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Pachypodanthium Staudtii Engl & Diels from Côte d'Ivoire: Composition of Leaf, Stem Bark and Roots Oils

Thierry Acafou Yapi
Laboratoire de Chimie Organique Biologique, UFR-SSMT
Université de Cocody-Abidjan, BPV 34 Abidjan, Côte d'Ivoire

Jean Brice Boti
Laboratoire de Chimie Organique Biologique, UFR-SSMT
Université de Cocody-Abidjan, BPV 34 Abidjan, Côte d'Ivoire

Tonzibo Zanaï Félix
Laboratoire de Chimie Organique Biologique, UFR-SSMT
Université de Cocody-Abidjan, BPV 34 Abidjan, Côte d'Ivoire

Antoine Coffy Ahibo
Laboratoire de Chimie Organique Biologique, UFR-SSMT
Université de Cocody-Abidjan, BPV 34 Abidjan, Côte d'Ivoire

Félix Tomi
Université de Corse-CNRS, UMR 6134 SPE, Equipe Chimie ET Biomasse
Route des Sanguinaires, 20000 Ajaccio, France

Ange Bighelli
Université de Corse-CNRS, UMR 6134 SPE, Equipe Chimie ET Biomasse
Route des Sanguinaires, 20000 Ajaccio, France

Abstract

The compositions of essential oil isolated from leaves, stem bark and roots of *Pachypodanthium staudtii* Engl. & Diels growing wild in Côte d'Ivoire, were investigated by GC (retention indices) and ^{13}C NMR. The oils were found to contain mostly hydrocarbons and aromatic compounds. The composition of root and stem bark oil were dominated by 2,4,5-trimethoxystyrene (42.7 and 47.9% respectively),□followed by Sabinene (15.4-15.8%)□. Conversely, β-elemene, E-β-caryophyllene, β-selinene, β-bisabolene, δ-cadinene, 2,4,5-trimethoxy-1-vinylbenzene were the major components of the leaf oil.

Keywords: *Pachypodanthium staudtii* Engl. & Diels, Annonaceae, terpenes hydrocarbons, 2,4,5-trimethoxystyrene.

1. Introduction

The Annonaceae is a large family of tropical and subtropical trees and shrubs, comprising about 135 genera and more than 2500 species distributed from Africa, Asia, Central and South America to Australia [1, 2]. *Pachypodanthium staudtii* is a widespread tree in west and central Africa, reaching 30 m high, growing in the evergreen forest from Sierra Leone to Nigeria and in Cameroun. It is characterized by its leaves oblong-lanceolate, attenuated the two extremity; 15 to 20 cm in length and 4 to 10 cm in width, with 10-15 pairs of lateral nerves climbing; petiole short and thick. The bark is used as a chest medicine [1], for tumours [1], toothache [2], bronchitis [3] oedemas and cancer [4]. It is added to arrow-poison mixture and considered as an excellent vermifuge [2]. Several papers regarding of *Pachypodanthium staudtii* phytochemistry have been reported. Solvent extracts have been reported to contain alkaloids [5-7], pachysontol [8], norlignans [9], lignans, bisnorlignans, and a potent antiviral flavonol (pachypodol) [4], 2,4,5-trimethoxystyrene [10]. A sample of bark oil of Gabon origin contained 2,4,5-trimethoxystyrene as major component [11]. The composition of two samples of bark oil from Cameroon was dominated by 2,4,5-trimethoxystyrene, β-caryophyllene and Δ-3-carene [12,13]. Furthermore, secondary metabolites (flavonoid [14], 2,4,5-trimethoxystyrene [15], alkaloids [16], lignans, bisnorlignans [17]), the essential oil composition and its anti-plasmodial activities [18], of *Pachypodanthium confine* have been reported in the literature.

Continuing our work on the characterisation of aromatic and medicinal plants growing wild in Côte d'Ivoire, through the chemical composition of their essential oils [19-23], the aim of the present study was to characterise the essential oils from *Pachypodanthium staudtii* and to observe an homogeneity or eventual variability in the composition. The essential oils obtained by hydrodistillation from the leaves, stem and roots bark were investigated by GC (RI) and ^{13}C NMR.

2. Materiel and Methods

2.1. Plant Materiel and Oil Isolation

The leaves (L), stem bark (SB) and roots (R) of *Pachypodanthium staudtii* Engl. & Diels were collected in South-Eastern Côte d'Ivoire, in the Yapo Abbé forest (near Adzopé, samples L1, SB1, R1), in Petit Yapo forest (near Agboville, samples L2 and L3), both forests are situated 40-50 km north of Abidjan, and in Bosse Matte forest (near Abengourou, sample L4) in Eastern Côte d'Ivoire in November 2008. Samples L1, SB1 and R1 belonged to the same plant. Plant material has been authenticated by Professor L. Aké Assi, from the Centre National of Floristique (CNF, Abidjan, Côte d'Ivoire).

