

Chemical compounds of essential oil of *Tagetes* species of Ecuador

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Received April 14, 2015; Accepted November 5, 2015

Abstract

It has limited information on the chemical composition of *Tagetes* species distributed in Ecuador, which limits its use as a biopesticide, for that reason, to advance the characterization of this plant genetic resource, in this work the oil composition described essential obtained by hydrodistillation of five species in situ plants. Using a procedure GC / MS the following major compounds were determined: anethole (66.997%), estragole (31.685 %) and anis aldehyde (1.495%) in *T. filifolia*; trans-tagetone (52.76%), 4-ethyl-4-methyl-1-hexene (25.56%), verbenone (3.32%), 1-verbenone (3%), β -ocimene (8.62%), β -linalool (1.19%) and cis-tagetone (6.21%) in *T. terniflora*; trans-tagetone (33.97%), 4-ethyl-4-methyl-1-hexene (13.85%), cariofilene (3.18%), β -ocimene 16.96%), trans-ocimene (3.73%), cis-tagetone (9.1 %), 1-verbenone (11.69%) and verbenone (16.57%) in *T. minuta*; trans-tagetone (17.89%), trans-ocimene (3.73%), 1-verbenone (13.89%), verbenone (24.34%), epoxy pinane 2.3 (0.44%) and 4-ethyl-4-methyl-1-hexene (39.69%) in *T. zypaquirensis*; and trans-tagetone (30.91%), trans-ocimene (25.66%) and valeric acid (43.43%) in *T. multiflora*.

Tagetes, Ecuador, essential oil, chemical composition.

Citation: ZAPATA-MALDONADO, Christian Iván, SERRATO-CRUZ, Miguel Ángel, IBARRA, Emmanuel and NARANJO-PUENTE, Blanca. Chemical compounds of essential oil of *Tagetes* species of Ecuador. ECORFAN Journal-Republic of Nicaragua 2015, 1-1: 19-26

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Introduction

The genus *Tagetes* (Compositae) appeared on the American continent 65 million years ago (Turner, 1997). So far they have referred 53 species distributed in the Americas from the southwestern United States to southern Argentina (Plant List, 2013).

Refer some species of *Tagetes* (Asteraceae) took advantage of Mesoamerica medicinal, ornamental and ceremonial purposes in ancient times (Kaplan, 1960; Neher, 1968; Estrada, 1986; cited by Serrato, 1999) In the Florentine Codex. In countries like Argentina, Chile, Peru, Bolivia and Colombia, the *Tagetes* even be used as a medicinal plant or food preparation and flavor (*T. minuta* and *T. terniflora*) and flavor (Ulloa, 2006). In Ecuador, *Tagetes filifolia* and *T. terniflora* are used as food additives (De la Torre et al., 2008), also used to dye clothing such as *T. minuta* (brown) and *T. filifolia* (yellow color); and anise (*T. filifolia*) an alcoholic beverage flavor is prepared and given to the chicha and humita (Bertero et al., 2009).

Today we seek further applications of these plants for their chemical properties. Specifically we are working with the essential oil and the aqueous portion of the distillation to leverage their chemical compounds as bactericides (*T. patula*) (Wan et al., 2015), antitripanosomal (*T. caracasana*, *T. zypaquirensis* and *T. heterocarpa*) (Escobar et al., 2009); Biopesticides (*T. caracasana* and *T. zypaquirensis*) (Barrientos et al., 2012) for its biological effect against fungi and nematodes (Tariq et al., 2010).

The low production costs of essential oil of this species (Serrato, 2003) and its organic origin, represent an important economic and ecological option compared to synthetic insecticides products, which, besides being a source of environmental pollution and damage to human health, are partly due to unprofitability of agricultural production systems.

There are few studies on endemic species *Tagetes* Ecuador in chemical composition. Therefore, this paper aims to provide knowledge about the chemical composition of species distributed in Ecuador, in addition to well protect the natural resources of this country.

