, split ratio 1/60, ionization energy 70 ev, scan time one sec., and mass range 40-300 amu.

Identification of compounds: The oil constituents were identified through comparison of their mass spectra with those in a computer library (LIBR-TR and Wiley-5 lib.) or with authentic compounds (Adams, 1995).

Antibacterial analysis: The feverfew, essential oil antimicrobial activity was determined against five Gram negative bacteria, four Gram positive bacteria and yeast. Microorganisms included *Bacillus cereus* (PTCC 1247), *B. subtilis* (PTCC 1023), *Micrococcus luteus* (PTCC 1169), *Staphylococcus aureus* (PTCC 1431), *Yersinia enterocolitica* (PTCC 1151), *Pseudomonas aeruginosa* (PTCC 1430), *Escherichia coli* (PTCC 1399), *Klebsiella oxytoca* (PTCC 1402), *Serratia marcescens* (PTCC 1187) and *C. albicans* (5027). These were obtained from the microbial collection of the Department of Biotechnology, Iran Research Organization of Science and Technology (IROST), Tehran, Iran.

The essential oil antibacterial activity was determined using disk diffusion method (European Pharmacopia, 1996) and the bacteria were cultured on Triptic Soy Agar medium (Merck, Germany). The bacteria were also suspended in a Tryptocase Soy Broth medium (Merck, Germany). With reference to the value 1 MacFarland standard, 0.5 mL standardized inocula was placed on the surface of the media and distributed uniformly. Oils were diluted with ethanol (1:5). Sterile paper disks (6 mm in diameter, prepared from Whatmann No. 42) were impregnated with 20 µL of essential oil already diluted with ethanol (1:5) and placed on the surface of each inoculated plate and incubated at 37ºC for 24 h. Tetracycline (30 µg) and gentamicin (10 µg) disks were used to compare antibacterial activity of essential oils. The zone of inhibition was measured 24 h after incubation.

RESULTS

Percentage of the oils extracted from the feverfew aerial parts (AP), stem/leaf (SL), inflorescence (IF), unripe and ripe seeds (US & RS) of both samples are given in Table I. It was proved that the amount of essential oil in vegetative parts of the plants was more compared with the seeds. As shown in Table I, the essential oil yields in all plant parts (except IF) of the Tehran samples were higher than those collected from Hamedan. The amount of essential oil extracted from stem/leaf of feverfew, was considerably higher compared with the other parts of the plants.

The essential oil compositions of different parts of feverfew plants were analyzed by GC and GC/MS (Table II). The number of compounds identified in AP, SL, IF, US and RS of Hamedan samples were 19, 20, 21, 15 and 20, respectively while they shown to be 17, 17, 18, 11 and 17 in Tehran samples. Three constituents including camphor, chrysanthenyl acetate and camphene were common in all the oils as three major compounds. Camphor was the major oil constituent in AP (53.39 & 52.98%), SL (47.90 & 49.64%), IF (11.61 & 11.52%), US (12.30 & 12.41%) and RS (11.40 & 10.35%) of the Hamedan and Tehran samples, respectively. Chrysanthenyl acetate and camphene were the major constituents in the aerial parts and stem plus leaf oils. Chrysanthenyl acetate in AP and SL oil was 22.54 and 21.15% in Hamedan and 21.12 and 22.28% in Tehran samples, while camphene of the same parts was 9.84 and 10.45% in Hamedan and 10.25 and 10.26% in Tehran

Table I: Percentage of volatile oils (w/w) in different parts of feverfew (*Tanacetum parthenium* L.) plants

Localities	AP	SL	IF	US	RS
Hamedan	3.80	6.01	3.61	0.49	0.31
Tehran	4.96	6.94	3.39	0.96	0.87

Table II: Percentage of volatile compounds identified in the essential oils of feverfew (*Tanacetum parthenium* L.) plants

