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Essential oil and phototoxic compounds in *Clibadium surinamense* L. and *Montanoa grandiflora* D.C. (Asteraceae)

(With 3 Tables)

Aceites esenciales y compuestos fototóxicos en Clibadium surinamense L. y Montanoa grandiflora D.C. (Asteraceae)

(Con 3 Tablas)

Pérez-Amador* MC, V Muñoz*, A Noyola*, F García-Jiménez**

Abstract. Two Asteraceae species, *Clibadium surinamense* L. and *Montanoa grandiflora* D.C., were analyzed to determine the composition of the essential oil and to search for phototoxic compounds.

Three parts of the plants were studied, inflorescence, stems and leaves. Intra and interspecific differences were found in the essential oil composition, which was determined by gas chromatography.

In the chromatographic profiles, run for phototoxic compounds, spots for these products were present in the Clibadium surinamense extracts, but were absent in *Montanoa grandiflora.*

From the hexane extract of *Clibadium surinamense* inflorescence 2 new polyacetylene esters were isolated, ichtyothereol capric ester and its derivate tetrahydroichthyothereol-7'-en-miristic ester.

Key words: Asteraceae, phototoxic compounds, essential oils.

Resumen. Se analizaron dos especies de Asteráceas, *Clibadium surinamense* L. y *Montanoa grandiflora* D.C., para determinar la composición de su aceite esencial y la presencia de compuestos fototóxicos.

Se analizaron 3 partes de la planta, inflorescencia, tallos y hojas. En la composición del aceite esencial, que se determinó por cromatografía de gases, se encontraron diferencias intra e interespecíficas.

En los perfiles cromatográficos de compuestos fototóxicos se observaron manchas de estos productos en *Clibadium surinamense* pero no en *Montanoa grandiflora.*

Del extracto hexánico de inflorescencia de *Clibadium surinamense* se aislaron dos esteres poliacetilénicos, el ester cáprico de ictiotereol y el ester-7'-en-mirístico de su derivado, el tetrahidroictiotereol.

Palabras clave: Asteráceas, compuestos fototóxicos, aceite esencial.

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INTRODUCTION

Several species of the Asteraceae family contain polyacetylenic and thiophenic compounds which are phototoxic and act as defense of the plants. On the other hand, these compounds could also be used as taxonomic markers of the family or of some of its genera. We have been studying phototoxic compounds with the aim to observe the frequency of their presence in the species. In continuation with this study, in this paper we report the analysis of *Clibadium surinamense* and *Montanoa grandiflora* two species not studied in this respect in Mexico.

The two genera, *Clibadium* and *Montanoa*, occur in southeastern Mexico, Central America and northern part of Southamerica, and comprise about 20 and 25 species, respectively (Arriagada, 1995; Funk, 1982; Mc Vaugh, 1948). Some South American species contain a polyacetylenic compound, ichthyothereol, a fish poison (Czerson et al., 1979). The main purpose of this study is to search for this compound in our mexican plants.

MATERIAL AND METHODS

The plants were collected in Cuetzalan, Puebla, Mexico. Voucher specimens were deposited at the National Herbarium, Instituto de Biología, UNAM (MEXU).

Essential oil.- The plant material was divided into three parts, inflorescence, stem and leaves. The essential oil of the three parts was obtained by steam distillation, then extracted with ether, dried with anhydrous sodium sulfate and the solvent was eliminated at reduced pressure. The residual oil was analyzed by gas chromatography; the *Clibadium surinamense* oil in a column AT Aquawax, 30 m x 0.25 mm and the *Montanoa grandiflora* oil in a column of Methyl Siloxan 25 m x 0.2 mm. Due to the different composition of the essential oils, the two mentioned columns were used to have a better resolution of the less volatile components of *Montanoa grandiflora*. An Agilent 6890 cromatographer was employed and the run conditions were: for the Methyl Siloxan column: carrier gas: helium; detector: FID; Temperature: 290ºC; flux: 1 ml/min. For the AT Aquawax column the conditions were the same, except a lower temperature was used (260ºC).

For the identifications of the compounds a reference of the original oils was used, run under the same conditions.

Phototoxic compounds. The 3 parts of the plants were extracted with hexane (3 x 24 h) at room temperature in darkness and the solvent was eliminated at reduced pressure. From the dry extracts TLC profiles were determined and the biological test carried out.