For Ecuador eight species are recognized: *T. terniflora*, *T. filifolia*, *T. verticillata*, *T. minuta*, *T. multiflora*, *T. zypaquirensis* and *T. dianthiflora* (Tropicos, 2015) they are found in the Andes, between the 2000-3500 meters, while introduced species and domesticated and *T. erecta* and *T. patula* covering an altitudinal range of 0-3000 meters (Tropicos, 2015).

Among the chemical compounds of *Tagetes* they are: thiophenes, phenols, flavonoids, coumarins and terpenes, which have biological activity nematicide, insecticide and acaricide (Nava et al, 2012.).

Several environmental factors are involved in the chemical composition of the essential oil, for example, nutrition, climate issues, water availability, amount and intensity of light and soil type; in mint, monoterpenes metabolism is influenced by environmental factors. (Burbott and Loomis, 1967). *T. filifolia* in different geographic areas of Argentina where it is distributed naturally variation of chemical compounds is observed in the essential oil (Maestri et al., 1991).

Whereas no background on the profile of essential oils Ecuador species and that the distribution of these species correspond to different geographic locations, the objective of this study was to identify the chemical constituents in the essential oil of plants and five species populations obtained in situ.

Methodology

Collection of biological material

To perform the essential oil extraction plant fresh tissue it was collected in five species of flowering *Tagetes* Ecuador.

In the case of *T. filifolia*, the samples are located in the provinces of Canar, Loja, Pichincha; *T. terniflora*, in the provinces of Canar, Chimborazo, Pichincha, Tungurahua; *T. minuta* in the province of Pichincha; *T. multiflora* in the provinces of Azuay, Canar, Chimborazo, Pichincha and *T. zypaquirensis* in the provinces of Bolivar, Canar, Carchi, Chimborazo, Cotopaxi, Imbabura, Pichincha (Table 1).

<i>Tagetes</i> species	altitude meters	coordinates
<i>filifolia</i>	3090	N 0°17'24,2" O 78°21'17,2"
<i>filifolia</i>	2497	S 00°15'42,1" O 78°22'59,9"
<i>filifolia</i>	2461	S 1°4'11,1" O 78°1'49"
<i>minuta</i>	2656	S 0°5'59,8" O 78°26'44,3"
<i>minuta</i>	2500	N 00°00'44,0" O 78°25'52,2"
<i>minuta</i>	2445	S 0°6'7,1" O 78°18'23,4"
<i>multiflora</i>	2445	S 0°6'7,1" O 78°18'23,4"
<i>multiflora</i>	2010	S 0°1'29,02`` 78°20'5,3`` O
<i>multiflora</i>	1935	S 0°4'11,8`` 78°22'23,89`` O

<i>terniflora</i>	2656	S 0°5'59,8" O 78°26'44,3"
<i>terniflora</i>	2600	S 1°25'57,4" O 78°30'52,7"
<i>terniflora</i>	2078	S 4°0,7'22,15`` 70°12'7,51`` O
<i>terniflora</i>	2010	S 0°1'29,02`` 78°20'5,3`` O
<i>zypaquirensis</i>	3154	S 0°41'56" O 78°5'2,8"
<i>zypaquirensis</i>	3090	N 0°17'24,2" O 78°21'17,2"
<i>zypaquirensis</i>	2490	N 0°16'46,7" O 78°14'48,3"
<i>zypaquirensis</i>	2078	S 4°0,7'22,15`` 70°12'7,51`` O

Table 1 Coordinates of biological material collected

Extraction of essential oil

The fresh tissue was cut into pieces of 3 cm and led to distillation in a Clevenger (Günther, 1948) series-connected computer, using round flasks of 1 L capacity; distillation of essential oils by hydrodistillation was a process that lasted for 45-60 min. Each species and population separately distilled. Once the extraction is completed, the amber oil was stored in base plates and cooling were carried.

Determination of chemical compounds

The chemical composition of the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS) on a Polaris Q Finnigan Trace GC Ultra equipped with Polaris Q mass detector, electron impact (70 eV). One diphenyl dimethyl polysiloxane 5MX RTX-column (5:95) of 30 x 0.25 mm D x 0.25 microns was used. The injector and detector were set at 250 and 300 ° C. The oven temperature started at 70 ° C, 1 min remained well and was programmed to reach 250 ° C at a rate of 20 ° C.min-1.