Fresh material (1.0-2 Kg) was submitted to hydrodistillation for 4 h using a Clevenger-type apparatus. The oils samples were dried over anhydrous sodium sulfate and stored in refrigerator before analysis.

2.2. Methods

2.2.1. Analytical GC

The GC analysis was carried out with a Perkin-Elmer Autosystem apparatus equipped with two flame ionisation detectors (FID), and fused capillary columns (50 m x 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polymethylsiloxane) and BP-20 (polyethylene glycol). Carrier gas, helium; linear velocity, 0.8 ml/min. The oven temperature was programmed from 60°C to 220°C at 2°C/min and then held isothermal (20 min). Injector temperature: 250°C (injection mode: split 1/60). Detector temperature: 250°C. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

2.2.2. ^{13}C-NMR Analysis

All NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.13 MHz for ^{13}C, equipped with a 5 mm probe, in deuterated chloroform ($CDCl_3$), with all shifts referred to internal tetramethylsilane (TMS). ^{13}C NMR spectra were recorded with the following parameters: pulse width (PW), 4 μs (flip angle 45°); acquisition time, 2.7 s for 128 K data table with a spectral width (SW) of 25000 Hz (250 ppm); digital resolution 0.183 Hz/pt. The number of accumulated scans was 3 000 for each sample (about 50mg of essential oil in 0.5 mL of $CDCl_3$).

2.2.3. Identification of Components

Identification of the individual components was based: (i) on comparison of their GC retention indices (RI) on apolar and polar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation ('Target Compounds' software of Perkin-Elmer), with those of authentic compounds, (ii) by ^{13}C-NMR spectroscopy, following the methodology developed and computerized in our laboratories, using home-made software, by comparison with spectral data of reference compounds compiled in a laboratory-built library.[24-26]. Each component which accounted for 0.4% at least was identified by ^{13}C NMR.

3. Results and Discussion

Hydrodistillation of the fresh plant materiel of *Pachypodanthium staudtii* produced a clear essential oil with following yields (*w/w* calculated on fresh weight basis): leaf oil = 0.037-0.083%; stem bark oil = 0.043% and root oil = 0.044%. Thirty eigth compounds (18 monoterpenes, 17 sesquiterpenes and 3 aromatic compounds) have been identified in the oil samples representing 93% to 86.5% of the whole composition (Table 1).

Leaf oil: The four essential oils were characterised by a high proportion of sesquiterpenes (17 identified compounds, 73.4-43.3%) with β-bisabolene present at medium to high content in almost all samples (19.7-5.8%) (Table 1). The oils were found to possess little differences in the chemical composition but considerable variation in the levels of the individual components. Samples L1 was characterized by the pre-eminence of δ-cadinene□ (13.6%), 2,4,5-trimethoxystyrene □(11.0%) and β-bisabolene (10.4%) while α-phellandrene (6.6%), Sabinene (6.0%) and β-phellandrene (5.9%) were present at appreciable amounts. Conversely, β-bisabolene (17.6%), (E)-□ β-caryophyllene (13.1%), and β-selinene (11.1%) were the major components of sample L2 beside β-pinene (6.7%) and caryophyllene oxide (5.4%).The major components of samples L3 and L4 were 2,4,5-trimethoxystyrene □ (44.8%) and β-elemene (47.3%) respectively. Sample L3 was characterized by containing higher concentration of β-bisabolene (19.7%), and relatively low concentration of germacrene-D (5.8%) while, sample L4 contained medium amount of germacrene-D (8.4%) and relatively low proportions of □-bisabolene (5.8%) and thymol (4.1%).

Stem and root bark oils. The composition of the essential oil extracted from stem and root bark differed drastically from those of the leaf oils (Table 1). The sesquiterpenes which dominated leaf oils were present at low contents. The composition of two samples, exhibited a high amount of 2,4,5-trimethoxystyrene (stem 42,7%) and (root 47,9%), and contained sabinene at the same appreciable amounts (15,4% and 15,8%). However, the oils could be distinguished from each other by the levels of α-pinene and β-selinene. Stem bark oil was characterized by containing appreciable concentration of β-pinene (6.7%), α-pinene (3.9%) and terpinen-4-ol (5.5%) while, root oil contained higher amount of terpinen-4-ol (8.7%) and smaller proportions of β-pinene and□ α-pinene (1.1 and 0.8% respectively). Conversely, β-elemene and δ-cadinene the major components of two samples of leaf

oil, and bicyclogermacrene present at appreciable amount of leaf oil were absent in the stem and root oils.

