SESQUITERPENE RICH VOLATILE SEED OIL OF TAGETES PATULA L. FROM NORTHWEST IRAN

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ABSTRACT

Hydrodistilled volatile seed oil composition of commonly growing ornamental *Tagetes patula* L. was analyzed for its constituents by GC/MS. Forty constituents were identified, comprising 94% of the total oil. Sesquiterpene hydrocarbons (52.7%) and oxygenated sesquiterpenes (15.8%) were the main subclasses of volatile oil components followed by monoterpene hydrocarbons (12.6%). The principle constituents of the volatile oil were (E)-caryophyllene (44.6%) caryophyllene oxide (14.8%), germacrene D (3.8%), (Z)-β-ocimene (3.8%) and limonene (3.7%). From chemical point of view, oxides (15.7%) were the predominant group of components with caryophyllene oxide as their main representative. α-terthienyl (3.8%) comprised partially large amount in the volatile oil content despite of its polar and less-volatile nature. Taking into account the volatile oil profile, the chemical composition of the volatile seed oil of commonly growing ornamental *T. patula* L. was characterized as sesquiterpene and α-terthienyl rich one probably with appreciable biocidal (Insecticidal and nematicidal) and pharmacological potential.

Keywords: *Tagetes patula* L., Asteraceae, volatile oil, sesquiterpene, (E)-caryophyllene, caryophyllene oxide, α-terthienyl

INTRODUCTION

Tagetes patula L. (Family: Asteraceae or Compositae, Tribe: Helenieae) commonly known as French marigold is native to South America but introduced and naturalized in most parts of the world and currently is a cosmopolitan ornamental plant [5]. *T. patula* L. is an annual bushy plant with typical morphological characteristics of Asteraceae family i.e. large capitula flowers and alternate leaves [6,17]. This plant reaches to height of 30-60 cm with upright/straight stem [17]. *T. patula* L. is a frost sensitive plant owing to its native tropical origin [6]. French marigold with beautiful brown and red corolla of ligulate capitula flowers is a commonly spring, summer and early autumn growing ornamental plant especially for boundaries and rock gardens in landscape design [7]. For therapeutic purposes, this plant and its preparations have been used as astringent, flatulent, diuretic, sedative, stomachic and to treat colics, diarrhea, vomit, fever, skin diseases and hepatic disorders [12,13,17]. Furthermore, divergent biological activities have been devoted to the active principles of this plant as antibacterial, antifungal, insecticidal, nematicidal and larvicidal [2,3,8,11,12,14]. Essential oils extracted from the above ground parts and roots as well as acetylenic thiophenes derived from roots are the most important secondary metabolites of French marigold [14]. Monoterpenes, sesquiterpenes and α-terthienyl have been reported as the main ingredients of above ground herb and roots of *T. patula* L. respectively [8,13,15,16]

The highlighted insecticidal and nematicidal activity of *T .patula* L. is due to the presence of high levels of sesquiterpenes and thiophene compounds in the essential oil and organic solvent extracts of roots [2]. α-Terthienyl rich root secretions of French marigold have shown a suppressive effect on the population of soil-born nematodes [8]. There is considerable research interest toward the compositional analysis of *T. patula* L. volatile oil and its organic solvent extracts [4,11,12,14,15,16]. Literature survey revealed that there is several reports concerning the profiling of volatile oil of different natural and in-vitro culture derived organs of *Tagetes patula* L. with their main constituents summarized in Table1

Despite these reports, information about the compositional analysis of *T. patula* L. volatile seed oil is lacking. Therefore, the present experiment was aimed to characterize the volatile seed oil composition of *T. patula* L. plants commonly growing as floriculture crops in Northwest of Iran for the first time.

MATERIAL AND METHODS

Plant material: The ripen seeds of *Tagetes patula* L. plants commonly growing in landscapes of Maragheh district in Northwest of Iran were collected in early-autumn of 2008. The plants and harvested material were verified by a plant taxonomist. A voucher specimen of the plant was deposited in the Herbarium of the Faculty of Agriculture, University of Maragheh. The harvested seeds from about twenty individual plants were air-dried to constant moisture content in a shaded place with ambient temperature of about 25ºC for 4-5 days. The air-dried plant materials were mixed and crushed to obtain a homogenous fine grade powder. The powdered material immediately subjected to hydrodistillation.

Volatile oil recovery: 50 grams of air-dried and grinded seeds was water-distilled by an all-glass Clevenger type apparatus for 3 hrs. The oil phase was trapped with 1 ml of hexane as organic collecting solvent. The solvent was allowed to evaporate under ambient room temperature. The oil was dried over anhydrous Na_2SO_4 and refrigerated in sealed tubes until analysis.