Helium as carrier gas was used at a flow rate of 1 ml min⁻¹. Diluted samples (1/100 in methylene v / v chloride) 1 µL injected manually, Split (scan) mode and three replicates of each species were made.

For a description of the major results only compounds relative percentages corresponded to more than 1% were considered.

Quantitative data were obtained from a percentage of peak area.

Identification of components was done by comparing retention indices and mass spectra relative to the NIST database system GC-MS and spectral data published by Allured Publishing Corp., Carol Stream, Illinois, (Adams, 2001).

Results and discussion

The retention times of the majority in the essential oil molecules species from Ecuador showed 4.4 to 10.6. The major species were composed as follows: anethole (66.9%), estragole (31.6%) and anise aldehyde (1.4%) in *T. filifolia*; trans-tagetone (52.7%), 4-ethyl-4-methyl-1-hexene (25.5%), verbenone (3.3%), 1-verbenone (3%), β-ocimene (8.6%), β-linalool (1.1%) and cis-tagetone (6.2%) in *T. terniflora*; trans-tagetone (33.9%), 4-ethyl-4-methyl-1-hexene (13.8%), caryophyllene (3.1%), β-ocimene 16.9%), trans-ocimene (3.7%), cis-tagetone (9.1 %), 1-verbenone (11.69%) and verbenone (16.5%) in *T. minuta*; trans-tagetone (17.8%), trans-ocimene (3.7%), 1-verbenone (13.8%), verbenone (24.3%), 2.3 epoxy pinene (0.4%) and 4-ethyl-4-methyl-1-hexene (39.6%) in *T. zypaquirensis*; and trans-tagetone (30.9%), trans-ocimene (25.6%) and valeric (43.4%) acid in *T. multiflora*. Secondary metabolites in *T. filifolia* correspond to phenylpropanoids, while in other species it is terpenes.

The results are described for the first *Tagetes* species of Ecuador, and give an idea of intra- and inter-specific variability that exists in the type of chemical and the concentration of the same partners phyto geographic area they inhabit such species.

Previously it reported some species composition: dill and estragole in *Tagetes filifolia* (. Maestri et al, 1991); tagetone, dihydrotagetone, ocimene and ocimenone in *T. terniflora*; tagetone and dihydrotagetone in *T. minuta* (Chamorro et al., 2008) in *T. zypaquirensis* dihydrotagetone; and tagetone, ocimenone and tagetenona in *T. multiflora*.

The next action research would be the assessment of the performance of oils by piloting as they have limited data of biomass production and oil extraction on an industrial level *Tagetes* (Saavedra et al., 2003) to determine the economic viability of this biotechnology (Serrato, Barajas & Diaz, 2007).

Specifically on the variability of essential oils (Figures 1, 2, 3 and 4) several trends: a) the relative abundance of molecules among populations of the same species differs depending on geographical origin, for example, in *T. filifolia* found in higher percentage anethole and estragole in both populations and *T. zypaquirensis* find molecules as trans-tagetone, verbenone, 1-verbenone, trans-ocimene, pinane 2.3 epoxy and 4-ethyl-4-methyl-1-hexane (Figures 1 and 4); b) molecules that occur in some people, not produced elsewhere and *T. minuta* (trans-tagetone (64.48%), trans-ocimene (11.2%) and cariofilene (9.56%)) and *T. terniflora* (verbenone (8.94%), 1-verbenone (9.07%) and 0.23% cariofilene)) (Figures 2 and 3).

These results match with those obtained by other authors (Giorgi et al., 2005; Badoni et al, 2009; Sarvari, 2009; Mahzooni et al, 2012; Mahzooni-Kachapi et al., 2014), who emphasize in different species the content of essential oil interacts with the geographic location, confirming that between individuals of the same species, chemical components, in some cases vary in concentration and some others disappear and others appear as changes in altitude that developed. In *T. filifolia* variability compounds in the essential oil is a function of the different phyto geographical areas of Argentina (Maestri, Zygadlo, Grosso, Abburra, & Guzman, 1991).

