

CHEMICAL CONSTITUENTS OF THE ESSENTIAL OIL FROM THE STEM BARK OF *Cleistopholis Patens*.

(ANNONACEAE)

by

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ABSTRACT

Twenty two Chemical Compounds (mainly terpenes) have been identified in the essential oils of stem barks of swamp and upland *Cleistopholis Patens*. The swamp *C. patens* gave much more essential oil than the upland plant though the oil from the latter was much more fragrant. Seven monoterpene derivatives were identified in the oil from the upland plant whereas no monoterpenes were detected in the essential oil of the swamp plant. Six of the identified compounds were earlier identified by other workers in the essential oil of the roots of *C. Patens* but α -copaene, guaiol, β -eudesmol, bulnesol, carvacrol, α -ionone, α -humulene, germacrene-D, linalool, γ -terpineol, δ -terpineol, geraniol, dimethylterephthalate and pachouli alcohol identified in our essential oils are new to

cleistopholis Patens.

INTRODUCTION

Before the work of Abd-El Atti and co-workers (1) published in 1982 in which they reported the isolation of some alkaloids from extracts of the stem bark, there was no report of any phytochemical work on *C. Patens* inspite of claims about its use in West African traditional medicine for the treatment of worm infestation, infective hepatitis, fever, skin diseases (2, 3) and as a cordial (4). In 1985, Waterman and Muhammad (5) reported the isolation of two novel methyl farnesoate derivatives and some new alkaloids from extracts of both the root and stem barks of *C. Patens*. About the same time Oguntimein and Erhun (6) reported that both

the extract and volatile oil of the stem bark of *C. Patens* exhibited appreciable antibacterial and antifungal action. These workers also identified α -terpinene and M-cymene in the volatile oil and made a partial identification of one of the components of the petroleum extract as a hydroazulene derivative.

Recently, Ekundayo and Oguntimein reported the identification of 15 major constituents of the essential oil of *C. Patens* roots (7) and noted that cadinol and bornyl acetate were the most abundant.

We now report the identification of 22 of the major constituents of the essential oil from the stem bark of *C. Patens* growing in both the swamp and upland areas of Amata town near Owerri in Eastern Nigeria.

EXPERIMENTAL

Materials

The stem bark from *C. Patens* growing in the swamp was collected in Amata town near Owerri on 24th December, 1984 whilst the stem bark from that growing upland was collected in the same town on 13th August, 1985 when rainy season flooding made it unsafe to attempt to get to the ones growing in the swamp. Both barks were air-dried and powdered.

Authentic compounds used for confirmation of identity of the constituents of the volatile oils were either purchased from reputable companies, synthesised in our laboratories, isolated from well documented sources or obtained from reputable researchers. The purity of each authentic compound was ascertained by capillary gas chromatography.

Authentication

The plant was identified as *Cleistopholis Patens*. (Benth) Engls and Diels (Annonaceae) by Dr. Z.O. Gbile of the Forestry Research Institute, Ibadan, Nigeria and a voucher specimen has been kept in the Department of Pharmaceutical Chemistry, University of Benin.

Preparation of the essential oils

600G of powdered stem bark from the swamp *C. Patens* was first extracted with petroleum spirit 60 – 80°C by percolation, (the more highly volatile petroleum spirit 40 – 60°C was found less adequate for extracting the large amount of resinous oily matter that tended to leave the highly porous powder almost at once). Removal of the solvent and subsequent steam distillation of the fragrant brownish oily extract furnished a viscid yellowish oil (4.5g) with a characteristic fragrance which crystallised in the refrigerator and remained

a crystalline semi-solid outside at room temperature, melting range 42–48°C. A methanolic extract of the marc gave on steam distillation a more fragrant yellowish oil (1.75g).

When 600g of powdered stem bark of upland *C. Patens* was similarly treated, a yellow very fragrant essential oil (1.91g) was obtained from the petroleum extract and a definitely more fragrant yellow essential oil (0.11g) was obtained from the methanolic extract.

All the essential oils were subjected to analytical capillary GC with and without internal hydrocarbon standards and to GC – MS measurements.

Analytical GC.

