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Volatiles Constituents and Anticonvulsant Activity of the Aerial Parts of *Dichrostachys Cinerea* L

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Abstract
GC/MS analysis of the volatile constituents of aerial parts of *D. cinerea* revealed the identification of 70 compounds representing (86.13%) of the total volatiles of the plant. α-Pinene (26.47%), nonanal (5.11%) and 8, 11, 14 eicosatrienoic acid (4.90%) were found to be the major compounds. Oxygenated compounds constituted (32.81%) mainly attributed to nonanal (5.11%), 8, 11, 14 eicosatrienoic acid (4.90%), hexadecanoic acid (2.95%), phytol (1.17%), β-ionone (1.45%), and methylisoeugenol (1.08%). Anticonvulsant activity of the total ethanol extract and successive extracts were investigated which revealed that highest activities were exhibited after 2 hours by100mg of total ethanol extract (80.87% potency), methanol extract (121.76% potency) and 100mg of ethyl acetate extract (74.15% potency) respectively.

Key Words: Volatile constituents, α Pinene, nonanal, anticonvulsant activity.

Introduction

The aim of the present study was to identify the volatile constituents and anticonvulcent activities of the aerial parts of *Dichrostachys cinerea. D.cinerea* belongs to Family Fabaceae the tree is up to 7 meter tall with very characteristic bi-coloured flowers (bottlebrush) and is native to South Africa. The plant was used in the traditional Indian system of medicine which is reported to be used in many cases, the bark of the plant is used to treat dysentery, tooth-aches and elephantiasis. The leaves are laxative and used to treat gonorrhoea, boils, stomache problems, can remove poison from snake-bites, as aphrodisiac and as astringent for scorpion bite. Root infusions are taken for leprosy, syphilis coughs, as anthelmentic, purgative and strong diuretic. The plant is used in veterinary medicine in India[(1)]. Joshi and Sharma[(2)] reported that triterpenoids and some other constituents from *Dichrostachys cinerea*, Friedelin, friedlan 3β-ol, β-sitosterol and α-amyrin were isolated from the bark of the plant. Heart wood of the plant contained octacosanol and sitosterol, the leaves containe hentricontanol ,and β–amyrin and β-sitosterol. The phenolic constituents of the aerial parts of the plant were studied, the plant was found to posses good anti-inflammatory, antiulcer and antioxidant activity[(3)].

Materials

Plant material

The aerial parts of *D. cinerea* (leaves, stems and flowers) were obtained from the Orman Botanical Garden, Giza, Egypt. The plant was authenticated by Mrs. Terase Labib, Taxonomist of Orman Garden and confirmed by the Taxonomist, Dr. M. El-Gebaly, National Research Centre (NRC). A voucher specimen (No.135-2003) was kept in the Herbarium of Pharmacognosy Department, NRC.

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Experimental animals

Adult Sprauge Dawely albino rats weighing 130-150 g were obtained from the Animal House Colony of the National Research Centre, Egypt. they were kept under the same hygienic conditions and well balanced diet and water. All animal procedures were performed after approval from the ethics committee of the National Research Centre and in accordance with the recommendations for the proper care and use of Laboratory animals (NIH Publication No. 85-23, revised, 1985).

Drugs

Carbamazepin (Tegretol, Swiss Pharma S.A.E., Cairo, Egypt), was used as a standard anticonvulsant drug.

Apparatus

1. Gas Chromatograph Coupled with a Mass Spectrometers GC / MS Finnigan mat SSQ 7000, Digital DEC EL eV 70 for GC/MS analysis of volatiles.
2. Modified Likens and Nikerson apparatus was used for preparation of the volatile constituents.

Methods

Preparation of the volatile constituents

Three kg of fresh aerial parts of *D.cinerea* were subjected to steam distillation in a modified Lickens and Nikerson apparatus[4], which allowed the simultaneous extraction of the volatile components in an organic solvent (n-pentane). The solvent was evaporated carefully after dehydration over anhydrous sodium sulphate. The yielded volatiles were kept in a refrigerator for GC/MS analysis.