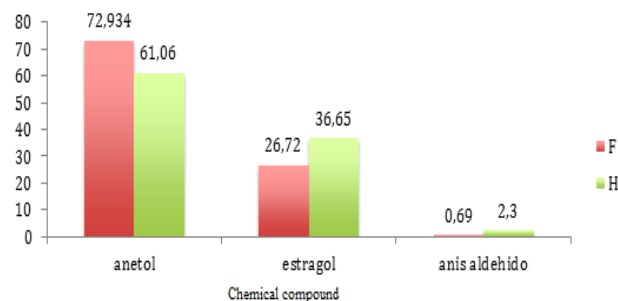


Figure 1 Chemical composition of *Tagetes filifolia*. Populations (F and H)

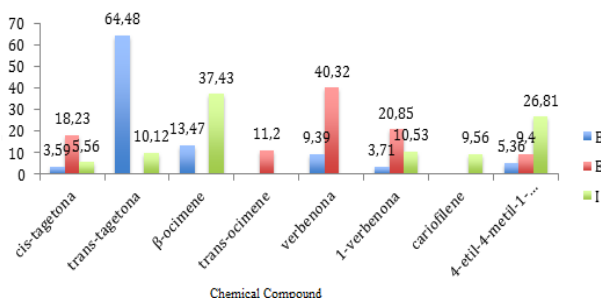


Figure 2 Chemical composition of *Tagetes minuta*. People (B, E and I)

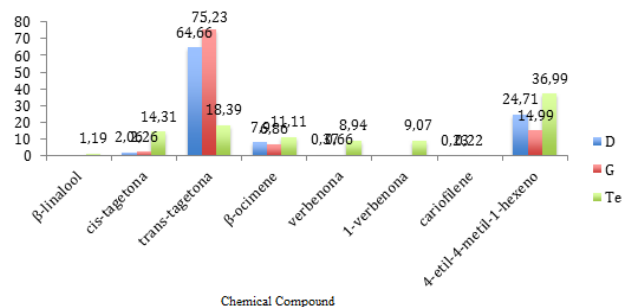


Figure 3 Chemical composition of *Tagetes terniflora*. Towns (D, G and I)

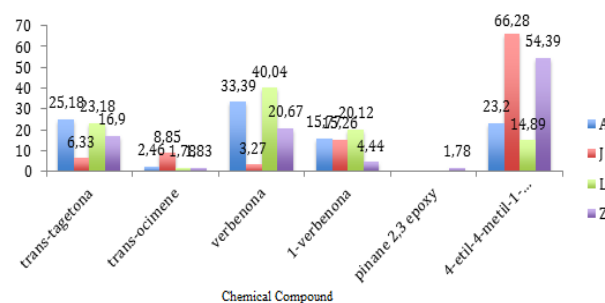


Figure 4 Chemical composition of *Tagetes zipaquirensis*. Populations (A, Y, L and Z)

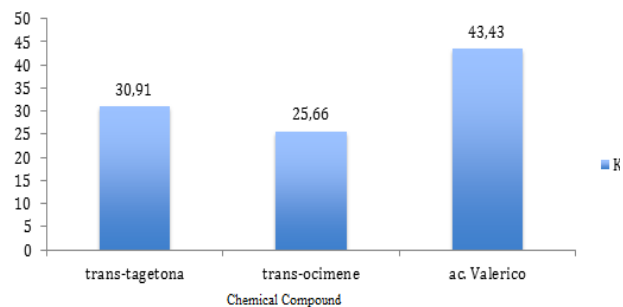


Figure 5 Chemical composition of *Tagetes multiflora*. Populations (K)

Conclusions

In the essential oil of the five species of **Tagetes** Ecuador phenylpropanoids and terpenes found in relative abundance the variability depending on geographical origin.

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