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GC-MS ANALYSIS OF THE ESSENTIAL OIL OF COSMOS BIPINNATUS Cav. GROWING IN EGYPT

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ABSTRACT

The composition of hydrodistilled oils of the flower-heads and leaves of Cosmos bipinnatus was investigated by capillary gas chromatography coupled with mass spectrometry. Based on interpreting and comparing the resultant spectra with the available reported data, sixteen components were identified in the flower heads amounting to 91.58% and six in the leaves amounting to 98.28%. Hydrocarbons were found predominent in both oils and alcohols are present in variable concentrations, but the oxides were found in the flower-heads oil only. Both oils showed pronounced antimicrobial activity against certain micro-organisms.

INTRODUCTION

Genus Cosmos Cav. is a rather small genus[1] of the tribe Helianthae (Astraceae), it comprises over 25 species distributed all over tropical America[2]. Plants belonging to this genus are characterized by their nicely coloured flowers[3]. Cosmos bipinnatus Cav. is an annual plant indigenous to Mexico[4] and grows wild in Bolivia and Arizona[3], now it is growing and flourishing in different localities in Egypt as an ornamental garden plant.

Cosmos species were previously reported[5] to possess cytotoxic activity and proved to be effective against human epidermoid carcinoma of nasopharynx.

Earlier examination of the essential oil[6] of Cosmos bipinnatus among other nine plants belonging to family Astraceae cultivated in Norway[6], reported the presence of a volatile hydrocarbon cosmene ($C_{10}H_{14}$), identified as 2,6-dimethyl-octa-1,3,5,7-tetraene,which showed a four-bands UV absorption spectrum. this compound (mp -2° C to - 1° C, B.p 30° C/33 mm), was found to be utterly unstable in air and light, and rapidly polymerises to a white, insoluble solid material. Hence cosmene was obtained when steam distillation was carried out in a stream of nitrogen and the distillate stored under hydroquinone[6,7]. On the basis of the uv spectrum of cosmene and the biogenetic considerations, a synthetic route to this compound had been postulated[6,8].

Concerning Cosmos bipinnatus Cav. growing in Egypt, a botanical study of its different organs, as well as, preliminary phytochemical and biological screenings were carried out[9].

On account of the study of the volatile oil of the plant, nothing was traced in the current literature except for the chemistry of cosmene. Thus, it was deemed of interest to analyze the essential oil of the flower heads and leaves of the plant and screen them for antimicrobial activity.

MATERIALS AND METHODS

Plant Material:

Fresh flower heads and leaves of Cosmos bipinnatus Cav. were collected from plants cultivated in the Experimental Station of Medicinal Plants of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University during July to October 1991. The plant was kindly identified by Dr. N. El- Hadidi, Professor of Plant Taxonomy, Faculty of Science, Cairo University, to whom the author is indebted.

Preparation of the Oils:

The oil from both fresh flower heads and leaves of C. bipinnatus Cav. was separately prepared by hydrodistillation [E.P. 1984] [10].

Gas Chromatography- Mass Spectrometry (GC/MS):

A Finnigan MAT- 5100 series, GC/MS apparatus was used. The analyses of the oils were performed on capillary columns: BD_1-methyl silicon, 30m x 0.22 mm. (i.d.) for flower heads oil and BD_5 -5% phenyl methyl silicon, 15 m x 0.15 mm(i.d.) for leaves oil. Helium was the carrier gas at a rate of 40 ml/min. The temperature programming range was 55-300° C increased at the rate of 5° C/min, then isothermal for 3min. Temperatures of the injector and interface were 220° C. The ion source was electron impact (E.I) at 70 e v and scan range 50-600.

Qualitative identification of the individual component was determined by interpreting their observed mass spectral data, as well as, comparing them with those reported in the literature [11-14]. The peak area method was adopted for quantitative determination of different components,and the data obtained are listed in tables (1 &2).

Screening for Antimicrobial Activity:

The prepared oil of the flower heads and leaves of C.bipinnatus Cav. at different concentrations; 100, 200, 300 and 400 µg/ml were used for testing their antimicrobial activity against the selected microorganisms (Table 3); adopting the disc- agar diffusion method[15]. Ampicillin was used for comparison.