The analytical GC. used for calculation of Retention Index (RI) was measured on an Arian 3700 instrument coupled to a 3390A Hewlett Packard integrator and fitted with a J & W Scientific Inc. fused silica capillary column (30M long, 0.25mm ID, 0.25 μ M film coated with DB5 – 3N). Initial temp. 50°C, programmed 4°C/min. to final temp. 230°C, carrier gas, helium (14 psi). Attenuation 2. Internal hydrocarbon standards C₈ – C₂₂. Retention Index (RI) was calculated for each peak using the established formula:

$$RI = 100.Z + \left(100. \frac{Rt_x - Rt_z}{Rt_{z+1} - Rt_z} \right)$$

Where

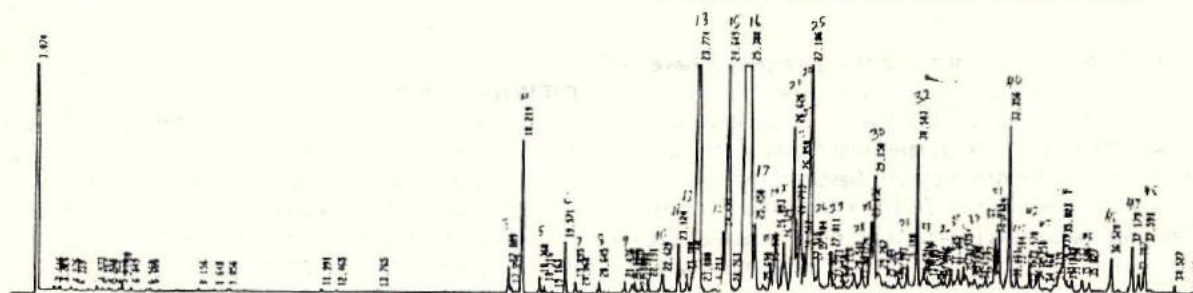
- Z = No. of C – atoms of the 1st standard
- Z+1 = No. of C – atom of the 2nd standard.
- X = Substance to be identified or authentic sample.
- Rt = Retention time.

GC – MS Analysis

This was carried out on a Finnigan/Mat GC – MS instrument model 1020B equipped with a J & W Scientific Inc. fused silica capillary column (30M long, 0.32mm I.D, 0.25 μ m film coated with DB – 5). Injection volume 1 μ l – split 1;20. Carrier gas and temp. programming were same as for analytical GC. MS conditions: ionisation energy 70eV, multiplier spanning 2000V, temp of ion source 180°C. MS was coupled directly to the GC and scanned from 35 to 320 AMU in one sec. The instrument also processed the mass spectral data and carried out computerised library searches.

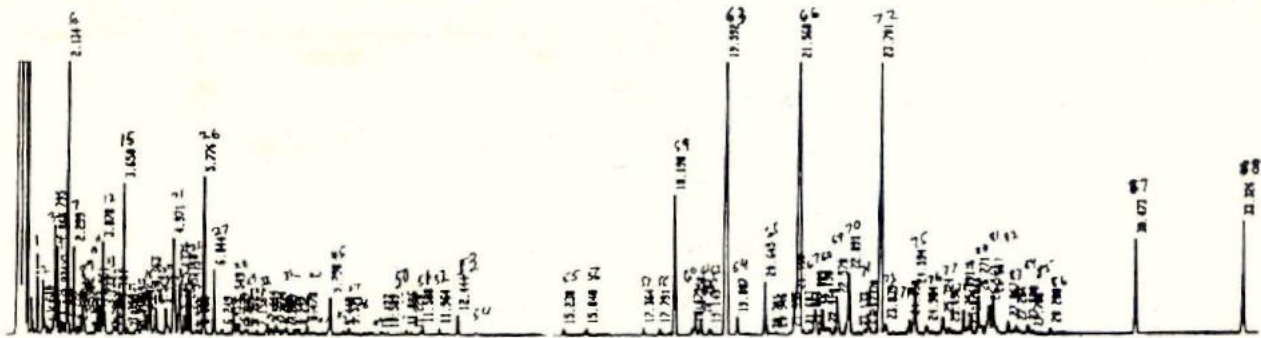
RESULTS AND DISCUSSION

The capillary gas chromatograms of the essential oils from the petroleum spirit extracts of the stem barks of *C. Patens* growing in the swamp and that growing upland are presented as figures 1 and 3 respectively and the compounds identified



LEGEND TO FIG. 1.

