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# Determining Chemical Composition and Antimicrobial Activity of Feverfew (Tanacetum parthenium L.) Essential Oil on Some Microbial Strains

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Article information	Abstract		
Article history: Received: 22 May 2011 Accepted: 6 May 2012	<b>Background:</b> Feverfew ( <i>Tanacetum parthenium</i> L.) is a herbal plant that has anti- septic, anti-microbial, anti-parasitic and anti-inflammatory effects. The main objective of this study is to evaluate the antimicrobial effect of shoot essential oil (essential oil of the aerial		
Available online: 29 Oct 2012 ZJRMS 2013; 15(6): 8-13 Keywords:	parts of the plant) of the feverfew on a number of microorganisms including gram- negative and gram-positive bacteria, filamentous fungi and yeasts.		
Feverfew Essential oils	<i>Materials and Methods</i> : In this empirical study, plant samples were collected at the full blooming stage. Shoot essential oil was extracted using hydro-distillation technique and		
Microorganism	Clevenger apparatus. Components of the extract were identified using GC and GC/MS apparatus and its antimicrobial properties were evaluated using diffusion in Agar method		
*Corresponding author at: Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Tehran,	(disk diffusion) and dilution in the well (Micro-broth dilution). <b>Results:</b> Among 35 compounds identified in the essential oil of the feverfew, camphor (45%), chrysanthenyl acetate (21.5) and camphene (9.6%), were the main components respectively. Essential oil showed very good antifungal effect which was stronger than its		
Iran. E-mail: maghaalikhani@modares.ac.ir	antibacterial effect. Gram-negative bacteria were less sensitive to the essential oil than gram-positive bacteria. The mean diameter of inhibition zone, in the bio-assessment of the effect of feverfew essential oil on gram-positive bacteria and fungi was respectively more		
	than the effect of vancomycin and amphotericin B and this effect on gram-negative bacteria was less than the effect of gentamicin. This effect is attributed to the high value of camphor, chrysanthenyl acetate and camphene found in the essential oil.		

*Conclusion*: Feverfew essential oil could be utilized as a sound and harmless substitute for the antibiotics.

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#### Introduction

edicinal plants have been used for centuries to treat diseases. Although a large part of the medications are chemical drugs, but it was estimated that at least one third of all medicinal products have plant origin or they have been altered after extraction from the plant [1]. Thus, given the diversity of the climate and a very diverse flora in Iran, it is possible to identify effective substances of the plants in different native plants of the country and to extract these substances in order to produce these materials in large quantities and at the industrial level. Evaluation of these capabilities especially in the case of plants native to Iran which were less studied is of special importance [2].

Feverfew (*Tanacetum parthenium* L.) is a perennial herbaceous plant belonging to the Asteraceae family which has scattered crack and a short and direct root. It also has a straight stem with longitudinal grooves in brown-red color. Its height is 30-80 cm, depended on the climatic conditions. The leaves have a long petiole, divided and oval-shaped lamina. Flowers are in two genera and appear during the months of June to August. Flowers have white color and their diameter is 0.6-1.6 cm [3, 4]. This plant is native to Kazakhstan, Central Asia and the Mediterranean region [5, 6] and has a wide

distribution range in Europe, Asia and America [5]. It is distributed in various regions of North, West, East and center of Iran and is found in the provinces of Golestan, Mazandaran, Gilan, Azarbaijan, Tehran, Hamedan, Markazi and Yazd as a wild herb [1]. According to the conducted studies, the major constituent of the essential oil of feverfew is camphor [7]. The active ingredients of this herb which is effective on the prevention, reduction and treatment of the migraine headaches are created and stored in all aerial organs and vegetative body of the plant especially in leaves and flowers [8]. The active ingredient of this herb is also used extensively in pharmaceuticals, cosmetics and healthcare industries.

Reports indicate that many vegetable oils contain significant inhibitory effects on pathogenic microorganisms [9, 10]. So, today, given the increasing resistance of microorganisms to the antibiotics, the use of antimicrobial compounds found in the plants, as the natural factors having lethal and inhibitory effects on pathogens, is further considered. People also are more willing to take herbal medicines instead of synthetic (chemical) drugs.

