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International journal of Advanced Biological and Biomedical Research

Volume 1, Issue 9, 2013: 1124-1128

Chemical constituents of the essential oils from the leaves, flowers and seeds of *TAGETS MINUTA* L. BY GC/MS

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ABSTRACT

Tagetes is a genus of 56 species of annual and perennial mostly herbaceous plants in the sunflower family (*Asteraceae* or *Compositae*).In this research our goal was recognizing the main components of the plant to extract useful ones. Essential oils of the leaves, seeds and flowers of *Tagets minuta* L., growing wild in Iran, were extracted by hydrodistillation and analyzed by GC and GC-MS. Identification of the constituents of the oils was done by comparison of their mass spectra and retention indices with those given in the literature and the authentic samples. Twenty-six components were identified in the essential oils of the investigated organs. The main components extracted from the leaf oils were dihydrotageton (45.9%), *cis*-*β*-ocimene (11.9%) and borneol (11.1%), and those of the seed oils included dihydrotagetone (21.0%) and benzoic acid-4-hydroxy-methyl ester (33.5%).Also, *trans*-ocimenone (27.0%), *cis*-*β*-ocimene (26.0%) and *cis*-ocimenone (17.6%) were the major constituents in the flower oils.

Key words: *Tagetes minuta* L., Hydro distillation, Dihydrotagetone, Essential oil, *Trans*-Ocimene, *cis*-*β*-Ocimene, GC/MS

INTRODUCTION

Tagetes is a genus of 56 species of annual and perennial mostly herbaceous plants in the sunflower family (*Asteraceae* or *Compositae*). The genus is native to North and South America but some species have become naturalized around the world. *Tagetes minuta* L. is considered a noxious invasive plant in some areas (Soule, 1996). Native to the southern half of South America, it is a tall upright marigold plant with small flowers, and is used as a culinary herb in Peru, Ecuador and parts of Chile and Bolivia, where it is called by the Incan term as "*huacatay*". It is also used as a flavorful tea for medical benefits such as a remedy for the colds, respiratory inflammations or stomach problems. The leaves when dried may be used as a seasoning. The sap of the plant may cause irritation to the skin as well as photo dermatitis (Soule,

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1993). It is considerable that *Tagetes minuta* L. has been studied previously in some other countries such as Argentina, UK. , South Africa, Egypt, Pakistan and Saudi Arabia (Chamorro et al., 2008, Senator et al., 2004, Shahzadi et al., 2010, Deeb et al., 2004).Moreover, there is another report on aerial parts essential oil analysis of this genus in Iran, Lorestan Province (Meshkatalsadat et al., 2010). This research reports the chemical composition of the essential oils hydrodistilled separately from the leaves, seeds and flowers of *Tagetes minuta* L. growing widely in the southeastern part of Iran (Kerman) and analyzed by GC and GC/MS.

MATERIALS AND METHODS

Plant material

The leaves, flowers and seeds of *Tagetes minuta* L. were collected from Mahan, Kerman Province (latitude 25 ° 55 / N, longitude 53° 26/ E, altitude 1755m), Iran, during the seed stage in April 2011. Dr. Peyman Rajaei identified the plants. A voucher specimen of *Tagetes minuta* L. (No.104) has been deposited in the Herbarium of Islamic Azad University, Kerman Branch. The plant material was air-dried at room temperature, protected from the light, for 1 week.

Isolation of the essential oils

Leaf, flower and seed essential oils of the plant were separately obtained by hydrodistillation in a Clevenger-type apparatus for 3 hours using n-hexane as collector solvent. The oils were separately dried over anhydrous sodium sulfate and their solvents were evaporated under a N_2 flow before analysis. Then they were stored in sealed vials protected from the light at 4˚C. Oil samples for each collection were prepared and analyzed by GC and GC-MS. The yield of the oils was calculated based on the dried weight of the plant materials.

GC and GC-MS analysis

GC analysis of the volatile components was carried out using a Hewlett-Packard 6890 instrument coupled to a flame ionization detector (FID).The components were separated on a HP-5MS capillary column (5% phenyl methyl poly siloxane, 30m × 0.25mm, film thickness 0.25 µ m). The temperature of the column was kept at 60˚C for 3 minutes and programmed to 220˚C at a rate of 5˚C/min. The temperature of both injector and detector was 270˚C and the flow rate of helium as a carrier gas was 1ml/min. A mixture of aliphatic hydrocarbons (C_8-C_{23}) in hexane was directly injected into the GC injector under the above temperature program in order to calculate the retention indices of each compound. The retention indices of all components were determined according to the Van Den Dool's method (Van Den Dool et al., 1963). GC-MS analysis was performed using a Hewlett-Packard 5973 mass spectrometer coupled to a Hewlett-Packard 6890 gas chromatograph equipped with a HP-5MS capillary column (5% phenyl methyl poly siloxane, 30m × 0.25mm, film thickness 0.25µm). The carrier gas was helium. All mass spectra were acquired in electron-impact (EI) mode with an ionization voltage of 70 e V. Identification of the components of the volatile oils was done based on the retention indices, computer matching with the Wiley 275.L library and by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Massada, 1976, Adams, 2004).