GC/MS analysis: The analysis of the volatile oil was performed using a GC (Agilent Technologies 6890N) interfaced with a mass selective detector (MSD, Agilent 5973B) equipped with an apolar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30m × 0.25 mm i.d. and 0.25μm film thickness). The carrier gas was helium with constant velocity of 1ml/min. Oven temperature was set at 50ºC for 2 min, then programmed until 110ºC at a rate of 10ºC/min with hold time of 3 min, once more time heated to 200ºC at the 5ºC/min rate with 2 min hold time and finally increased at the rate 20ºC/min to 280ºC, isothermal at the temperature for 2 min. Injector and detector temperatures were 300ºC and 200ºC respectively. Injection mode, split; split ratio 1:100, volume injected, 4μl of the pure oil. The MS operating parameters were as follows: Ionization potential, 70 eV; interface temperature, 200ºC and acquisition mass range; 50-800.

Identification and quantification of volatile oil components: Relative percentage amounts of the volatile oil components were evaluated from the total peak area (TIC) by apparatus software. Identification of components in the volatile oil was based on the comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature [1,4,11,12,14-16]

RESULTS AND DISCUSSION

Hydrodistillation of the seeds of *T. patula* L. afforded a dark yellow liquid with a yield of 0.15% (V/W) based on dry weight. The results obtained from the compositional analysis of the volatile oil of *T. patula* L. i.e. the chemical profile of the studied oil, percentage content of the individual constituents, their retention indices and the main classes, subclasses, and chemical groups of the identified constituents of the volatile oil are shown in Tables 2 & 3 respectively. In total, forty components were identified in the seed essential oil of *T. patula* L. comprising 94% of the total oil (Tables 2 and 3). Sesquiterpene hydrocarbons (52.7%) were the main subclass of identified components with (E)-caryophyllene (44.6%), germacrene D (3.8%) and α-humulene (1.1%) as highlighted ones. Oxygenated sesquiterpenes (15.5%) were the second subclass of compounds followed by monoterpene hydrocarbons (12.6%). Caryophyllene oxide (14.8%) was the main compound of oxygenated sesquiterpenes followed by (Z)-β-ocimene (3.8%), limonene (3.7%), meta-cymenene (1.4%) and allo-ocimene (1.1%) as notable constituents of monoterpene hydrocarbons. Piperitenone (1.5%) and p-cymen-8-ol (1.1%) were the only representatives of oxygenated monoterpenes (5.8%) with minor proportion in the total identified components compared with major subclasses. In particular, (E)-caryophyllene and its oxide (Sum 59.4%) comprised 63% of the identified components (Table 2).

Considering the mono and sesquiterpenoidal profile of the studied volatile oil, there was significant qualitative and quantitative differences between the data from the present experiment and reports of other scientists from elsewhere (Tables 1, 2 and3) [4,11,12,14-16]. Isoaromadendrene epoxide (1.2%) was a newly identified compound from this plant with notable amounts compared with most of the mono and sesquiterpene components (Table 2). From chemical point of view, oxides (15.7%), ketones (3.2%) and alcohols (2.2%) were the main groups of chemicals with caryophyllene oxide and piperitenone oxide (~1%) as main oxides and, (Z)-β-Ocimene and piperitenone as major ketones. P-cymen-8-ol was the only member of alcohol compounds with >1% amount. Regarding the high content of monoterpene hydrocarbon limonene, it seems that there is a chemotaxonomic relationship between *T. patula* L. and members of *Citrus* and to some-extent Lamiaceae family. Furthermore, presence of carvone and thymol chemotaxonomically relates this plant to the other members of Asteraceae and Lamiaceae family respectively. α–terthienyl [$(C_4H_3S)C_4H_2S$], a member of the newly identified sulfur containing phototoxic compounds with great biocidal (Insecticidal, nematicidal and larvicidal) activity [9] was contained markedly large amounts in the volatile oil content of *T. patula* L. as well (Figure 1).

This is in spite of several reports that water and steam distillation were ineffective methods for α–terthienyl and other thiophene compounds extraction due to the polar and less-volatile nature of these compounds compared with commonly occurring mono and sesquiterpene compounds [13,14,16]. Furthermore, to the best of our knowledge the present experiment is the first report on the occurrence of α–terthienyl from seed volatile oil of *T. patula* L. because all previous reports were largely from roots and hairy root cultures of this plant [4,11,12,14-16]. It seems that, the different plant material used was the main reason for the difference between the chemical profile of the present experiment and previous reports. Comparative overview of the results showed that there were great differences between monoterpene, sesquitepene and thiophene compounds profile of different studies [4, 11-16]. Apart from plant material used, it seems that probably the different climatological, geographical and plant growing conditions had an inevitable effect on the plant growth and its secondary metabolites biosynthesis and accumulation. In total, results of the present experiment were in weak accordance with the findings of other scientists from other parts of the world. These chemical profile differences, indicating that diverse plant growing and environmental conditions and different plant parts beside other technical and operational factors such as: sample extraction method and instrumental parameters strongly affect volatile oil and chemical profile of *T. patula* L. plant from seed origin. Meanwhile, volatile seed oil content and composition of ornamental *T. patula* L. was comparable with those of other plant parts from previous studies. In conclusion, high content of α–terthienyl in the volatile seed oil of *T. patula* L. plant could be a promising source for producing of home and agricultural insecticidal and nematicidal compounds owing to this compound rapid decomposition with ~4hrs half-life and non-residual environment-friend nature [9]. Furthermore, this in-expensively extracted oil can be used as an initial source material for the formulation of valuable antibiotic, antifungal and anti HIV drugs [9].