Conditions of GC/MS analysis

Capillary column; DB-5 fused silica (5% phenyl methylpolysiloxane), 30 m length, 0.25 mm id and 0.25 µm thickness; Carrier Gas was Helium at 13 psi.; oven temperature was programmed at 60°C isothermal for 3 min. then heating to 260°C at a rate of 4°C /min. then isothermal at 260°C for 5 min; Injector Temperature 220 °C; Ionization Energy, 70eV; Volume Injected was 1µl.

Identification of the constituents was carried out by comparison of their retention times, and mass spectral fragmentation patterns with those of the available database librarie (Wiley Int. USA) and NIST (Nat. Inst. St. Technol., USA)], and/or published data[1,3]. Eight Peaks Index of Mass Spectra[5] and Jennings and Shibamato[6]. Quantitative determination was carried out based on peak area integration.The identified compounds of the volatile constituents in Table 1.

B. Investigation of biological activities

Anticonvulsant activity test:

This activity was carried out according to the method reported by Rizzo, et al [7].

Seventy two adult male albino rats, divided into twelve groups, each of six animals were orally treated with two doses(50,100 mg/kg.bwt) of each extract, carbamazepin (positive control, 100mg/ kg.bwt), or saline (negative control). Electrical stimulation was applied to the rat ear by using 515 Master Shocker (Lafayette Inst. Co.). The increase in voltage required to induce an electrical shock in treated animals is taken as a measure of anticonvulsant activity. Results are compiled in Table 3.

Conflict of Interest statement: The authors are declaring no conflicts of interest.

Table 1: Results of GC/MS analysis of the volatile constituents of the aerial parts of *Dichrostachys cinerea* L.

No.	Compound	RRt	B.P.	M^+	Rel.%	M. formula
1	α- Pinene	1.00	93	136	26.47	$C_{10}H_{16}$
2	β- Pinene	1.19	93	136	0.21	$C_{10}H_{16}$
3	Myrcene	1.29	93	136	1.07	$C_{10}H_{16}$
4	2-Carene	1.36	93	136	2.54	$C_{10}H_{16}$
5	3-Carene	1.49	93	136	1.05	$C_{10}H_{16}$
6	cis -Ocimene	1.54	43	136	0.06	$C_{10}H_{16}$
7	γ- Terpinene	1.644	43	136	0.14	$C_{10}H_{16}$
8	Terpinolene	1.805	121	136	0.97	$C_{10}H_{16}$
9	n-Undecane	1.86	43	156	0.16	$C_{11}H_{24}$
10	n-Nonanal	1.97	41	142	5.11	$C_9H_{18}O$
11	Orthonitrophenol	2.10	43	139	0.07	$C_6H_5O_3N$
12	trans- Pinocarveol	2.27	41	152	0.22	$C_{10}H_{16}O$
13	Methylsalicylate	2.45	120	152	0.98	$C_8H_8O_3$