Fig. 1 Capillary gas chromatogram of the essential oil from the petroleum spirit extract of the stem bark of *C. Patens* growing in the swamp.



LEGEND TO FIG. 3.
Fig. 3. Capillary gas chromatogram of the essential oil from the petroleum spirit extract of the stem bark of upland *C. Patens*.

or indicated in both oils are respectively recorded in tables I and III.

TABLE I
Constituents of the steam-volatile fraction of the petroleum spirit extract of swamp *C. Patens* stem bark

Peak No.	Retention Index (R.I.)		Identity of constituent		Approximate % abundance
	Calculated for Peak	Calculated for authentic sample	Confirmed	Indicated	
4	1370.60	1370.60	α -copaene		2.2
6	1412.60	1412.0	Caryophyllene		0.7
11	1525.9			Sesquiterpene epoxide mw 220	0.8
13	1545.43	1545.30	Elemol		14.1
14	1570.48			Ferrocyl acetate	0.5
15	1575.35	1575.30	Dihydrocaryophyllene, 1, 2-epoxide		5.8
16				Guzol	41.1
19	1625.30			Sesquiterpene alcohol mw 222	0.8
21	1642.74	1642.70	β -Eudesmol		2.7
23	1650.96			7, 13-Dimethyltetradec-2, 11-enediol	1.9
25	1661.57			Sesquiterpene alcohol mw 222	5.9
32	1702.15	1702.10	Methyl ferrocylate		2.2
47	2043.76			Mono-olefinic primary alcohol mw 254	0.9
48	2060.06			Mono-olefinic primary alcohol mw 254	0.9

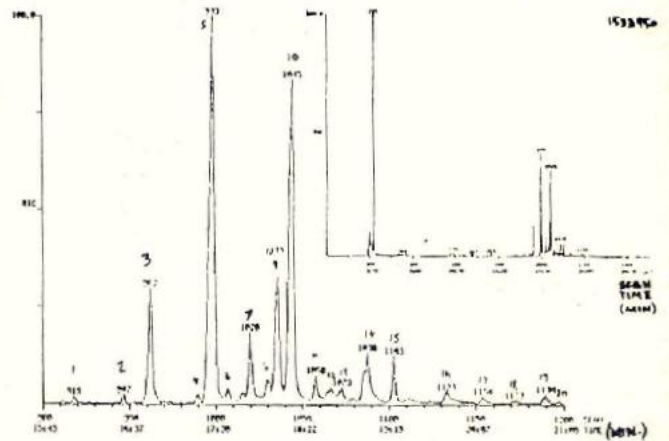
TABLE III
Constituents of the steam-volatile fraction of petroleum spirit extract of upland *C. Patens* stem bark.

Peak No.	Retention Index (R.I.)		Identity of constituents		Approximate % abundance
	Calculated for Peak	Calculated for authentic sample	Confirmed	Indicated	
24	970.82	971.00	β -pinene		0.8
55	1281.75	1281.70	Bornyl acetate		0.13
56	1300.03	1300.00	Carvicol		0.3
59	1370.63	1370.60	α -copaene		3.6
63	1413.06	1412.90	Caryophyllene		14.1
64	1423.04	1422.90	α -ionone		0.5
65	1446.96	1447.20	α -Humulene		1.6
66	1475.42	1475.10	Germacrene-D		13.53
68	1492.58			sesquiterpene hydrocarbon mw 204	1.0
69	1508.46			sesquiterpene epoxide mw 220	1.2
70	1518.96	1319.20	γ -Cadinene		2.0
72	1545.50	1545.30	Elemol		6.4
75	1575.47	1575.30	Dihydrocaryophyllene, 1, 2-epoxide		1.4
78	1615.30			sesquiterpene hydrocarbon mw 204	0.5
79	1625.22			sesquiterpene alcohol mw 222	0.13
80	1632.57			sesquiterpene epoxide mw 220	0.52
81	1642.60	1642.70	β -Eudesmol		1.04
82	1646.05			sesquiterpene alcohol mw 222	1.11
87	1782.35	1782.10	Methyl ferrocylate		2.6