Chromatographic profiles. The TLC profiles were run on silica gel plates using as a mobile phase hexane-AcOEt 85:15 and detection by UV radiation (365 nm).

Bioassay. The test was carried out with *Bacillus subtilis* ATCC-6051 using the Daniel's method (1965), of paper disc with the extract (0.1, 0.25 and 0.5 mg/ml) on a Petri dish with agar containing a bacterial concentration of 107 UFC, and incubated at 37ºC for 24h. Two series of experiments were run, for comparison, one in the darkness and the other exposed to U.V. light (365 nm).

Compound isolation. The inflorescence extract of *Clibadium surinamense,* that presented the most intense spot in the TLC, indicating the greatest concentration of compounds of the three parts of the plant, was chromatographed in a silica gel Merck G-60 column, eluted first

with hexane and then with hexane : ethyl acetate (90:10), (85:15) and ethyl acetate. From the fractions eluted with hexane : ethyl acetate (90:10) two compounds were obtained which were analyzed by ^{1}H, ^{13}C-NMR, IR, MS and UV spectroscopy.

RESULTS AND DISCUSSION

Essential oils. The essential oil composition in species that grow in the same place is characteristic and may serve to differentiate them acting as interspecific marker (Pérez-Amador et al., 1994).

In this work the results indicate different essential oil composition for each species, this difference being an interspecific marker. Besides, as the plant in both species was divided in 3 parts, leaves, stem and inflorescence, in order to analyze the different distribution of compounds, the results also show intraspecific differences for the essential oils (Table 1).

Essential oil	*Montanoa grandiflora*				*Clibadium surinamense*			
Name	Percentage			R.T.	Percentage			R.T.
	Inflorescence	Stem	Leaves		Inflorescence	Stem	Leaves	
α-pinene	2.70	4.43	4.27	3.63				
β-pinene	---	0.41	---	5.91	35.12	62.68	80.17	5.57
α-phellandrene	0.82	0.33	---	6.51				
β-phellandrene	6.63	7.74	4.22	6.85				
Benzaldehyde	---	0.31	---	4.25				
Camphene	0.48	1.06	0.79	4.35	---	2.72	---	4.50
Limonene	4.91	4.80	2.37	7.72	9.69	2.72	0.36	7.44
Eucalyptol	1.24	1.56	0.94	7.85	0.83	---	4.72	7.77
Menthol	---	0.33	---	18.28	---	0.44	0.40	18.51
Linalool					---	0.25	---	20.45
Citronellal	2.48	3.50	2.82	21.29	---	0.49	0.46	21.35
Geraniol					0.59	---	---	27.66
Dodecanol					3.02	---	0.28	28.85
Terpineol	0.58	---	1.80	19.85				
Geranyl acetate	1.13	---	---	21.65				
Benzyl acetate	---	0.31	---	12.12				
Guayacol	---	---	1.99	24.98				
Methyl eugenol	---	---	0.78	27.66				
Thymol	---	0.31	---	32.05				
Cedrenol	---	---	0.55	32.48				
Terpenil acetate	0.64	---	---	32.74				
Manol	---	0.95	0.77	34.49				

Table 1. Essential oil composition of two species of Asteraceae

Phototoxic compounds. One of the properties of the phototoxic compounds is to produce, on exposure to sun light, skin dermatitis, when touching the plant, a fact that attracted the attention of several researches, among them Bohlmann (1962) and Towers (1977).

Towers and his group analyzed several *Tagetes* species where they found phototoxic compounds which could serve as markers of the genus.

In our study of Asteraceae species searching for phototoxic compounds, we analyzed several Mexican species of *Tagetes* (García Jiménez et al, 1990; Pérez-Amador et al., 1994), *Simsia amplexicaulis* (Pérez-Amador et al., 2000), *Dyssodia* (Pérez-Amador et al., 2004) and now in our search for phototoxic compounds we chose *Clibadium surinamense* and *Montanoa grandiflora* of the state of Puebla.

C. surinamense has been studied in central and South America (Arriagada, 1995) and the purpose of this study is to determine the influence of the region and the climate on the production of ichthyothereol and similar other compounds; *M. grandiflora* has not been studied and is not reported in the literature.