The study conducted on the feverfew essential oil collected from natural habitats in Turkey, showed that the

oils extracted from this plant has 49% camphor, 22.1% chryzantenil acetate and 9.4% camphen [11]. Polatoglu et al. examined the antibacterial effect of the essential oil of this herb on gram-positive and gram-negative bacteria and showed that the oil will prevent the growth of aureus, staphylococcus and bacillus subtilis bacteria [11]. The purpose of this study is to evaluate the qualitative and quantitative characteristics of the feverfew essential oils collected from the natural habitats in Hamedan and the level of camphor and other components found in this essential oil and the antimicrobial effect against pathogenic microorganisms including bacteria, fungi and yeast during in vitro condition.

# **Materials and Methods**

This study was conducted in 2010 in Oral Diseases Department, School of Dentistry of Hamadan University of Medical Sciences. Shoot feverfew (aerial parts of the feverfew) were collected at the full blooming stage from its natural habitat near the city of Hamadan, Then it was dried in the shade at proper temperature.

For the essential oil extraction, plant materials were primarily milled, and then the essential oil of 100 grams of milled plant was extracted for 3 h in a Clevenger apparatus using hydro-distillation method and its moisture was absorbed by sodium sulfate [12].

A sample of the plant was sent to the Herbarium of the School of Pharmacy of Hamadan University of Medical Sciences and was confirmed with the scientific name of Tanacetum parthenium L. Before doing bio-assessments for determining antibacterial properties, gas chromatography devices and gas chromatograph connected to the mass spectrometer were used for isolating and identifying the essential oils compositions. The prepared samples were primarily injected into the gas chromatography and the most appropriate plan for column temperature for the complete separation of the oil composition was obtained. Percentage of the essential oil components were calculated for each sample.  $C_8$ - $C_{28}$ series of normal alkenes were injected into the device for the calculation of retention index (RI) components of the essential oil. Retention index of the components were calculated using a computer program. Then the essential oils were injected into the gas chromatography apparatus attached to the mass spectrograph and mass spectra of the compounds were obtained.

Ultimately, components of the essential oil were identified through comparing obtained mass spectra with the standard mass spectra existed in the Wiley 2000 electronic library of Labsolution software of the gas chromatography apparatus connected to the mass spectrometer; then the calculation of the standard retention index of  $C_8$ - $C_{28}$  alkanes series and their comparison with the standard numbers of references were performed and determined [13, 14].

In this study, the Shimadzu gas chromatograph and also 9A gas chromatograph quipped with a DB-5 column in 30 m length and 0.25 mm diameter with the thickness of 0.25 micrometers at the stationary phase were used, where the helium gas with a purity of 99.99 was used as the mobile

phase. Temperature program of the column started from 60°C and after 5 minutes stopping in this temperature, it gradually increased with the rate of 4 degrees and reached to the 210°C. The temperature of injection chamber was 300°C. FID was the detector used in the apparatus and its temperature was set at 270°C.

Gas chromatography apparatus connected to the Varian mass spectrometer, model 3400 was used in order to analyze and identify the composition of essential oil. Analysis conditions and specifications of this apparatus include: DB-5 column with the length of 30 m, diameter of 0.25 millimeters and thickness of 0.25 micrometers for the stationary phase. Thermal planning of the column was similar to the planning of column in the gas chromatography apparatus. Only the final column temperature was raised to 250°C.

Injection chamber temperature was set at10 degrees higher than the final temperature (260°C). Carrier gas was helium which was moving with the speed of 31.5 cm/s along the column. The scan time was one second, the ionization energy was 70 electron volts, and the mass area was from 40 to 340. Microorganisms studied in the bio-assessment of the antimicrobial effect of the essential oil of feverfew (Table 1) were gram (+) cocos, gram (+) and gram (-) bacills, fungi and yeast.

Bacterial strains were cultured overnight at 37°C in an appropriate medium (nutrient agar, blood agar). Fungi and veast were inoculated on Sabouraud dextrose agar at 30°C. Antimicrobial effect of the essential oil of feverfew were evaluated using diffusion in agar method (disk diffusion) and dilution in the wells (Microbroth dilution) [15]. In the diffusion in agar method, two or three colonies of bacteria culture were inoculated in the sterile saline. Under studied fungi and yeast were suspended from 24 and 72 h of culture on Sabouraud dextrose agar medium in RPMI 1640 (Sigma) which was buffered with 0.165 molar morpholin propane sulfonilic acid and turbidity of each suspension was regulated with half-McFarland standard. (Approximate number of bacteria was 1.5×108 m1/ CFU, and number of yeast and fungi was 1x105-106CFU/ml) using a sterile swab, from above prepared microbial suspension, on the Hinton medium agar (non-fastidious bacteria) and Todd Hewitt Agar enriched with 0.5% of the yeast extract and 0.1000% of Tween 20 (enriched THA (Streptococcus) and fungal and yeast suspension was cultured on Sabouraud dextrose agar. Sterile discs with 2.5, 5, 7.5 and 15 ml of the essential oil diluted in 10 ml of DMSO dimethyl sulfocside were added to the medium. Sterile disks were provided from Tehran Padtan Teb Co. The antibiotic disks and disk containing dimethyl DMSO were used as the positive and negative control. Bacterial cultures were incubated at 37°C for 24 hours and fungal culture was incubated at 30°C for 48 hours. Diameter of growth inhibition zone was calculated in millimeters. Discs of gentamicin, vancomycin and amphotericin B were used respectively in 10 gu, 30 gu and 100U doses. Above experiments were repeated three times for each strain in order to be sure of the results obtained for the considered essential oil.

