ISSN 0972-060X

# Chemical Composition of Essential Oils and Toxicological evaluation of *Tagetes erecta* and *Tagetes patula* from Venezuela

**Rossana Martínez [2], Beth Diaz [1], Luís Vásquez [1], Reinaldo S. Compagnone [2], Stephen Tillett [1], Dilsia J. Canelón [3], Fátima Torrico [1] and Alírica I. Suárez [1]***

[1] Facultad de Farmacia, Universidad Central de Venezuela, Apdo. 40109, Caracas, Venezuela
[2] Escuela de Química, Facultad de Ciencias, Universidad Central de Venezuela
[3] Escuela de Bionalisis, Facultad de Medicina, Universidad Central de Venezuela

Received 04 February 2008; accepted in revised form 12 December 2008

**Abstract:** The essential oils from the flowers of *Tagetes erecta* and *Tagetes patula,* two plants widely used in traditional medicine of America and Africa countries, were obtained by hydrodistillation. The oil composition was analyzed by GC-MS. Twenty five compounds were identified in the oil of *T. erecta,* of which linalool (22.5 %), 2-hexyl-1-decanol (18.3 %), piperitone (13.4 %), 4-terpinyl acetate (7.8 %) and caryophyllene (6.6 %) are the main components. In the essential oil of *T. patula* 21 compounds were identified, and α-terthienyl (43.1 %), pentatriacontane (23.9 %), and 2-ethyl-1-dodecanol (7.9 %) are the major constituents. The essential oils were tested for their toxicological potential on mice, the results showed that the oils of these species have a low toxicity with a $TD_{50}$ 99.6 mg/kg for *T. erecta* and 112 mg/Kg for *T. patula.*

**Keywords:** *Tagetes erecta*; *Tagetes patula*, Essential oils; Toxicological evaluation; chemical composition.

**Introduction:** The genus *Tagetes* (Asteraceae) includes 56 species many of which have been reported to be used in traditional medicine in America, Africa and Asia countries. In Venezuela only five species have been reported [1]. The two species herein reported *Tagetes erecta* L. and *Tagetes patula* L., both known in our country as "flor de muerto", are native from Mexico but widely spread in tropical and subtropical regions of the world. It doesn't matter which, they are both used in the traditional medicine to treat gastric ulcers, conjunctivitis, bronchitis, fever and intestinal and stomach diseases [2]. The flowers of *T. erecta* are sold in some markets of our country instead the well known medicinal plant *Calendula officinalis*. Significant differences have been reported on the essential oil composition of *Tagetes* especies around the world [3-6]. In spite of the large number of studies

*Corresponding author (Alírica I. Suárez)
E- mail: < alirica.suarez@ciens.ucv.ve >

carried out worldwide on *T. erecta* and *T. patula*, the composition of the essential oils of theses species from Venezuela have been scarcely studied. The toxicological evaluation of the aqueous extract, which contains the essential oil of these flowers, was undertaken with the purpose of investigate the toxicity or the possible collateral effects, which the administration of these plants can produce.

The aim of this paper is to obtain the chemical composition of the two oils samples extracted from the flowers of *T. erecta* and *T. patula,* and compare sameness and differences, as well the toxic effects produced by the administration in mice.

**Experimental**

**Plant material:** The fresh flowers of *T. erecta* were purchased in June 2005, from a local market in Maracay, Aragua State in Venezuela and *T. patula* flowers were collected in San Jose de los Altos, Miranda State in May 2005, both plants were identified by Dr. Stephen Tillett. Voucher specimens of each plant with codes (MYF 24879) for *T. erecta* and (MYF 24243) for *T. patula*, are deposited in the Herbarium Victor Manuel Ovalles of the Faculty of Pharmacy, Universidad Central of Venezuela.

**Isolation of volatile components:** Fresh flowers of *T. erecta* (0.250 Kg) and *T. patula* (0.250 Kg), were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus.The oils were dried over anhydrous sodium sulphate and stored at 5°C until analyzed. Essential oil yields were 1.00 % for *T. erecta* and 0.88 % *T. patula*, based on weight of fresh samples.