Table 1: The main volati le oil constituents of Tagetes patula L. from different countries

Origin (Country)	Plant part	Main compound(s)	Reference
India	Capitula	Limonene (24.5%), Terpinene (12.1%) (Z)-β-Ocimene (10.4%), (E) and (Z)-Tagetone (9.3%)	Garg et al (2005)
Hungary	Flowers	β-Caryophyllene (53.5%)	Szarka et al (2006)
	Leaves	Terpinolene (21.1%)	
	Hairy roots	5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) (28.5%)	
	Intact plants	5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) (44%)	
Hungary	Intact plant flowers	β-Caryophyllene (50.2%)	Szarka et al (2007)
	Intact plant roots	5-(3-buten-1-ynyl)-2,2'-bithiophene (BBT) (47%)	
		2,2':5',2"-terthiophene (α-terthiophene) (19.8%)	
	Hairy root cultures	5-(3-buten-1-ynyl)-2,2'-bithiophene (BBT) (25%)	
		2,2':5',2"-terthiophene (α-terthiophene) (14.3%)	
		5-(4-acetoxy-1-butynyl)-2,2'-bithiophene (BBTOAc) (13.7%)	
India	Leaves, capitula and total above ground herb	Limonene (6.2-13.6%), (Z)-β-Piperitone (6.1-11.9%), Dihydrotagetone (4.5-8.1%), Terpinolene (0-11.2%), p-Cymen-8-ol (3.4-11%)	Vidya et al (2005)
Venezuela	Aerial parts	Piperitone (33.77%), trans-β-Ocimene (14.83%), Terpinolene (13.83%)	Rondon et al (2006)
India	Capitula	Piperitone (24.7%), Piperitenone (22.93%), Terpinolene (7.8%), Dihydrotagetone (4.91%)	Romagnoli et al (2005)

Table 2: Chemical composition of the volatile oil of Tagetes patula L. identified and quantified by GC/MS

No	Compound	Retention index	%
1	α-Pinene	0939	0.3
2	Sabinene	0975	0.4
3	α-Phellandrene	1003	0.2
4	Limonene	1029	3.7
5	Δ-3-Carene	1031	0.6
6	(Z)- β-Ocimene	1037	3.8
7	(E)- β-Ocimene	1050	0.6
8	γ-Terpinene	1060	0.2
9	meta-Cymenene	1085	0.3
10	Terpinolene	1089	1.4
11	Linalool	1097	0.4
12	endo-Fenchol	1117	0.3
13	allo-Ocimene	1132	1.1
14	p-Cymen-8-ol	1183	1.1
15	(Z)-Ocimenone	1229	0.6
16	(E)-Ocimenone	1238	0.4
17	Carvone	1243	0.2
18	Thymol	1290	0.4
19	Piperitenone	1343	1.5
20	Piperitenone oxide	1369	0.9
21	β-Cubebene	1388	0.4
22	Cyperene	1399	0.4
23	n-Tetradecane	1400	0.6
24	(E)-Caryophyllene	1419	44.6
25	(Z)-Thujopsene	1431	0.3
26	2-Pentadecanone	1451	0.7
27	α–Humulene	1455	1.1
28	Germacrene D	1485	3.8
29	Bicycloelemene	1495	0.5
30	Bicyclogermacrene	1500	0.9
31	β-Bisabolene	1506	0.2
32	γ-Cadinene	1514	0.2
33	Δ-Cadinene	1523	0.3
34	Germacrene B	1561	0.5
35	Caryophyllene oxide	1583	14.8
36	n-Hexadecane	1600	0.5
37	Isoaromadendrene epoxide	1612	1.2
38	n-Nonadecane	1900	0.3
39	n-Docosane	2200	0.5
40	α–Terthienyl	2243	3.8
	Total		94

Table 3: Main classes, subclasses and chemical groups of Tagetes patula L. volatile seed oil constituents from Iran.

Class, subclass and chemical group of compounds	%
Monoterpenes	18.4
Monoterpene hydrocarbons	12.6
Oxygenated monoterpenes	5.8
Sesquiterpenes	68.2
Sesquiterpene hydrocarbons	52.7
Oxygenated sesquiterpenes	15.5
α-Terthienyl	3.8
Others	3.6
Total identified	94
Chemical groups	
Alcohols	2.2
Ketones	3.2
Oxides	15.7

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