A source of almost pure methyl chavicol: volatile oil from the aerial parts of *Tagetes lucida* (Asteraceae) cultivated in Costa Rica

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Abstract: The plant *Tagetes lucida* Cav. (syn. *T. florida* Sweet, *T. schiedeana* Less.) is an aromatic herb distributed naturally from Mexico to Honduras, at elevations between 1 000 and 2 000 m. It is used as a spice, for medicine, as insecticide and as ornamental plant. It is cultivated commercially in Costa Rica as a spice herb; it contains an oil having an anise-like odor, and the fresh aerial parts of this plant are sold in the supermarket as a substitute of tarragon (*Artemisia dracunculus* L.). The essential oils isolated from aerial parts bought, at May and October, in a supermarket in San José (Costa Rica). Fresh flowering aerial parts, flowers and leaves plus stems, were subjected to hydrodistillation for 3 hr using a modified Clevenger-type apparatus. The distilled oils were collected and dried over anhydrous sodium sulphate and stored in a freezer (0-10°C). The light yellow green oil yield was about 0.07% (v/w). GC/MS analyses were performed using a Shimadzu GCMS-QP5050 apparatus and CLASS 5000 software with Wiley 139 computer database. Identification of the components of the oil was performed using the retention indices, which were calculated in relation to a homologous series of hydrocarbons, and by comparison of their mass spectra with those published in the literature or those of our own database. Thirty compounds were identified, of which methyl chavicol (95-97%) was the major constituent. From flower oil, two bithienyls were detected as minor constituents. Rev. Biol. Trop. 52(4): 853-857. Epub 2005 Jun 24.

Key words: *Tagetes lucida*, Asteraceae, essential oil, phenylpropanoid, methyl chavicol, bithienyls.

The genus *Tagetes* belongs to the Asteraceae family (tribe Tageteae) and consists of approximately 40-50 species (Strother 1977, Lawrence 1985). The plant *Tagetes lucida* Cav. (syn. *T. florida* Sweet, *T. schiedeana* Less.) is commonly known in Mexico as “pericón”, “anisillo” and “flor de Santa María” (Guzmán and Manjarrez 1962). It is cultivated commercially in Costa Rica as a spice herb; it contains an oil having an anise-like odor, and the fresh aerial parts of this plant are sold in the supermarket as a substitute of tarragon (*Artemisia dracunculus* L.). The main constituent of tarragon oil is methyl chavicol (estragole) which may make up 60-75% of the oil (Masada 1976, Bauer *et al.* 1990).

T. lucida is an aromatic herb distributed naturally from Mexico to Honduras, at elevations between 1000 and 2000 m. It has sessile, glabrous, oblong-lanceolate and opposite leaves (5-10 cm long) with yellow terminal flowers.

In Mexico, it is used medicinally as an infusion, and also as an insecticide and as ornamental plant. The infusion is used as a tonic, as a remedy for coughs, headaches, fevers, colic, abdominal pain, gastrointestinal ailments, body ache, and to speed birth (emmenagogue) (Browner 1985, Bye 1986). In Guatemala extracts of this plant are sold as infusion, tincture and elixir (Cáceres 1996). These products are used for stomach pains, gastritis, menstrual pains (Girón *et al.* 1991), to treat infections (Cáceres *et al.* 1993a) and diarrhoea (Cáceres *et al.* 1990, 1993b).

This plant is cultivated in the United States, France and England as a flowering herb

(Morton 1981). In Nottinghamshire (England), at the beginning of the twentieth century, the plant was used in place of tarragon in soup (Morton 1981). Today, this plant is used in the Southern states of USA, as well as in Costa Rica, as a spice for food.

In continuation of our work on the screening of Costa Rican aromatic plants of the genus *Tagetes* (Vila *et al.* 2000), the volatile oil from aerial parts of *T. lucida* cultivated in Costa Rica was examined by GC/MS.

