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CHEMICAL CHARACTERIZATION OF VOLATILE COMPONENTS OF *TAGETES MINUTA* L. CULTIVATED IN SOUTH WEST OF IRAN BY NANO SCALE INJECTION

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The essential oil of cultivated *Tagetes minuta L*. have been obtained by hydrodistillation and analyzed by GC–MS. A total of 27 compounds constituting 92% of essential oil of aerial part were identified. The main components were limonene (13.0%), piperitenone (12.2%), α-terpinolene (11.0%), piperitone (6%), (E)-tagetone (5.7%) and (Z)-ocimenone (5.1%).

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1. Introduction

The new world genus *Tagetes* (Asteraceae) includes the popular garden marigold, *Tagetes erecta* L. Other members of the genus are equally easy to cultivate, and have a long history of human use as beverages, condiments, ornamentals, as medicinal decoctions, and in ritual [1]. *Tagetes erecta* is used as a beverage in South Carolina [2] and in parts of the southern United States [Linares and Bye 1987]. *Tagetes lucida* Cav. is a popular beverage in Mexico and Guatemala, where it is also used as a medicinal tea and in ritual [3]. *Tagetes minuta* L., a species native to southern South America, is used as a condiment, as a refreshing beverage, and for medicinal purposes [4]. In each case, leaves, stems, and flowers are utilized. In recent years, there has been an increasing interest in using the herbal products of indigenous peoples [5]. *Tagetes minuta* could be another new herb brought to the world market*Tagetes minuta* is native to the temperate grasslands region of southern South America, including the countries of Argentina, Chile, Bolivia, Peru, and in the Chaco region of Paraguay [6]. *T. minuta* is often found growing in disturbed areas during early successional stages. This affinity for disturbed sites has allowed the species to colonize many areas around the world. Since the time of the Spanish Conquest, it has been introduced into Europe [7], Africa [8], India [9] and Hawaii [10].

Tagetes minuta is an erect annual herb reaching 1 to 2 m leaves are slightly glossy green, and are pinnately dissected into 4 to 6 pairs of pinnae. Leaf margins are finely serrate. Four or five fused involucre bracts surround each head. There are typically 3 to 5 yellow-orange ray florets, and 10 to 15 yellow-orange disk florets per capitula. The heads are small, 10 to 15 mm long, and including ray florets, 10 to 20 mm in diameter. The heads are borne in a clustered panicle of 20 to 80 capitula. The dark brown achenes are 10 to 12 mm long, with a papas of 1 to 4 tiny scales and 0 to 2 retrosely serrulate awns which are 1 to 3 mm long.

Tagetes minuta is used as a condiment in Chile and Argentina. It is popular in rice dishes and as a flavoring in stews. *Tagetes minuta* is commercially grown and harvested for its essential oils which are used in the flavor and perfume industry as "Tagetes Oil." The oil is used in perfumes, and as a flavor component in most major food products, including cola beverages, alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins, puddings, condiments, and relishes [11]. Brazil is one major producer of *T. minuta* for Tagetes Oil [12]. Worldwide production of the oil was around 1.5 tonnes in 1984 [13]. *Tagetes minuta* is rich in many secondary compounds, including acyclic, monocyclic and bicyclic monoterpenes, sesquiterpenes, flavonoids, thiophenes, and aromatics [14]. There is evidence that the secondary compounds in *Tagetes* are effective deterrents of numerous organisms, including: fungi pathenogenic on humans [15], bacteria [16], round worms in general [17], trematodes [18], nematodes [19], and numerous insect pests through several different mechanisms [20]. Many closely related plant secondary compounds have demonstrated medicinal value in humans [21] In vivo human studies of the secondary compounds of *T. minuta* have not been reported, although other *Tagetes* species have proven medically safe and efficacious [22]. Many studies have been reported on volatile components of Tagetes plants [23-25]. The chemical composition of *T. minuta* oil produced from plants grown in different parts of the world has also been reported [26]. From these analyses, it could be seen that the composition oils varied according to the harvesting location [27], growth stage, [28] plant parts [27] and chemotypes [29].

2. Experimental

2.1 Plant material

Tagetes minuta L is not a native plant of Iran. The seeds of *T. minuta* were provided from Namibia in Africa. The seeds were sown on clay loam soil in April 2007 at the experimental farm of Azad University located in Khoramabad Iran. Spacing in the rows were 50 cm. Cultivated Tagetes minuta L. was harvested on university farm by cutting the bushes at full bloom stage to the ground and then dried in laboratory.

2.2 Isolation of the Essential oil

Air-dried aerial parts were subjected to hydro-distillation for 2 h using a Clevenger-type apparatus to produce oil in 1.2% yield according to the method recommended in British Pharmacopoeia [30]. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 °C until analysis.