In the method of dilution wells, the minimum inhibitory concentration (MIC) for the growth and the Minimum Bactericidal Concentration (MBC) of the essential oil on microorganisms were determined. Also, the essential oil was diluted in 10% DMSO dimethyl-sulphoxside, so that the essential oil concentration should be  $0.0125-8 \mu$ l/ml. RPMI 1640 (fungi and yeast), Muller Hinton Broth (nonfastidious bacteria) and enriched THB (Streptococcus) were used in the broth medium [18-20]. 100 micro liters of each dilution were added to the wells of the each of 96 plates. Concentration of microbial suspension was diluted so that the number of bacteria reached to 105-106 CFU/ml and the number of fungi and yeasts reached to 104-105. 100 micro liter of each organism was added to the each series of dilution; and bacterial and yeast plates were incubated at 37°C for 24 hours; and fungal plates were incubated for 48 hours at 30°C.

The minimum inhibitory concentration (MIC) for the growth and minimum bactericidal concentration (MBC) was determined. Statistical analysis of the experimental data was performed using SPSS-13 software and p<0.05 was considered as the minimum level of difference between the means.

#### Results

In this experiment, essential oil of the feverfew had 35 compounds which had formed 95.8 percent of the entire essential oil. Camphor (45%), chrysanthenyl acetate (21.5) and camphene (% 9.6) are the main components of the essential oil among identified compounds. Percentage of the oil was 4.5% (based on one hundred grams of dry weight). Three compounds of para-semen, alpha-pinene and burnyl acetate, with values of 4.15, 3.55 and 2.88 percent respectively, had significant values in comparison with other compounds (Table 2).

Results of the antimicrobial activity of the essential oil showed that in the agar diffusion method, the average mean diameter of inhibition zone (Means $\pm$ SD) of the essential oil on gram-positive bacteria (per mm) in a concentration of 5 ml is 0.36 $\pm$ 23.00 ml and of vancomycin is 22.40 $\pm$ 0.80 mm.

Gram-positive Staphylococcus aureus bacteria and Staphylococcus epidermidis are sensitive to the essential oil of feverfew (p=0.001) and are resistant against Bacillus subtilis (Table 3). The average mean growth inhibition of the essential oils of feverfew on gramnegative bacteria is less than gram-positive bacteria (19.30±0.92 versus 30.70±0.70 mm in 15 microliters concentration) (p=0.027). E. coli (25923), E. coli (157), S. flexneri and Klebsiella pneumonia are sensitive to the essential oil of this herb (p=0.016) and are resistant to the Salmonella typhimurium (p=0.041).

Based on the results of growth diameter of inhibition zone and minimum concentrations of growth, gramnegative bacteria are more resistant against essential oil of this herb than gram-positive bacteria (p=0.003). Also, the antifungal effect of the essential oil of feverfew is more than its antibacterial effects (p=0.001). Candida albicans yeast is more sensitive to the essential oil compared to the

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Aspergillus nijer and Candida cruise (p=0.035). MBC of candida cruise is several times the growth minimum inhibitory concentration.