4. Conclusion

Although some quantitative differences could be observed, 2,4,5-trimethoxystyrene was the major component of the investigated sample root oil and those already reported from gàbon [11] and from Cameroon [12,13]. The composition of *Pachypodanthium confine* bark oil from Cameroon contained mainly cyperene (15.5%), δ-cadinene (8.1%), caryophyllene oxide (7.2%), α-copaene (7.1%) and α-cedrene (6.0%) while 2,4,5-trimethoxystyrene was at very low proportion (0.4%) [18]. Our results which describe the first analysis of *Pachypodanthium staudtii* essential oils from Côte d'Ivoire showed a chemical variability of this plant and it should be of interest to carry out a large sampling. A screening of its potential insecticidal activities may be undertoock. Indeed, 2,4,5-trimethoxystyrene extracted from the plant is used as an insecticide againt *Acanthoscelides obtectus* [27].

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Table 1: Composition of the essential oils from leaves, stem and root bark of *Pachypodanthium staudtii*

				Adzopé			Azaguié	Agboville	Abengouro u
No	**RIa**	**Rip**	**Identified Compounds**	**Stem**	**Root**	**L1**	**L2**	**L3**	**L4**
1	922	1028	α -thujene	2,3	2,5	0.6	0,4		
2	930	1028	α -pinene	3.9	0.8	3.7	3.3		0.5
3	966	1125	Sabinene	15.4	15.8	6.0	3.4	0.4	0.4
4	971	1115	β-pinene	6.7	1.1	4.7	6.7	0.4	0.5
5	980	1162	Myrcene	0.6	0.4	1.3	0.6		
6	997	1168	α -phellandrene	0.8		6.6	0.5		
7	1004	1151	Δ-3-Carene				1.2		
8	1009	1183	α -terpinene	1.1	0.9	0.2	0.5		0.5
9	1011	1274	p-cymene	0.8	0.7	1.6	1.4	0.4	0.5
10	1020	1204	Limonene*	0.5	0.5	2.1	0.8	0.5	0.7
11	1020	1211	β-phellandrene*	1.9	3.0	5.9	0.4	0.4	0.4
12	1020	1213	1.8-cineol*	2.5	2.6	0.4	1.2	0.4	0.4
13	1024	1237	(Z)-□β-Ocimene			0.4	0.8		
14	1035	1249	(E)-□β-Ocimene			0.3			
15	1048	1251	γ-terpinene	2.2	2.3	0.3	0.8		0.5
16	1078	1288	Terpinolene	0.5	0.5	0.4	0.3		
17	1162	1605	Terpinen-4-ol	5.5	8.7	0.8		0.7	0.6
18	1265	2190	Thymol	0.5			0.4		4.1
19	1376	1494	α-copaene			1.8	1.8	0.6	0.4
20	1388	1593	β -elemene		0.4	1.8	1.6	2.0	47.3
21	1399	1873	2,5-dimethoxy-p-cymene					0.3	1.1
22	1418	1600	(E)- β -caryophyllene	0.7		3.5	13.1	2.8	1.1
23	1432	1587	(E)- α -bergamotene				0.4	0.4	0.5
24	1451	1674	α -humulene			1.7	1.5	2.3	0.4
25	1478	1712	Germacrene D	0.5		1.5	2.0	5.8	8.4
26	1482	1724	β -selinene	0.7		4.3	**11.1**	3.8	2.2
27	1491	1728	bicyclogermacrene			3.9	4.2	2.2	2.3
28	1500	1730	β -bisabolene	2.3	1.2	10.4	17.6	19.7	5.8
29	1514	1765	δ -cadinene			13.6	0.9	1.8	1.0
30	1521	2337	2,4,5-trimethoxystyrene	42.7	47.9	11.0	1.6	44.8	1.5

31	1534	2081	β -elemol					1.4	1.2
32	1572	1987	Caryophyllene oxide			0.4	5.4	0.4	0.4
33	1585	2091	guaiol						0.8
34	1643	2238	α -cadinol			0.5			0.5

Table 1: Composition of the essential oils from leaves, stem and root bark of *Pachypodanthium staudtii* - continued

35	1643	2235	Selina-11-en-4α -ol						0.6
36	1653	2141	β -bisabolol				0.5		0.6
37	1726	2615	benzyle Benzoate				0.5		2.8
38	2098	2604	trans Phytol			1.1	1.2		
			Monoterpenes hydrocarbons	36.7	28.5	34.1	21.1	2.1	4.4
			Oxygenated monoterpenes	8.5	11.3	1.2	1.6	1.1	5.1
			Sesquiterpenes hydrocarbons	4.2	1.6	42.5	54.2	41.4	69.4
			Oxygenated sesquiterpenes	0	0	2.0	7.1	1.8	4.1
			Aromatic compounds	42.7	47.9	11.0	2.1	45.1	5.4
			TOTAL	**93.0**	**90.1**	**91.6**	**86.5**	**91.2**	**88.3**

L: leaves

Order of elution and percentages are given on apolar column (BP-1), except for compounds with an asterisk (*), percentage on BP-20. Components having their percentage indicated with bold letters were identified by GC/RI and ^{13}C-NMR spectroscopy. The others were identified by comparison of their retention indices on two columns (BP-1 and BP-20) with those of the components reported in Table 1.

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