14	n-Dodecane	2.47	43	170	0.61	$C_{12}H_{26}$
15	Pulegone	2.60	41	152	0.25	$C_{10}H_{16}O$
16	4-Methyl-2-nitro-phenol	2.75	153	153	0.12	$C_7H_7O_3N$
17	1-Decanol	2.81	41	158	0.17	$C_{12}H_{26}O$
18	5-Methyl dodecane	2.91	43	184	0.27	$C_{13}H_{28}$
19	n-Tridecane	3.06	43	184	0.13	$C_{13}H_{28}$
20	Cumine alcohol	3.11	135	150	0.47	$C_{10}H_{14}O$
21	4- Methyl tetradecane	3.13	43	212	0.49	$C_{15}H_{32}$
22	3-Methoxy benzoic acid	3.24	152	152	0.48	$C_8H_8O_3$
23	6-Tridecen-4-yne	3.27	79	178	0.70	$C_{13}H_{22}$
24	1,2,3,4-Tetrahydro,1,1,6-trimethylnaphthalene	3.31	159	174	0.38	$C_{13}H_{18}$
25	α -Copaene	3.43	41	204	0.32	$C_{15}H_{24}$
26	2,4- Dodecadienal	3.46	55	180	0.15	$C_{12}H_{20}O$
27	cis-Jasmone	3.56	79	164	0.98	$C_{11}H_{16}O$
28	n-Tetradecane	3.58	57	198	0.13	$C_{14}H_30$
29	β -Caryophyllene	3.71	41	204	1.01	$C_{15}H_{24}$
30	α -Santalene	3.72	93	204	0.13	$C_{15}H_{24}$
31	Hexylresorcinol	3.77	123	194	0.13	$C_{12}H_{18}O_2$
32	Nerylacetone	3.86	43	194	0.43	$C_{13}H_{22}O$
33	α -Humulene	3.88	93	204	0.26	$C_{15}H_{24}$
34	cis -Methylisoeugenol	3.90	135	178	0.68	$C_{11}H_{14}O_2$
35	β- Ionone	4.02	177	192	1.45	$C_{13}H_{20}O$
36	2-Methyl tetradecane	4.04	43	212	0.42	$C_{15}H_{32}$
37	Tran- Methylisoeugenol	4.19	178	178	1.08	$C_{11}H_{14}O_2$
38	Tran-Nerolidol	4.42	41	222	0.25	$C_{15}H_{26}O$
39	Spathulenol	4.50	43	220	0.20	$C_{15}H_{24}O$
40	n-Hexadecane	4.56	57	226	0.61	$C_{16}H_{34}$
41	Methyl-8(2furyl)octanoate	4.68	81	224	0.16	$C_{13}H_{20}O_3$
42	1-Tetradecanol	4.89	43	214	0.15	$C_{14}H_{30}O$
43	2-Methyl hexadecane	5.02	43	240	0.40	$C_{17}H_{36}$
44	n-Heptadecane	5.04	57	240	1.31	$C_{17}H_{36}$
45	Hexadecanal	5.07	41	240	0.49	$C_{16}H_{32}O$
46	1-Pentadecanol	5.16	43	228	0.11	$C_{15}H_{32}O$
47	2-Methyl heptatadecane	5.21	43	254	0.72	$C_{18}H_{38}$
48	n-Octadecane	6.33	57	254	1.45	$C_{18}H_{38}$
49	1-Eicosyne	5.64	43	278	1.03	$C_{20}H_{38}$
50	6,10,14Trimethyl-2-pentadecanone-	5.65	43	268	0.93	$C_{18}H_{36}O$
51	Neophytadiene	5.66	82	278	0.39	$C_{20}H_{38}$

52	9-Eicosyne	5.81	81	278	0.46	$C_{20}H_{38}$
53	Methyl 8,11,14-heptadecatrienoate	5.85	79	278	0.79	$C_{18}H_{30}O_2$
54	n-Nonadecane	5.87	43	268	1.05	$C_{19}H_{40}$
55	Methylhexadecanoate	6.00	74	270	0.94	$C_{17}H_{34}O_2$
56	Bis(2-ethylbutyl)phthalate	6.12	149	334	1.59	$C_{20}H_{30}O_4$
57	Hexadecanoic acid	6.30	256	256	2.95	$C_{16}H_{32}O_2$
58	n-Eicosane	6.35	57	282	0.47	$C_{20}H_{42}$
59	Methyl-9-octadecenoate	6.67	55	296	3.78	$C_{19}H_{36}O_2$
60	Phytol	6.78	71	296	1.17	$C_{20}H_{40}O$
61	8,11,14-Eicosatrienoic acid	7.00	41	306	4.90	$C_{20}H_{34}O_2$
62	n-Docosane	7.09	43	310	0.88	$C_{22}H_{46}$
63	n-Tricosane	7.43	57	324	1.82	$C_{23}H_{48}$
64	n-Tetracosane	7.77	57	338	1.00	$C_{24}H_{50}$
65	n-Pentacosane	8.09	57	352	1.09	$C_{25}H_{52}$
66	Bis(2ethylhexyl)phthalate	8.21	149	390	1.65	$C_{24}H_{38}O_4$
67	n-Hexacosane	8.41	57	366	0.75	$C_{26}H_{54}$
68	n-Heptacosane	8.75	57	380	1.44	$C_{27}H_{56}$
69	n-Octacosane	9.17	57	394	0.24	$C_{28}H_{58}$
70	Squalene	9.19	69	410	0.70	$C_{30}H_{50}$
	Total identified constituents	--	--	--	86.13	--
	Unidentified constituents	--	--	--	13.87	--

RRt = retention time relative to α -Pinene (Rt=6.22 min)

Table 2: Percentage of different chemical classes in the volatiles of aerial parts of *Dichrostachys cinerea* L.