Compounds		Hamedan sample					Tehran sample				
	RI[a]	AP[b]	SL[c]	IF[d]	US[e]	RS[f]	AP	SL	IF	US	RS
α-tujene	928	0.53	0.43	0.34	-	0.07	0.61	0.39	0.25	-	0.03
α-pinene	940	2.04	2.55	0.10	-	-	-	-	-	-	-
Camphene	955	9.84	10.45	5.11	6.45	4.45	10.25	10.26	5.46	5.38	4.16
Benzaldehyde	965	0.05	0.03	-	-	-	0.06	0.01	-	-	-
Sabinene	977	0.25	0.35	-	0.24	0.21	0.30	0.34	-	-	0.19
β-pinene	984	-	-	1.24	1.03	0.98	-	-	1.20	1.10	-
Myrcene	995	-	-	-	36.04	42.07	-	-	-	45.02	50.12
α-phellandrene	1004	-	0.27	0.20	0.18	0.21	0.02	0.48	0.29	0.16	0.29
α-terpinene	1021	0.16	0.15	0.58	0.48	-	0.23	0.19	0.68	-	0.02
p-cymene	1025	4.15	4.23	4.18	2.36	3.10	-	-	-	-	-
Limonene	1032	0.86	1.05	0.89	0.91	0.96	0.89	0.95	1.04	1.16	1.23
γ-terpinene	1061	-	-	0.23	-	-	-	-	0.34	-	-
Camphor	1143	53.39	47.90	11.61	12.30	11.40	52.98	49.64	11.52	12.41	10.35
Pinocarvone	1159	0.39	0.25	0.32	0.44	0.38	0.23	0.22	0.34	0.29	0.39
Borneol	1166	0.23	0.22	0.34	-	0.29	0.64	0.34	0.39	-	0.44
terpinene-4-ol	1177	0.42	0.68	-	-	-	0.51	0.75	-	-	-
α-terpineol	1193	0.14	0.12	0.15	-	-	0.13	0.16	0.22	-	-
Myrtenal	1196	-	0.09	0.16	0.10	0.20	0.01	0.16	0.19	0.18	0.21
chrysanthenyl acetate	1236	22.54	21.15	8.85	6.29	6.23	21.12	22.28	7.63	5.12	4.32
bornyl acetate	1283	1.70	2.05	0.48	0.08	0.36	-	-	-	-	-
Thymol	1294	-	-	-	0.08	0.02	-	-	-	0.04	0.05
Carvacrol	1303	0.17	-	0.10	-	0.08	-	-	0.13	-	0.12
β-caryophylene	1411	0.36	0.33	0.25	-	0.23	0.31	0.37	0.23	-	0.19
(E)-*β*-farnesene	1459	0.64	0.53	0.40	0.11	0.15	0.91	0.87	0.49	0.09	0. 13
valencene	1491	-	-	34.26	-	0.02	-	-	42.96	-	-
β-bisabolene	1511	0.20	0.15	0.08	-	0.02	0.33	0.13	0.18	-	0.08
Total identified		98.06	92.98	69.87	67.09	71.43	89.53	87.54	73.54	71.95	72.32

a: Retention Index, b: Aerial parts, c: Stems and leaves, d: inflorescence, e: Unripe seeds, f: Ripe seeds

Table III: Antimicrobial activity of feverfew (*Tanacetum parthenium* L.) essential oils obtained from Hamedan and Tehran plant samples*

Microorganism	Tehran sample					Hamedan sample						
	AP[a]	SL[b]	IF[c]	US[d]	RS[e]	AP	SL	IF	US	RS	TET[f]	GEN[g]
Pseudomonas aeruginosa	10	7	9.5	0	0	10.5	7.5	6.5	8.5	9.5	nt	14
Klebsiella oxytoca	0	0	0	0	0	0	0	0	0	0	nt	18.5
Serratia marcescens	0	0	0	0	0	0	0	0	0	0	nt	27.5
Escherichia coli	0	0	0	0	0	0	0	0	0	0	15	nt
Yersinia enterocolitica	7	8	0	10	10	0	11	0	0	0	nt	15
Bacillus cereus	19	17.5	12.5	12.5	10	32.5	27.5	25	27.5	18.5	36	nt
Micrococcus luteus	10	8	12.5	9	0	8.5	9.5	10	10.5	9.5	35	nt
Bacillus subtilis	0	0	0	0	0	0	9	10	0	8	22.5	nt
Staphylococcus aureus	12	8.5	11.5	8.5	3	18	13.5	11	10	11	30	nt
Candida albicans	14	13	12	15	8	15	10	0	0	0	nt	nt

*: The data show the diameter of inhibition zone growth in mm

a: Aerial parts, b: Stems and leaves, c: inflorescence, d: Unripe seeds, e: Ripe seeds, f: Tetracycline, g: Gentamicin

samples. Bornyl acetate, α-pinene and *p*-cymene were found in Hamedan samples only (Table II).

Feverfew essential oils affected on the growth of bacteria and *C. albicans* that potentially causes infection. Our data showed that there was no uniform response among tested bacteria. Significant difference was observed between Gram positive and Gram negative bacteria in terms of their susceptibility, so that Gram positive bacteria were more sensitive to antimicrobial activity of feverfew essential oil (Table III).