**Gas Chromatography-Mass Spectrometry:** Analysis of the oils by GC-MS were carried out on a Varian Saturn 2000 Spectrometer instrument using an ionic trap detector. A fused capillary column coated with crosslinked dimethylpolysiloxane (CP-Sil 8CB), length 30 mts and 0.25 mm of diameter. The analytical conditions were: carrier gas; helium; flow rate, 1mL/min, injector temperature, 250°C; split rate; 20:1; temperature program, 5 min. at 60°C rising to 290°C at a rate of 5°C/min. Spectra were recorded at 70 eV.

Individual components were identified by comparison of the retention indices (Kovats indices) and mass spectra provided by WILEY and NIST libraries in the data system. The retention indices were determined using homologous series of n-alkanes ($C_6$-$C_{24}$).

**Preparation of the aqueous extract:** *T. erecta* and *T. patula* aqueous extracts containing the essential oils were obtained by decoction of the flowers in distilled water for 30 min. Solution were filtered and then dried by lyophilization. At the time of use, extract were reconstituted in distilled water at the required concentrations.

**Animals:** Animals used for the assessment of the acute toxicity were male albino mice NMRI strain, weighing 30-35 g. The animals were housed in standard polypropylene cages and maintained under standard conditions, 12 h light and 12 h dark cycle at 25 ±. 2°C for a period of 10 days. Before and during the experiments, the mice were maintained on standard pellet diet with free access to food and water, except the day of experiment. Animals were treated according international standards of animals' welfare (NIH, 1996).

**Acute toxicity ($TD_{50}$ and $LD_{50}$):** The $LD_{50}$ and $TD_{50}$ of the aqueous extracts were determined according to Litchfield and Wilcoxon method [7]. 45 animals were randomly divided into nine groups of 5 animals each. One group was treated with physiological solution and considered as control and the remaining groups with increasing doses (0.03, 0.09, 0.18, 0.36, 0.75, 1.5, 3.0 and 12.0 g/kg) of *T. erecta* and *T. patula* aqueous extract (i.p.), in a requisite volume of 0.1 ml/kg. Behavioral changes were observed thoroughly during the 10, 30, 60, 90 minutes and 24 h after treatment.

**Results and Discussion**

**Analysis of Essential Oil Composition:** The relative amounts (%) of the components of *T.* erecta and *T. patula* essential oils, calculated based on GC peak areas, are reported in Table 1, according to their retention indices on the column. The main components of the two oils were different, but they share some minor components with different relative contents in both samples. In particular the main components of *T.* erecta and *T. patula* essential oils were linalool (22.5 %), and. α- terthienyl (43.1 %), respectively. The essential oil from *T. erecta* showed to be rich in monoterpenes, accounting them for a 46.3 % of the total composition; the predominant monoterpenes components were linalool (22.5 %), piperitone (13.4 %), terpinyl acetate (7.8 %). The second major groups of compounds were oxygenated aliphatic hydrocarbons from which 2-hexyl-1-decanol (18.3 %) was the most abundant. The sesquiterpenes caryophyllene (6.6 %) and β-cubebene (3.0 %) were also present in the oil from *T. erecta.* Compounds such as tagetones and ocimenones reported in this specie from other countries were not found in our analysis [8-10]. A compara-tive analysis with the literature indicated that the qualitative composition in some cases is similar, but the contribution of the certain major components is completely different. The major constituents of the essential oil from the flowers of *T. patula* were found to be aromatic thiophenes structures; the main volatile component was α-terthienyl (43.1 %), followed by 5-(3-buten-1-ynyl)-2-2'-bithienyl (BBT) (23.9 %). Other characteristic components of this oil were: 2-ethyl-1-decanol (7.9 %), 2-hexyl-1-octanol (4.2 %), spathulenol (2.4 %), caryophyllene oxide (1.6 %), pentatriacontane (1.2 %); other minor compounds were: terpinolene (0.9 %), linalool (0.4 %), piperitone (0.2 %).