RESULTS AND DISCUSSION

The oil yield obtained by hydrodistillation of the flower heads and leaves of Cosmos bipinnatus Cav. was up to 0.1% and 0.05% v/w respectively. Both oils possessed characteristic persistant aroma and faint yellow colour with white deposits adhearing to the walls of the apparatus. The oils were freely soluble in ether, chloroform, absolute alcohol and 90% alcohol, while the white solids were insoluble in all organic solvents and water.

(Table 2) : GC - MS analysis of the essential oil of the leaves of Cosmos bipinnatus (Cav.)

Peak No	R_t	Component	M^+	B.P	Rel.%
1	5 : 26	(-) -2 Carene	136	59	2.29
2	6 : 06	(+) -4 (10) Carene	136	93	41.58
3	6 : 28	Unidentified *	--	--	0.44
4	7 : 09	Ocimene	136	93	31.32
5	7 : 20	Unidentified *	--	--	0.46
6	8 : 26	p-mentha-1,3,8- triene	134	91	9.87
7	9 : 11	Terpinene - 4 - ol	154	71	6.37
8	12: 18	Unidentified *	--	--	0.48
9	13:19	β - gurjunene	204	161	6.85
10	15: 40	Unidentified *	--	--	0.30

* Concentration in the oil is not sufficient to be analysed by the mass spectrometer, R_t = retention time, M^+ = molecular ion, B.P. = Base peak, Rel.% = relative percentage.

(Table 3) : Results of antimicrobial screening of the flower heads and leaves of C. bipinnatus (Cav.)

Tested microorganism	Conc. in ug/ml								Amp.
	100		200		300		400		
	F. H.	L.	F. H.	L.	F. H.	L.	F. H.	L.	
Staphylococcus aureus	-	-	-	-	-	-	+	+	+
Bacillus subtilis	-	-	-	-	-	-	+	+	+
Streptococcus epidermides	-	-	-	-	-	-	+	+	+
Proteus vulgaris	-	-	-	-	-	-	+	+	+
Pseudomonas aerogenosa	-	-	-	-	-	-	-	-	+
Escherichia coli	-	-	-	-	-	-	-	-	+
Candida albicans	-	-	-	-	-	-	-	-	--

+ means zone of inhibition > 10 mm.
conc = concentration ; F. H. = flower heads ; L. = leaves ; Amp. = ampicillin.

Results of GC/MS analyses of the oils (Table 1 &2) demonstrated the occurrence of at least 25 and 10 components in each of the flower heads oil and leaves oil, respectively. Only 19 components from the flower heads oil and 6 from the leaves oil were present in sufficient concentrations to be analysed by the mass spectrometer.

The rest of components failed to be analyzed and identified because they were present in trace amounts.

The analysis of the flower heads oil (Table 1) allowed the identification of 16 components (accounting to 91.58 %) of those analyzed by GC/MS (19 components). Hydrocarbons represented the most abundant group of the oil (52.13 %) followed by the alcohols (34.23 %) and lastly the oxides (5.22 %). Among the identified hydrocarbons, methyl bornene was the major (24.95%), followed by β-elemene (4.86 %), (+)-4(10) carene (4.6%), γ-gurjunene (3.82%), 2,8-dimethyl-3-isobutenyl-5-isopropyl dihydronaphthalene (3.14%), dekalin (3.13%), caryophyllene (2.90%), longifolene (2.57%) and bulnesene (2.16%). Sabinol alcohol was found to be the second major component of the oil (20.07%), followed by trans- nuciferol (6.44%), pinocarveol (3.55%), Cis-nuciferol (2.36%) and α-terpineol (1.81 %). Linalool oxide (3.06 %) and caryophellene oxide (2.16 %) could also be identified.

On the other hand, six components from the oil of the leaves were identified (Table 2), represented 98.28% of the oil. Hydrocarbons which constituted the bulk of the oil, being 91.91%, the most prominent compound was (+)-4(10) carene (41.58%), followed by ocimene (31.32%), p-mentha 1,3,8 triene (9.87%), β- gurjunene (6.85%) and (-)2-carene (2.29%). Terpinene -4-ol (6.37%) could also be identified.