2.3 Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC/MS)

GC-FID analyses of the oil were conducted using a Thermoquest-Finnigan instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of 1.1 ml/min. The oven temperature was raised from 60 ºC to 250 ºC at a rate of 5 ºC/min. The injector and detector (FID) temperatures were kept at 250 ºC and 280 ºC, respectively. The amount of the sample injected was 1.0 nL (diluted 1.0 μL of sample in 1000 ml of n-pentane, v/v) in the splitless mode [31-34]. GC-MS analysis was carried out on a Thermoquest-Finnigan Trace GC-MS instrument equipped with the same column and temperature programming as mentioned for GC. Transfer line temperature was 250 ºC. Helium was used as the carrier gas at a flow rate of 1.1 ml/min with a split ratio equal to 1:50. The quadrupole mass spectrometer was scanned over the 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA.

2.4 Identification of bioactive and fragrant components

The constituents of the volatile oils were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C6–C24) and the oil on a DB-1 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [35]. Quantitative data was obtained from FID area percentages without the use of correction factors. The components of the oil were identified by comparison of their mass spectra with those of a computer library search and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature (Table 1).

Table 1. Chemical composition of essential oil of cultivated Tagetes minuta L. from south west of Iran

No	compounds[a]	RT[b]	RI[c]	%[d]
1	α-Pinene	9.06	931	0.5
2	Sabinene	9.93	966	0.8
3	Limonene	11.39	1021	13.0
4	Dihydrotagetone	11.37	1033	1.2
5	1-methyl-4-isopropenylbenzene	12.82	1073	1.0
6	α-Terpinolene	12.98	1078	11.0
7	cis-Epoxy ocimene	13.84	1109	2.6
8	trans-Epoxy ocimene	14.14	1120	1.2
9	Z-Tagetone	14.22	1123	3.2
10	E-Tagetone	14.41	1129	5.7
11	p-Cymene-8-ol	15.27	1160	2.0
12	Linalyl propionate	15.64	1173	0.9
13	Verbenone	16.55	1205	3.7
14	Z-Ocimenone	16.76	1213	5.1
15	Piperitone	17.19	1229	6.0
16	Piperitenone	19.49	1312	12.2
17	Piperitone oxide	20.06	1334	1.2
18	β-Caryophyllene	22.45	1424	2.5
19	Spathulenol	26.24	1563	0.7
20	Caryophyllene oxide	26.43	1576	3.0
21	Heptadecane	31.62	1795	0.7
22	Neophytadiene	32.45	1832	0.6
23	Octadecane	33.8	1894	0.9
24	Nonadecane	35.89	1993	1.2
25	Heneicosane	39.96	2095	2.5
26	Docosane	42.34	2286	5.0
27	Tricosane	45.17	2390	4.2

[a]Compounds listed in order of their RI
[b]Retention Times
[c]RI (retention index) measured relative to n-alkanes (C_6–C_{24}) on the DB-1 capillary column
[d]%, Relative percentage obtained from peak area

3. Results and discussion

The oil of *T. minuta L.* has been investigated by a number of workers [29] who have identified (Z)-β-ocimene, dihydrotagetone, (Z)- and (E)-tagetone, and (Z)- and (E)-tagetenone [(Z)- and (E)-ocimenone] as the major components. The results of our analysis show that oil of *T. minuta L* was particularly rich in limonene (13.0%), piperitenone (12.2%), α-terpinolene (11.0%), piperitone (6%), (E)-tagetone (5.7%) and (Z)-ocimenone (5.1%). The oil of *T. minuta L* consisted mainly of monoterpene hydrocarbons (28.3 %), oxygenated monoterpenes (45.2 %), sesquiterpene hydrocarbons (2.5 %) oxygenated sesquiterpenes (3.7 %), oxygenet diterpene (0.6 %) and other compounds (17.2 %). A survey of the literature [13, 36] reveals that no oil of any *Tagetes* species has been found in which verbenone was the main constituent so its identification of potential importance to the genus cultivated in Iran. However, there are reports showing that percentage of minor constituents varies. For example, thymol has been found a minor component in the oil *T. minuta* [25], while it occurred in appreciable amounts (7.4%) in oil from Brazil [12]. Also, α-phellandrene and o-cymene were the major oil components from Argentina as reported by Gill et al. 2000 [29]. The variations in chemical composition could be due nature of the soil [37], the amount of sunlight and temperature variations and the occurrence of chemotypes [29]. Thus, we believe that intrinsic and external factors could have affected the content and composition of the oil of *T. minuta*.

Herein, we illustrate biological properties and application of some important components from essential oil of *Tagetes minuta L.*:

Limonene: is used as fragrance material for perfuming household products and as component of artificial essential oils.

Piperitenone: is used in the creation and/or manufacturing of fragrance and flavor concentrates of all types. Piperitenone is a valuable intermediate for the synthesis of menthol which is much sought after for cosmetic and pharmaceutical purposes.

α-Terpinolene: has floral, sweet, and pine-like aroma notes.

Piperitone: is used as the principal raw material for the production of synthetic menthol and thymol. The primary source of D/L-piperitone is from *Eucalyptus dives*, produced mainly in South Africa [38].

(E)-tagetone: affected the function of GABAA receptor in a complex way: on the one hand it impaired FNTZ binding; on the other hand tagetone improved both the coupling between FNTZ and GABA binding sites and it enhanced GABA-induced chloride permeability. Changes in the geometrical and electrostatic properties of the self-organized membrane structure may account for these effects of tagetone. [39]

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