The composition found in the essential oil of *T. patula*, resembles the composition of this specie from other countries [10-12], thiophene compounds and sesquiterpenes as caryophyllene have been reported in other latitudes, however a recent report of this specie harvested in a mountain place of our country, showed different compounds, piperitone which was found in our search as minor component, was the major one in this report, and no one thiophene compounds were reported in this work [13]. The results obtained in this investigation when are compared with the literature chart, showed that the chemical composition of the essential oils from different species of *Tagetes*, differs depending either on the geographical situation, extraction method, growth conditions, environmental factors, and genetics.

**Acute toxicity evaluation:** As a common way of preparation, flowers of these plants are used in traditional medicine as decoction in water. The toxicological evaluation assays

were made on the base of essential oil and water decoction can be linked, in spite of that, we are aware that other compounds could be present in the aqueous extract.

Acute toxicity study indicated that water extracts of *T. erecta* and *T. Patula* produce only reversible toxic effects such as intestinal contortion, piloerection and lethargy in mice. Intestinal contortion was chosen to determine the $DT_{50}$, because it was the most representative toxic effect observed in this test, no mortality was observed. The median lethal dose ($LD_{50}$) was determined to be higher than the highest dose tested, 12.0 g/kg., for *T. erecta*. The results indicated that for *T. patula* the $LD_{50}$ was 3.57 g/kg. No mortality occurred in both species during the period of observation. The results obtained showed that the oils have a low toxicity with a $TD_{50}$ 99.6 mg/kg for *T. erecta* and 112 mg/kg for *T. patula.* No noteworthy signs of toxicity were noted in this study of these two *Tagetes* species.

**Acknowledgements:** The financial support provided by FONACIT Grant G2005-00389 is gratefully acknowledged.

**References**

1. **Schnee, L. (1984).** Plantas Comunes de Venezuela, Ed. Biblioteca, Universidad Central de Venezuela.
2. **Serrato, C., Miguel, A., Quijano M. (1993).** Usos de algunas especies de *Tagetes*. Proceeding of Ist International Symposium on Sustainable Agriculture. Mexico, 228-238.
3. **Krishna, A., Kumar, S., Mallavarapu, G.R., Srinnivasaiyer, R. (2004).** Composition of the essential oils of the leaves and flowers of *Tagetes erecta* L. J. Essent Oil. Res., 16: 520-522.
4. **Kaul, P.N., Bhattacharya, A.K., Rajeswara Rao, B.R. Syamasundar, K.V., Ramesh, S. (2005).** Essential Oil Composition of *Tagetes minuta* L. fruits. J. Essent Oil. Res., 17: 184-185.
5. **Marotti, M., Piccaglia, R., Biavati, R., Marotti, I. (2004).** Characterization and yield evaluation of essential oils from different *Tagetes* species. J. Essent Oil. Res., 16: 440- 444.
6. **Perez-Gutierrez, R.M., Hernández-Luna, H., Hernández-Garrido, S. (2006).** Antioxidant Activity of *Tagetes erecta* Essential Oil. J. Chil. Chem. Soc., 51: 883-886.
7. **Litchfield, J., Wilcoxon, F., (1949).** A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther., 28: 99-108
8. **Senatore, F., Napolitano, F., Mohamed, M.A-H., Harris, P.J.C., Mnkeni, P.N.S., Henderson, J. (2004).** Antibacterial activity of *Tagetes minuta* L. (Asteraceae) essential oil with different chemical composition. Flav. Frag. J. 19: 574-578.
9. **Sefidkon, F., Salehyar, S., Mirza, M., Dabiri, M. ( 2004).** The essential oil of *Tagetes erecta* L. occurring in Iran. Flav. Frag. J. 19: 579-581.
10. **De Feo, V., Urrunaga Soria, E., Urrunaga Soria, R, Pizza, C., (2005).** Composition and *in vitro* toxicity of the essential oil of *Tagetes terniflora* HBK. (Asteraceae).