The chemical composition of *Tagetes lucida* (*T. florida*) oil has been the subject of previous studies. French chemists in 1938 identified the phenylpropanoid estragole (methyl chavicol) in high concentration (Anonymous 1938). More recently, the major constituents of this oil were determined to be methyl eugenol (80%) and methyl chavicol (12%) (Guzmán and Manjarrez 1962) or anethole (23.8%), eugenol (24.3%) and methyl chavicol (33.9%) (Bichi *et al.* 1994). From a methanol extract of the entire plant collected in Mexico, four coumarins were isolated (Ríos and Flores 1976). Also, three bithienyls [5-(3-buten-1-ynyl)-2,2'-bithienyl, 5-(4-hydroxy-1-butynyl)-2,2'-bithienyl and 5-(4-acethoxy-1-butynyl)-2,2'-bithienyl] and α-terthienyl were isolated from roots of this plant (at that time known as *T. lucidus*) (Bohlmann *et al.* 1973). Four flavonoid glycosides were identified from aerial parts of the plant collected in Argentina (Abdala 1999). More recently, Aquino *et al.* (2002) identified a new flavonol glycoside and two new phenolic acids from the air-dried leaves of the plant collected in Guatemala.

MATERIALS AND METHODS

Aerial parts of *Tagetes lucida* were bought, at May and October, in a supermarket in San José (Costa Rica). A voucher specimen was deposited at the Herbarium of the University of Costa Rica at the School of Biology (USJ 76083).

Fresh flowering aerial parts, flowers and leaves plus stems, were subjected to hydrodistillation for 3 hr using a modified Clevenger-type apparatus. The distilled oils were collected and dried over anhydrous sodium sulphate and stored in a freezer (0-10°C). The light yellow green oil yield was about 0.07% (v/w).

General analytical procedures: The GC/MS analyses were performed using a Shimadzu GCMS-QP5050 apparatus and CLASS 5000 software with Wiley 139 computer database. The data were obtained on a bonded 5% phenyl methyl silicone fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 μm). Operation conditions were: carrier gas He, flow 1.0 mL/min; oven temperature program: 60°-240°C at 2°C/min; sample injection port temperature 250°C; detector temperature 260°C; ionization voltage: 70eV; ionization current 60 μA; scanning speed 0.5 s over 38-400 amu range; split 1:70.

Identification: Identification of the components of the oil was performed using the retention indices, which were calculated in relation to a homologous series of hydrocarbons, and by comparison of their mass spectra with those published in the literature (Mc Lafferty 1993, Adams 1995, 2001) or those of our own database.

RESULTS

The composition of the oils is summarized in Table 1. Methyl chavicol (1) (Fig. 1) was found to be the major constituent of the oils

1
2
3

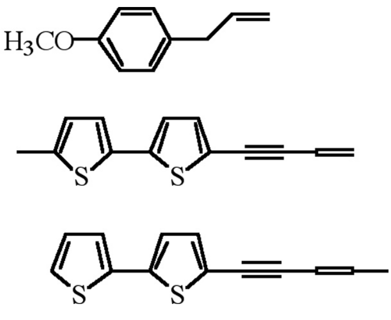

Fig. 1. Essential oils isolated from the aerial parts of *Tagetes lucida*: (1). Methyl chavicol; (2). 5'-methyl-5-(3-buten-1-ynyl)-2,2'-bithienyl; (3). 5-(3-penten-1-ynyl)-2,2'-bithienyl.

TABLE 1

Percentage composition of Tagetes lucida *oils*

Compound[a]	RI	Aerial parts (October)	leaves + stems (May)	Flowers (May)	Method[c]
n-hexanal	802		t		1, 2
ethyl, 2-methylbutanoate			t		1, 2
2*E*-hexenal	855		0.1		1, 2
3*Z*-hexenol	859	t[b]			1, 2
α-pinene	939		x		1, 2
myrcene	991	0.6	1.8	1.2	1, 2, 3
α-phellandrene	1003		t		1, 2
3*Z*-hexenyl acetate	1005	t			1, 2
α-terpinene	1017		t		1, 2
p-cymene	1025	t	t	t	1, 2
limonene	1029	t	0.1	t	1, 2, 3
1,8-cineole	1031	t	t		1, 2, 3
(*Z*)-β-ocimene	1037	t	t	t	1, 2
(*E*)-β-ocimene	1050	0.2	0.2	0.2	1, 2
γ-terpinene	1060	t	t		1, 2
linalool	1097	0.3	0.2	0.2	1, 2, 3
dill ether	1187	t			1, 2
methyl chavicol	1196	97.3	97.1	95.4	1, 2, 3, 4
α-copaene	1377			t	1, 2
(*Z*)-isoeugenol	1407	t			1, 2
β-caryophyllene	1419	0.2	t	0.3	1, 2, 3
α-*trans*-bergamotene	1435	t	t	t	1, 2
α-humulene	1455	t	t	t	1, 2
(*E*)- β-farnesene	1457	0.3	t	0.9	1, 2
germacrene-D	1485	0.5	0.2	0.5	1, 2, 3
(*E*, *E*)-α-farnesene	1506	0.3		0.1	1, 2
δ-cadinene	1523			0.1	1, 2
5’-methyl-5-(3-buten-1-ynyl) -2,2’-bithienyl				0.1	1, 2
5-(3-penten-1-ynyl)-2,2’-bithienyl				0.8	1, 2
n-heneicosane	2 100			t	1, 2