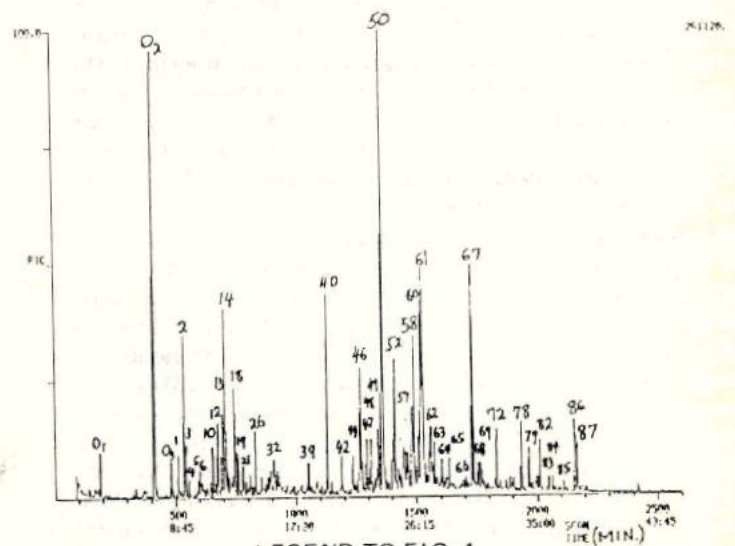
Figures 2 and 4 are the reconstructed ion currents (RIC) respectively of the essential oils from the methanolic extracts of the swamp and upland *C. Patens* and tables II and IV respectively record the compounds identified or indicated in the two oils. In each case, the peak numbers written in the tables correspond to the numbering in the respective chro-

matograms. The recorded approximate percentage abundance was computed by the Hewlett packard integrator.

Individual components of the essential oils were identified by interpreting their MS, comparing these with the MS of the same compounds in the literature where possible and with the



LEGEND TO FIG. 2.
Fig. 2. Reconstructed ion current (RIC) of the essential oil from the methanolic extract of the stem bark of *C. Patens* growing in the swamp.



LEGEND TO FIG. 4.
Fig. 4. Reconstructed ion current (RIC) of the essential oil from the methanolic extract of the stem bark of upland *C. Patens*.

MS of an authentic sample run on the same instrument. The identities of the compounds were further verified by comparative GC and Co-chromatography with authentic compounds. Final confirmation of identity in the case of the terpenes was claimed when the retention index (RI) of the essential oil component corresponded to that of the indicated authentic sample as calculated from capillary GC measured on the same instrument, under the same conditions. In the case of guaial (Peak 16, table I and Peak 5, table II) and dimethylterephthalate (Peak 46, table IV), the compounds were isolated by column chromatography on silica gel and identity established in the usual way by a combination of MS, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, IR and UV.

TABLE II

Constituents of the steam-volatile fraction of the methanolic extract of swamp *C. Patens* stem bark.

Peak No.	Retention Index (R.I.)		Identity of constituent		Approximate % abundance
	Calculated for Peak	Calculated for authentic sample	Confirmed	Indicated	
3	1545.29	1545.30	Elemol		9
5	1593.85		Guaial		33
7	1612.15	1612.20	β -Eudesmol		5
9	1618.21	1618.30	α -Eudesmol		9
10	1625.18	1625.21	Bulnesol		28
11	1630.05			sesquiterpene epoxide mw 220	1
14				sesquiterpene epoxide mw 220	6

TABLE IV

Constituents of the steam-volatile fraction of the methanolic extract of *C. Patens* stem bark.