Flav. Frag. J., 20: 89-92.

11. **Szarka, S., Héthelyi, É., Lemberkovics, E., Kuzovkina, I.N., Bányai, P., Szöke, É. (2006).** GC and GC-MS studies on the essential oil and thiophenes from *Tagetes patula* L. Chromatographia, 63: 67-73.
12. **Romagnoli, C., Bruni, R., Andreotti, E., Rai, M.K., Vicentini, C.B., Mares, D. (2005).** Chemical characterization and antifungal activity of essential oil of capitula from wild Indian *Tagetes patula* L. Protoplasma, 225: 57-65.
13. **Rondón, M., Velasco, J., Hernández, J., Pecheneda, M., Rojas, J., Morales, A., Carmona, J., Diaz, T. (2006).** Chemical composition and antibacterial activity of *Tagetes patula* L. (Asteraceae) collected from the Venezuela Andes. Revista Latinoamericana de Quìmica, 34: 32-36.

**Table 1. Composition of the essential oil of *Tagetes erecta* and *Tagetes patula***

| Compound | RI | Peak area (%) *T. erecta* | Peak area (%) *T. patula* | Identification |
|---|---|---|---|---|
| 1-Octanol | 1054 | 1.0 | 1.6 | - |
| E-ocimene | 1034 | tr | 2.2 | RI, MS |
| Guaine | 1082 | tr | nd | RI, MS |
| β-Cubebene | 1068 | 2.9 | nd | RI, MS |
| Linalool | 1081 | 22.5 | tr | RI, MS |
| Piperitone | 1093 | 13.4 | tr | RI, MS |
| Menthone | 1123 | 1.2 | 1.7 | RI, MS |
| Borneol | 1140 | 0.5 | 1.0 | RI, MS |
| Terpinen-4-ol | 1147 | 2.6 | tr | RI, MS |
| 4-Terpinyl acetate | 1206 | 7.8 | nd | RI, MS |
| Piperitenone | 1301 | 4.8 | 2.4 | RI, MS |
| Germacrene B | 1347 | 1.2 | tr | RI, MS |
| Caryophyllene | 1411 | 6.6 | 1.1 | RI, MS |
| β-Caryophyllene | 1414 | - | 2.7 | RI, MS |
| *allo*-Aromadendrene | 1458 | 0.2 | tr | RI, MS |
| α-Caryophyllene | 1480 | 0.5 | 0.3 | RI, MS |
| 1,4-Naptoquinone | 1501 | 6.5 | tr | RI, MS |
| Bisabolene | 1520 | nd | 0.8 | RI, MS |
| β-Cadinol | 1526 | 0.2 | tr | RI, MS |
| 5-(3-Buten-1-ynyl)-2-2'-bithienyl (BBT) | 1547 | 1.6 | 4.2 | RI, MS |
| 1,4-Naptoquinone | 1501 | 0.7 | tr | RI, MS |
| Spathulenol | 1566 | 0.3 | 0.4 | RI, MS |
| Caryophyllene oxide | 1579 | - | - | RI, MS |
| β-Terthienyl | 1608 | nd | 43.1 | RI, MS |
| Cedrol | 1638 | nd | 5.4 | RI, MS |
| Globulol | 1650 | 0.1 | tr | RI, MS |
| τ-Muurolol | 1635 | tr | 0.2 | RI, MS |
| 2-Hexyl-1-decanol | 1656 | 18.3 | 7.8 | RI, MS |
| Pentatriacontane | 1664 | nd | 23.9 | RI, MS |
| Cubenol | 1669 | nd | 1.0 | RI, MS |
| δ-Cadinol | 1672 | tr | nd | RI, MS |
| T-Cadinol | 1684 | tr | 2.3 | RI, MS |
| Tricosane | 1982 | 1.1 | 2.6 | RI, MS |

nd: not detected
tr: peak area less than 0.05