RESULTS AND DISCUSSION

The yield of the essential oils obtained from the leaves, seeds and flowers of *Tagetes minuta* L. was 0.9, 0.5 and 0.7 % (w/w), respectively. The composition together with the percentage and retention indices of the leaf, seed and flower oils of *Tagetes minuta* L. are shown in Table 1. As shown, 13 compounds

(96.9%) were identified in the leaf oil of *Tagetes minuta* L.; the main constituents were dihydrotagetone (45.9%), *cis*-*β*-ocimene (11.9%) and borneol (11.1%). Among the 17 compounds (97.6%) identified in the flower oil of *Tagetes minuta* L. *trans*-ocimenon (27.0%), *cis*-*β*-ocimene (26.0%) and *cis*-ocimenone (17.6%) were found as the major components. In addition, dihydrotagetone (21.0%) and benzoic acid-4-hydroxy-methyl ester (33.5%) were the main constituents among the 14 compounds (95.0%) characterized in the seed essential oil of *Tagetes minuta* L. The results also showed that the leaf oil of *Tagetes minuta* L. consisted of three oxygenated sesquiterpenes (47.1%), five oxygenated monoterpenes (35.4%), four monoterpene hydrocarbons (14.2%) and one sesquiterpene hydrocarbons (0.4%) , whereas the seed oil of *Tagetes minuta* L. contained two terpene esters (51.1%), three oxygenated sesquiterpenes (23.6%), five oxygenated monoterpenes (15.7%), three sesquiterpene hydrocarbons (3%) and one monoterpene hydrocarbon (1.6%).Furthermore, five oxygenated monoterpenes (53.4%), five monoterpene hydrocarbons (30.8%), one oxygenated sesquiterpene (5.5%), three nonterpenoids (4.6%),two sesquiterpene hydrocarbons (2.5%) and one terpene ester (0.8%) were identified in the flower oil of *Tagetes minuta* L. The results are shown that the plant is applicable for extracting dihydrotagetone, Ocimene and Benzoic acid-4-hydroxy-methyl ester. In addition the essential oil is rich in oxygenated terpenes in leaves, flowers and seeds of *Tagetes minuta L*. The main component of the leaf oil of our plant is dihydrotagetone (45.9%) as the essential oil of the plant, which was analyzed in England (54.1%) (Senator et al., 2004). The main yield of the essential oil obtained from the aerial parts of *Tagetes minuta* L. in Egypt and South Africa was *cis*-*β*-ocimene (32%, 50.9%,respectively) (Senator et al. 2004). As one of the major components that we found in our plant was *cis*-*β*-ocimene (flower 26%, leaf 11.9% and seed 1.6%). The main components characterized in another research in Saudi Arabia were tagetone (11.52%) and 5 octyn-4-one 2, 7-dimethyl (11.52%) (Deeb et al., 2004) but we just found tagetone in our research. In addition, we could not identify the main constituents such as limonene (13%) and piperitonone (12.2%), which were identified in a research in the southwest of Iran, Lorestan Province (Meshkatalsadat et al., 2010).

CONCLUSION

The environmental, ecological and geographical significantly affect the type and amount of the essential oil of the plant, analyzed in this research, in contrast with other papers that were mentioned (Senator et al., 2004, Deeb et al., 2004, Meshkatalsadat et al., 2010). This is also true about other specious of *Tagetes*.

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Table 1 . Chemical composition of the volatile oils from leaves, seeds and flowers of *Tagetes minuta* L.

Compounds [a]	**R I** [b]	**Flower (%)**	**Leaf (%)**	**Seed (%)**
α-Pipene	939	0.5	0.5	-
Camphene	959	-	0.4	-
Sabinen	979	1.1	1.4	-
Octanal	1000	0.6	-	-
cis-β-Ocimene	1035	26.0	11.9	1.6
trans-β-Ocimene	1045	0.9	-	-
Dihydrotagetone	1052	5.5	45.9	21.0
allo-Ocimene	1130	2.3	-	-
trans-Tagetone	1145	1.8	7.2	2.0
cis-Tagetone	1152	5.8	9.8	4.6
Borneol	1117	-	11.1	-
2,4,6-Trimethyl-1,3-benzendiamine	1121	1.4	-	-
cis-Ocimenone	1236	17.6	4.4	2.3
trans-Ocimene	1246	27.0	2.9	0.7
Carvacrol	1309	1.2	-	6.1
α-Terpinenyl acetate	1356	0.8	-	-
Nicotin amide	1400	2.6	-	-
β-Caryophyllene	1444	1.6	0.2	1.7
Benzoic acid-4-hydroxy-methyl ester	1455	-	-	33.5
Germacrene D	1495	-	-	0.7
Bicyclogermacrene	1510	0.9	-	-
δ-Cadinene	1530	-	-	0.6
Spathulenol	1588	-	0.4	1.7
β-Atlantol	1613	-	0.8	-
Caryophyllene oxide	1615	-	-	0.9
Benzoic acid-4-hydroxy-propyl ester	1623	-	-	17.6
Total identified	-	97.6	96.9	95.0

[a] Compounds are listed in order of their elution from the HP-5 capillary column.

[b] Retention indices ,RI, on the HP-5 column, experimentally determined using homologous series of C_8-C_{23} alkanes.