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Microorganism	Code	Type of
	abbreviation	microorganism
Staphylococcus aureus	ATCC 25923	Gram positive cocci
Staphylococcus epidermidis	ATCC 14990	Gram positive cocci
Bacillus subtilis	ATCC 6051	Gram positive bacilli
E. coli	ATCC 25923	Gram negative bacilli
E. coli	ATCC 157	Gram negative bacilli
Klebsiella pneumonia	ATCC 10031	Gram negative bacilli
S. flexneri	ATCC 1234	Gram negative bacilli
Serratia marcescens	ATCC 1111	Gram negative bacilli
Salmonella typhimurium	ATCC 19430	Gram negative bacilli
Candida albicans	ATCC 5027	fungus
Aspergillus nijer	ATCC 16404	fungus
Candida cruise	Clinical isolates	yeast

**Table 2.** Chemical compositions and their values in the essential oil of feverfew (Tanacetum parthenium L).

Compounds	Retention	Amount(%)		
-	index			
tricyclene	919	0.50		
α-tujene	921	0.25		
α-pinene	930	3.55		
Camphene	946	9.66		
benzaldehyde	957	0.01		
Sabinene	970	0.21		
β-pinene	981	1.37		
2-octanol	989	0.04		
myrcene	995	0.01		
n-decane	1000	0.01		
α-phellandrene	1005	0.14		
α-terpinene	1014	0.19		
P-cymene	1024	4.15		
limonene	1031	1.16		
γ-terpinene	1053	0.52		
camphor	1139	45.01		
pinocarvone	1159	0.42		
borneol	1162	0.29		
terpinene-4-ol	1179	0.02		
α-terpineol	1193	0.12		
myrtenal	1196	0.21		
chrysanthenyl acetate	1229	21.54		
bornyl acetate	1285	2.88		
thymol	1294	0.04		
carvacrol	1302	0.10		
tetradecane	1395	0.30		
β-caryophylene	1416	0.17		
(E)- $\beta$ -farnesene	1442	1.62		
valencene	1499	0.05		
(Z)-α -bisabolene	1510	0.04		
β-bisabolene	1513	0.15		
γ-cadinene	1557	0.10		
germacrene B	1559	0.14		
Caryophylene oxide	1563	0.66		
spathulenol	1593	0.17		

Microorganis m	Average of inhibition diameters (mm)						Minimum growth concentration(µl/ml)		
	Essential oils of feverfew(µl)				Vancomycin	Gentamicin	Amphotricin B	Feverfew	
	2.5	5	7.5	15	30 µg	10 µg/disc	100 U/Disk	MIC	MBC
S. aureus	18.5±0.0	34.0±0.7	39.0±0.7	42.5±0.0	28.5±0.8	-	-	1	2
S. epidermidis	10.5±0.0	23.5±0.0	28.5±0.4	31.6±0.7	18.5±1.2	-	-	0.5	1
B. subtilis	$6.0{\pm}1.0$	$11.5\pm0.4$	15.5±0.7	$18.0{\pm}1.4$	$20.2\pm0.4$	-	-	2	4
Means±SD	11.66±0.46	23.00±0.36	27.66±0.60	30.70±0.70	$22.40\pm0.80$	-	-		
E. coli(25923)	13.0±0.0	14.0±0.0	15.5±0.7	22.5±1.4	-	19.0±0.0	-	2	2
E. coli(157)	14.0±0.0	$16.8\pm0.4$	19.5±0.7	20.0±0.7	-	$18.8 \pm 1.0$	-	1	2
K. pneumonia	13.0±0.0	14.5±0.0	15.0±0.7	$24.0{\pm}1.4$	-	20.0±0.0	-	2	2
S. flexneri	13.0±0.0	15.8±0.7	$18.5 \pm 0.0$	19.5±0.4	-	17.6±1.0	-	1	2
S.	7.5±0.0	8.5±0.0	9.0±0.7	10.5±0.7	-	20.7±0.6	-	16	16
typhimurium									
Means±SD	12.10±0.0	13.92±0.22	15.50±0.56	$19.30\pm0.92$	-	$19.22 \pm 0.52$	-		
A. nijer	10.5±0.6	12.0±0.0	19.5±0.4	34.0±0.0	-	-	11.8±1.0	1	2
C. cruise	13.0±0.0	21.5±0.7	$40.0{\pm}1.4$	44.5±0.0	-	-	10.8±1.7	0.5	4
C. albicans	26.0±1.2	35.0±0.0	41.5±0.0	49.0±0.7	-	-	15.1±1.4	0.5	0.5
Means±SD	$16.50 \pm 0.60$	22.83±0.23	33.66±0.60	42.5±0.23	-	-	12.56±1.36		

Table 3. Evaluation of the antimicrobial effects of the feverfew essential oil (Tanacetum parthenium L.)