DISCUSSION

Iran is one of the richest countries of the world in terms of having a substantial number of different medicinal plants species grown in various ecological conditions (Zargari, 1999). Investigation of antibacterial properties of these plants has brought the opportunity of producing natural-based and environment friendly new drugs that could be replaced with the existing chemical ones to control bacterial infections without unpleasant side effects (Finnemore, 1926). There are many plant species that control different kinds of infections through various pathogen controlling mechanisms. These plants can easily reduce the growth of pathogens and therefore are essential to be more studied (Eloff, 1998).

In this study, the plants collected from both Hamedan and Tehran regions showed various amounts of essential oil ranging between 0.31 to 6.94% (W/W). The amount of essential oil extracted from stem/leaf of feverfew plants was considerably higher compared with the other parts of the plants. The oil yield of *T. argyrophyllum* aerial parts has been 3.3% (w/w) through steam distillation and 3.13-10.67% by supercritical extraction method (Askari & Mirza, 1998; Rodrigues *et al*., 2003). Comparison of the number and amount of essential oil chemical compositions in Hamedan and Tehran samples showed that the genetic constitution and environmental conditions could affect the yield and composition of volatile oil produced by feverfew plants. The influence of environmental and ecological conditions on plants is huge; so that similar plant species may show quite different pharmaceutical properties when grown in various ecological conditions. Difference between the populations of a single plant species grown in various ecological regions is a natural phenomenon (Agrawal, 2003). So that the number of 19, 20, 21, 15 and 20 chemical compositions were obtained from Hamedan samples, while they were 17, 17, 18, 11 and 17 in Tehran plants. Camphor, chrysanthenyl acetate and camphene were also the main constituents of essential oils. The highest amount of camphor were measured in AP, IF and RS of Hamedan samples. These samples also contained more chrysanthenyl acetate in AP, IF, Us and RS and camphene in SL, US and RS.

Some investigators have shown that the major constituents of the essential oils extracted from aerial parts of *T. parthenium* have been camphor (56.9%) followed by camphene (12.7%) and *p*-cymene (5.2%) (Akpulat *et al*., 2005). Camphor exists in the aerial parts of *T. aucheranum* (11.6%), *T. hiliophyllum* (28.1%), *T. argenteum* (14%) and *T. argyrophyllum* (22.3%) (Gören *et al*., 2002; Akpulat *et al*., 2005; Salamci *et al*., 2007; Tabanca *et al*., 2007; Omidbeigi, 2007; Askari, 2008).

Significant difference was observed between Gram positive and Gram negative bacteria in terms of their susceptibility, so that Gram positive bacteria were more sensitive to antimicrobial activity of feverfew essential oil. The higher sensitivity of Gram positive bacteria may be explained according to their cell wall structure. Most studies reporting the action of essential oils against food spoiling organisms and food borne pathogens agree that essential oils are relatively more active against Gram positive than Gram negative bacteria (Lambert *et al*., 2001). Deans and Ritchie (1987) and Imelouane *et al.* (2009) observed that the susceptibility of Gram positive and Gram negative bacteria to plant volatile oils had a little influence on growth inhibition. It was often reported that Gram negative bacteria were more resistant to the essential oils present in plants (Smith-Palmer *et al*., 1998; Mann *et al*., 2000). The cell wall structure of Gram negative bacteria is constituted essentially with Lipopolysaccharides (LPS). This constituent avoids the accumulation of the oils on the cell membrane (Bezić *et al*., 2003). In addition, differences in susceptibility among the microorganisms to the antimicrobial activity of essential oils may also be explained by inherited genes on plasmids (Dorman *et al*., 2000).

The antimicrobial activity of AP essential oil was more than that of other parts and the antimicrobial activity of samples collected from Hamedan was more compared with those from Tehran, possibly because of the high percentage of camphor. Camphor has been reported to have significant antimicrobial activity (Salamci *et al*., 2007).

In conclusion, essential oil of feverfew showed significant antimicrobial activity. camphor, chrysanthenyl acetate and camphene were common in all the oils as three major compounds. The results suggest that feverfew essential oils possess some compounds with antimicrobial properties, which can be used as antimicrobial agents in new drugs for treatment of infectious diseases. Moreover, the findings of this study demand further research on the evaluation of antimicrobial properties of several phytochemicals and in particular camphor.

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