[a] Compounds listed in order of elution from 5% phenyl methyl silicone fused silica column.

RI = Retention Indices on DB-5 (Adams 2001).

[b] t = Traces (<0.05%).

[c] Method 1 = Retention Index; 2 = MS spectra; 3 = standard; 4 = IR, ^{1}H-NMR, ^{13}C-NMR.

(95-97%), with minor quantities of the monoterpenes β-myrcene, (*E*)-β-ocimene and linalool. The sesquiterpene hydrocarbons amount only about 1-2%. β-caryophyllene, (*E*)- β-farnesene, germacrene-D and (*E*, *E*)-α-farnesene are the major ones.

DISCUSSION

The results of the samples from Costa Rica differ from those obtained previously from plants growing in Mexico by Guzmán and Manjarrez, 1962 (where methyl eugenol totals 80% and methyl chavicol only 12%) and from those from Peru obtained by Bichi *et al.* (1994) (where methyl chavicol amounts *ca*. 34% with the presence of anethole, *ca*. 24%, and eugenol, *ca*. 24%).

Like *T. filifolia* from Costa Rica, which contains methyl chavicol (61.2%) and *E*-anethole (33.1%) as main constituents (Vila *et al.* 2000), the oil of *T. lucida* also is rich in one phenylpropanoid and it is almost lacking in ocimenes. This oil does not contain ocimenones, tagetones and dihydrotagetone that are characteristic compounds of the majority of the *Tagetes* oils studied previously (Lawrence 1985, 2000, Zygadlo *et al.* 1993a, b, Tucker and Maciarello 1996, Garg *et al.* 1999).

The flower oil contains as minor constituents two acetylenic thiophenes: 5'-methyl-5-(3-buten-1-ynyl)-2,2'-bithienyl (2) (Fig. 1) and 5-(3-penten-1-ynyl)-2,2'-bithienyl (3). These types of compounds are characteristic secondary metabolites of the Asteraceae family (Bohlmann *et al.* 1973, Sørensen 1977) with interesting biological activities (Chan *et al.* 1979). These two compounds were identified previously from the flower oil of *T. patula* cv. *nana* (Bicchi *et al.* 1992). Compounds containing sulfur are not commonly encountered constituents of essential oils.

It is of interest to mention that methyl chavicol (estragole) is a common constituent of several aromatic plants used as food additives like anise, basil, fennel and tarragon. Biological studies with mice revealed that methyl chavicol is a naturally occurring genotoxic carcinogen after chronic exposure or after few repeated doses (De Vincenzi *et al.* 2000); for this reason, a limit of 0.05 mg/kg is recommended in food.

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RESUMEN

Los aceites esenciales extraídos de las partes aéreas de la planta *Tagetes lucida* Cav. cultivada en Costa Rica y utilizada como condimento, fue estudiado mediante la técnica de GC/MS en combinación con los índices de retención. Se identificaron treinta compuestos. El componente mayoritario resultó ser metil chavicol (estragol) en un 95-97%. En el aceite de las flores se detectaron e identificaron dos compuestos minoritarios que resultaron ser bitienilos no informados anteriormente como constituyentes de esta planta.

Palabras clave: *Tagetes lucida*, Asteraceae, aceites esenciales, fenilpropanoides, metil chavicol, bitienilos.

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