#### Discussion

Results indicate that the camphor, chrysanthenyl acetate and camphene are three major compounds of the essential oil of feverfew and the main part of the essential oil of this herb contains disinfectant camphor. Accordingly, the quantity and quality of the ingredients of feverfew essential oil of Hamadan region is different from the reported cases of Germany and Italy, so that 1 and 8 cineol is the main compound in Wurzburg region in Germany and Riva Del Garda in Italy with the values of 59.9% and 37.3% respectively; alpha-pinene and camphor with the values of 19.6% and 14% respectively in Germany and 10.3% and 9.9% in Italy were identified as the second and third major compounds; and chrysanthenyl acetate and camphene were not found in the essential oil [21, 22].

Also the research conducted by Saharkhiz et al. in Tehran showed similarities in terms of composition of the main constituents of this herb and differences in terms of the percentage of these compositions [23].

Therefore it can be acknowledged that the chemical composition of the feverfew essential oil is different depended on the type of variety, growth stage, time of collection and climatic conditions of habitat. Therefore, evaluation of the essential components of the plant populations with ecological and genetic differences can be influential and important in identifying the diversity of the essential oil within the population of a species and the introduction of chemo types.

The results of the antimicrobial effects of the essential oil of aerial parts of the herb showed that the essential oil of this herb has considerable antimicrobial effect on *Staphylococcus aureus* and *Staphylococcus epidermidis* gram-positive bacteria, *S. flexneri* gram-negative bacteria, *Klebsiella pneumonia* and *Escherichia coli* (*E. coli*) strains with 25923 and 157 numbers and all tested fungi. Antimicrobial effect of this herb on bacteria, *Bacillus subtilis* and *Salmonella typhimurium* is not significant.

Evaluation of the resources showed that antimicrobial properties of the essential oils and extracts of medicinal plants on different microorganisms have been reported in several areas [24]. For example Kapadia and Talib have studied the antimicrobial effect of the essential oil of feverfew (*Matricaria chamomilla* L.) on six grampositive and gram-negative bacteria and have used the antibiotics gentamicin, erythromycin and chloramphenicol as positive case group. Their studies showed that the essential oil of this herb has significant antimicrobial effect on *Staphylococcus epidermidis* grampositive bacterium and *E. coli* and *Serratia marsns* gramnegative bacteria in comparison with the gentamicin, whereas its effect was very weak compared to the erythromycin and chloramphenicol [25].

In another study, the effect of antimicrobial essential oils of *Calendula persica* (*Calendula officinalis* L.) on standard strains of *E. coli* and Staphylococcus has been proved. However, in the report delivered by Saharkhiz et al. essential oil of this herb had no effect on *S. aureus* [23]. It seems that different soil and climatic conditions (longitude and latitude, altitude, mean daily temperature, precipitation, humidity, etc.) in natural habitat of feverfew were effective on the type, amount and chemical properties of the essential oils evaluated in this study and in the study conducted by Saharkhiz et al.

In support of this assumption, further studies showed that the levels of chrysanthenyl acetate and p-symene in the feverfew tested in this study were more than samples used in the study conducted by Saharkhiz et al. Other researchers have also shown that an increase in the level of chrysanthenyl acetate and p-symene increases the effectiveness of the essential on gram-positive bacteria [27]. Although the essential oil components alone may not have any antimicrobial effect, but studies show that sometimes the major composition of the essential oil has less antimicrobial effects than the entire composition of the essential oil [28]. This result is consistent with the results of the experiments conducted by Polatoglu et al. on the inhibitory effects of the essential oil of this herb on Staphylococcus aureus bacterium and report prepared by Izadi et al. on the bacteria Staphylococcus aureus and Bacillus subtilis [11, 29].

Reports have shown that the gram-positive bacteria are more sensitive to the antimicrobial activity of this herb [29-31]. The results of this study indicate the inhibitory and germicidal power of feverfew essential oil. Considering the fact that camphor makes up 45% of the essential oil of feverfew which has disinfecting effects [7-8], 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 this component. 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.

And studies have shown that the antimicrobial properties of the essential oils in the herbs or plants such as sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and marjoram (*Origanum vulgare* L.) increase due to the synergistic effects of the minor constituents of the essential oil with other components existed in this essential oil [28].

According to the results of this study and increasing constraints in the use of anti-microbial chemicals such as

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side effects and drug resistance, the need to replace these ingredients with the natural ingredients and essential oils is felt which could be a ground for further applied studies to replace these materials in the line with the preservation of foodstuff and disease control. Thus feverfew essential oil is a powerful source of biologically active compounds which can be used as a useful source of new antifungal and anti-bacterial agent.

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# **Authors' Contributions**

All authors had equal role in design, work, statistical analysis and manuscript writing.

## **Conflict of Interest**

The authors declare no conflict of interest.

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