Chemical class	Relative area percentage	Chemical class	Relative area percentage
I. Oxygenated Compounds		II. Non-Oxygenated Compounds	
a. Terpenoids	5.409		
b. Long chain	23.708	a. Terpenoids	34.345
c. Aromatic	3.536	b. Long chain	18.595
d. Heterocyclic	0.157	c. Aromatic	0.380
Total	32.81	Total	53.32

Table 3: Anticonvulcent activity of total ethanol and successive extracts of the aerial parts *Dichrostacys cinerea* L.

Group	Dose (mg \kg b.wt.)	Volts needed before treatment (zero time)	Volts needed after single oral dose					
			One hour			Two hours		
			Mean±S.E.	%of change	potency	Mean±S.E.	% of change	potency
Control	1 ml saline	75.9±2.6	76.1±2.3	0.26	0.22	75.8±2.1	0.31	0.23
Ethanol	50	77.1±	112.8±5.1*	46.30	40.34	123.6±5.3*	60.31	43.94
	100	78.2±2.4	121.5±5.6*	55.37	48.24	141.7±8.1*	81.20	59.16
Pet.ether	50	75.1±1.5	98.4±3.9*	31.02	27.02.	105.7±3.2*	40.74	29.68
	100	75.1±2.2	120.7±2.9*	60.71	52.90	127.8±3.7*	70.17	51.12
Chloroform	50.	76.9±1.8	117.3±4.5*	52.53	45.76	121.2±3.6*	57.60	41.97
	100	73.6±3.1	129.5±3.2*	75.95	66.18	142.1±4.8*	93.07	67.81
Ethyl acetate	50	77.3±1.8	88.3±2.9*	14.23	12.40	104.2±5.2*	34.80	25.35
	100	72.9±2.8	136.1±5.6*	86.69	75.53	147.1 ±3.6*	101.78	74.15
Methanol	50.	73.5±2.1	115.9±4.8*	57.69	50.26	118.6±3.8*	61.36	44.70
	100	74.1±3.2	131.1±4.8*	76.92	67.02	146.4 ±4.8*	97.57	71.09
Carbamazepin	100	76.5±2.1	164.3±5.6	114.77	100	181.5±6.2*	137.25	100

*Significantly different from zero time at $p<0.01$

Results and Discussion

GC/MS analysis of the volatile constituents of *D. cinerea* revealed the identification of 70 compounds representing (86.13%) of the total volatiles of the plant. The major compounds were α pinene(26.47%), nonanal(5.109%) and 8,11,14 eicosatrienoic acid (4.898%).

Oxygenated compounds constituted (32.81%) mainly attributed to nonanal(5.109%), 8,11,14 eicosatrienoic acid (4.898%), hexadecanoic acid(2.954%), phytol(1.169%), β-ionone(1.448%), methylisoeugenol (1.080%), methyl salisylate(0.982%) and 3-methoxy benzoic acid (0.477%).

Non oxygenated compounds represent 53.32% atributed to α - pinene(26.47%), carene2(2.541%), tricosane(1.816%), heptacosane, (1.444%), heptadecane(1.311%), nonadecane(1.05%), carene-3 (1.049%), caryophyllene(1.006%), tetracosane(1.001%) and terpinolene (0.970%).

GC/MS analysis of the volatile constituents of *Dichrostachys cinerea* was done for the first time in this study.

The highest anticonvalcent activity was exhibited after 2 hours by100mg of ethyl acetate extract(74.15% potency) followed by 100mg of methanol extract (71.09%) followed 100mg of chloroform extract(67.81%) and in comparison with 100mg of carbamazepine (100%potency).

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