Comparing the composition of the two oils as represented by GC/MS: hydrocarbons were found in a prominent concentration in both oils (52.13% and 91.91% in the flower heads and leaves respectively). The oil of the flower heads was characterized by the presence of high percentage of alcohols (34.23%) while that of the leaves was relatively low (6.37%). Oxides were detected only in the oil of the flower heads. The only common

(Table 1) : GC - MS analysis of the essential oil of the flower heads of Cosmos bipinnatus (Cav.)

Peak No	R_t	Component	M^+	B.P	Rel.%
1	3 : 25	(+) -4 (10) Carene	136	93	4.6
2	4 : 01	Unidentified *	--	--	0.55
3	4 : 12	Methyl bornene	136	80	24.95
4	5 : 00	Sabinol	152	92	20.07
5	5 : 23	Linalool Oxide	170	59	3.06
6	5 : 41	Pinocarveol	152	91	3.55
7	5 : 50	α - Terpineol	154	59	1.81
8	6 : 06	Dekalin	138	67	3.13
9	6 : 28	Unidentified *	--	--	0.56
10	7 : 10	β- Elemene	204	81	4.86
11	7 : 25	Caryophellene	204	69	2.90
12	7 : 34	β - Bulnesene	204	108	2.16
13	7 : 55	γ - Gurjunene	204	105	3.82
14	8 : 01	Longifolene	204	91	2.57
15	8 : 12	Unidentified *	--	--	0.34
16	8 : 44	Caryophellene Oxide	220	79	2.16
17	9 : 11	Unidentified *	--	--	0.06
18	9 : 39	2,8- dimethyl, 3- isobutenyl, 5- isopropyl dihydronaphthalene	268	253	3.14
19	10: 16	Unidentified	208	73	1.20
20	10: 50	Unidentified	253	156	1.11
21	11: 14	Cis- Nuciferol	218	119	2.36
22	11: 38	Trans- Nuciferol	218	119	6.44
23	12: 03	Unidentified	198	135	2.09
24	13: 13	Unidentified *	--	--	0.85
25	15: 24	Unidentified	--	--	0.87

* Concentration in the oil is not sufficent to be analysed by the mass spectrometer, R_t = retention time, M^+ = molecular ion, B.P. = Base peak, Rel.% = relative percentage.

component identified in both oils was (+) -4(10) carene; its concentration in the leaves being almost ten times that in the flower heads (41.58 and 4.6% respectively). The variations in the other components of both oils may be attributed to the difference in the nature of the organs from which the oils were obtained.

However, cosmene which was detected in a previous investigation failed to show-up under experimental conditions adopted, a fact which may be attributed to its very high volatility at low temperature and its great instability[6].

Screening for the antimicrobial activity of the oils (Table 3) showed pronounced activity against S. aureus, B. subtilis, S. epidermides and Pr. vulgaris at a concentration of 400 µg/ml.

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تحليل الزيت الطيار لنبات كوزموس بيبناتس(كاف)الذى ينمو فى مصر باستعمال كروماتوجرافيا الغاز المتصلة بمطياف الكتلة

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تم فى هذا البحث دراسة الزيت الطيار لكل من هامات وأوراق نبات كوزموس بيبناتس(كاف) وذلك بإستخدام كروماتوجرافيا الغاز المتصلة بمطياف الكتلة .

وقد أمكن التعرف على ستة عشر مركبا فى الهامات وستة أخرى فى الأوراق والذى تمثل فى كل منهما حوالى ٩١٫٥٨٪ و ٩٨٫٢٨٪ على الترتيب . وقد وجد أن المواد الهيدروكربونية تمثل أعلى نسبة فى كل من الزيتين ولكن المواد الكحولية توجد فيهما بدرجات مختلفة التركيز . أما الأكاسيد فقد لوحظ أنها توجد فقط فى الزيت الطيار للهامات .

وقد وجد أن للزيت الطيار لكل من الهامات والأوراق تأثير فعال ضد بعض الميكروبات التى وقع عليها الإختيار .