Peak No.	Retention Index (R.I.)		Identity of constituents		Approximate % abundance
	Calculated for Peak	Calculated for authentic sample	Confirmed	Indicated	
03	8.907			2-Ethylhexan-1-ol	10.0
12	1097.89	1171.60	Linalool		3.5
12	1171.60	1171.60	γ -Terpineol		2.0
14	1185.70	1185.40	α -Terpineol		4.0
18				Benzoic acid, 4-methyl-hydrazide	3.0
26	1252.45	1252.50	Geraniol		2.0
40	1412.87	1412.90	Caryophyllene		6.0
46			1,4-Benzenedicarboxylic acid dimethyl ester, [Dimethylterephthalate]		6.0
50	1575.46	1575.30	Dihydrocaryophyllene-1,2-epoxide		14.0
58	1625.42			sesquiterpene alcohol mw 222	5.0
60	1642.84	1642.70	β -Eudesmol		8.0
61	1648.30	1648.10	Pachouli alcohol		7.0
67	1782.20	1782.10	Methyl farnesoate		7.0

Generally the swamp *C. Patens* gave by far more essential oil than the upland one but the essential oil from the upland plant generally had a more pleasant and delicate fragrance. The fragrance of the essential oil from the methanolic extract of the swamp plant was by far more pleasant and delicate than that from the petroleum extract of the same plant. The higher fragrance of the essential oils from the upland *C. Patens* is to be expected from the presence of 7 odoriferous monoterpenes (β -pinene, bornyl acetate, carvacrol, linalool, γ -terpineol, α -terpineol and geraniol) especially the oxygenated ones. It is significant that monoterpenes or their derivatives were detected only in the oils from the upland *C. Patens*. The two novel esters, methyl-(-)-(trans)-10, 11-dihydroxyfarnesoate and methyl-(+)-10-hydroxy-6, 11-cyclofarnes-7(14)-enoate identified by Waterman and Muhammad (5) as being responsible for the fragrance of their barks were not found in our essential oils. We however found methyl farnesoate (Peak 32, table I; Peak 87, table III and Peak 67, table IV) in the oils of both the swamp and upland *C. Patens*. Ekundayo and Oguntimein (7) also failed to detect the odoriferous novel farnesoates from their root essential oil and we believe that the difficulties in detecting them in the essential oil may have to

do with low steam-volatility of these compounds, especially the dihydroxyfarnesoate whereas the cyclic hydroxyfarnesoate which might be expected to be slightly more steam volatile exists only in extremely small quantities. This assumption is supported by the fact that Waterman and Muhammad isolated these substances in extremely small quantities (0.096% and 0.13% respectively) from solvent extracts and the high retention index (1782.10) and retention time (30.50 mins.) of the non hydroxylated methyl farnesoate in our essential oil give an indication of how much less volatile these hydroxylated oils would be expected to be.

We could not detect α -terpinene and M-cymene identified by Oguntimein and Erhun (6) in their essential oil from the stem bark of the plant growing in Ife but this is perhaps explainable from previously published data which show that the biosynthesis of essential oils is susceptible to influence by the soil conditions, time of harvest and the climate (8 and refs.) leading to significant variations in the amount and composition of essential oils.

Apart from the monoterpenes already mentioned, α -ionone, α -humulene, germacrene-D, γ -cadinene, dimethylterephthalate and pachouli alcohol were present only in the oils from the upland *C. Patens*. On the other hand, guaial, γ -eudesmol, α -eudesmol and bulnesol were found only in the essential oil from the swamp *C. Patens*. It is possible that differences in the soil conditions, season and time of harvest could have contributed to the observed differences in the composition of the essential oils. So far, there has been no incontrovertible scientific proof that *C. Patens* does what the herbalists claim it does and knowledge of the active principles responsible for each of these actions is even more remote. In the circumstance, it is premature to pass judgement as to which of the plants is likely to be more potent solely on the basis of the composition of their essential oils but the preference of the Amata herbalists for the swamp plant is shown by the much larger area of removed stem barks on the swamp trees and the fact that the upland plants are used only when it is impossible to get to the swamp trees.

Caryophyllene, elemol, dihydrocaryophyllene, 1, 2-epoxide, B-pinene, bornyl acetate and γ -cadinene identified in our oils were also identified in the essential oil of the roots by Ekundayo and Oguntimein (7). However, α -copaene, guaial, β -eudesmol, methyl farnesoate, γ -eudesmol, α -eudesmol, bulnesol, carvacrol, α -ionone, α -humulene, germacrene-D, linalool, γ -terpineol, α -terpineol, geraniol, dimethylterephthalate and pachouli alcohol identified in our oils are new to *Cleistopholis Patens*.

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