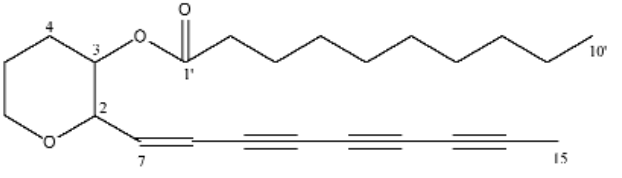
Chromatographic profiles. The chromatographic profiles of *M. grandiflora* did not show spots of phototoxic compounds. The lack of them was confirmed by the absence in the U.V. spectrum of the characteristic maxima at 200 and 300 nm and by the negative bacteriological test. This is the first specie of the Asteraceae we have analyzed that doesn't have these compounds.

The chromatographic profiles of *C. surinamense* in stead showed 2 spots with the characteristic blue fluorescence of the phototoxic compounds with Rfs 3.7 and 2, and gave as well a positive bacteriological test.

Bioassay. *Clibadium surinamense*, after irradiation with UV light (365 nm), showed phototoxic activity against *Bacillus subtilis* in all extracts. The inflorescence was more active than the leaves and stems (85, 64 and 40 % of bacterial growth inhibition, respectively), because it contains a bigger amount of the phototoxic compounds. Montanoa grandiflora did not present any activity.

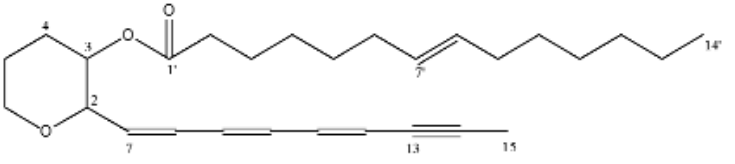
Compound isolation. The inflorescence hexane extract, which is the one that shows a larger amount of these compounds, was chromatographed in a silica gel column and eluted with hexane; hexane : ethyl acetate (90:10), (85:15) and ethyl acetate, 2 compounds of interest were isolated. These compounds were identified, by 1H, 13C-NMR, IR, MS and UV spectroscopy, as the capric ester of ichthyothereol, (Table 2) and the 7'-en-miristic ester of tetrahydroichthyothereol (Table 3), both new compounds isolated from *Clibadium surinamense*.

Table 2. Spectroscopic data of Ichthyothereol capric ester



C		^{1}H NMR (δ)	^{13}C NMR (δ)	
C-2	CH	3.77	129	
C-3	CH	4.5		
C-4	CH_2	1.45, 2.04		UV: 342, 318, 248 y 246 nm IR: 2927.64, 2854.85, 1726.87, 1602.31 y 1464.74 cm-1 MS: 368 m/z p.f.: 50-52 °C
C-5	CH_2	1.5, 1.7		
C-6	CH_2	3.4, 3.9		
C-7	–CH	5.8	129.99	
C-8	–CH	6.29	128.05	
C-9	C	-	68.88	
C-10	C	-	64.39	
C-11	C	-	62.08	
C-12	C	-	62.10	
C-13	C	-	64.38	
C-14	C	-	64.38	
C-15	CH_3	1.94	0.3	
C-1'	CO		174	
C-2'	CH_2	1.63	34	
C-8'	CH_2	1.25	31.91	
C-9'	CH_2	1.30	22.68	
C-10'	CH_3	0.96	14.10	

Table 3. Spectroscopic data of Tetrahydroichthyothereol 7-en-miristic ester



C		^{1}H NMR (δ)	^{13}C NMR (δ)	
C-2	CH	4.32	77.20	
C-3	CH	4.17	72.10	
C-4	CH_2	2.03	27.20	
C-5	CH_2	1.60	22.68	
C-6	CH_2	3.77	62.09	UV: 342, 318, 248 y 246 nm IR: 2927.64, 2854.85, 1726.87, 1602.31 y 1464.74 cm-1 MS: 368 m/z p.f.: 50-52 °C
C-7	–CH	5.97	29.68	
C-8	–CH	6.48	24.82	
C-9	=CH	6.51	31.89	
C-13	C	-	32.75	
C-14	C	-	22.67	
C-15	CH_3	1.80	14.10	
C-1'	CO	-	172.84	
C-2'	CH_2	2.31	37.32	
C-7'	=CH	5.32	130.01	
C-4'	CH_2	1.30	29.68	
C-14'	CH_3	1.0	14.10	

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