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Constituents, antibacterial activities and toxicological assay of essential oils of *Artocarpus communis* Forst (Moraceae) and *Sphenocentrum jollyanum* (Menispermaceae)

S. A. ABOABA* and O. EKUNDAYO

Department of Chemistry, University of Ibadan, Ibadan, Nigeria.
** Corresponding author, E-mail: sa.aboaba@mail.ui.edu.ng; Tel: 2348038011394.*

ABSTRACT

The volatile composition of the leaf of *Artocarpus communis* Forst (Moraceae) and root of *Sphenocentrum jollyanum* (Menispermaceae) were studied. The essential oils were obtained by hydrodistillation in a modified Clevenger-type apparatus while the analyses were performed by GC and GC-MS. The main constituents of *A. communis* (leaf) were α-pinene (14.1%), 6-methyl-5-heptene-2-one (7.0%), α-phellandrene (8.9%), α-ionone (6.1%), E-nerolidol (8.3%) and 1,8-cineole (5.5%). The main compounds identified in the root oil of *S. jollyanum* were α-eudesmol (26.1%), α-pinene (11.2%), isocaryophyllene (7.7%), 1.8-cineole (6.3%) and β-pinene (5.3%). The yields of the oils were 0.15% and 0.20% for *S. jollyanum* and *A. communis* respectively. The toxicity of both oils to the brine shrimp indicated that *A. communis* had LC_{50} of 24.50 µg/mL and *S. jollyanum* 84.87 µg/mL. *A. communis* showed very weak activity to *P. mirabilis* while *S. jollyanum* was active against *B. subtilis* and *P. aeruginosa*.

Keywords: Gas chromatography-Mass spectrometry, α-pinene, α-eudesmol, *Artemia salina*, Anti-bacterial activity.

INTRODUCTION

Artocarpus communis Forst (Moraceae) is a large tree that grows to a maximum height of 20 m. It is also known as *Artocarpus altilis* Parkinson Fosberg. *A communis* exists in two varieties as the seedless cultivar and the seeded form (Burkill, 1997). The seeded form is commonly called 'breadnut' or 'white man's groundnut' found in Puerto Rico (Kennard and Winters, 1960; Reeve, 1973) while the seedless cultivar is known as breadfruit. It is widely cultivated in the West Indies and South Pacific Island where it serves as a staple for many. It also has a wide distribution in most tropical countries. The seeded form was used for this study. The leaves of *A. communis* are used as folder for animals, and the extract has been found useful for lowering blood pressure and relieve from asthma (Graham and Bravo, 1981).

The extracts of *A. communis* have demonstrated considerable pharmacological actions which included antinephritis activities and enzyme inhibition (Patil et al., 2002; Shen-Ching et al., 2003; Fukai et al., 2005). Flavonoids and triterpenoids were isolated from various parts of this plant (Hano et al., 1980; Lin et al., 1996). This might represent a first attempt at characterizing the essential oil composition of *A. communis*.

Sphenocentrum jollyanum (Menisper-maceae) is an erect shrub of about 1.40 m

high which grows in the West African belt from Cote D'Ivoire to Southern Nigeria. The plant is used locally as a standard tonic and it has capacity for male sexual stimulation (Burkill, 1997). The yellow roots have acid taste but causes things eaten thereafter to taste sweet. It is also known to possess some anti-inflammatory properties (Dalziel, 1955). Herbalists in Ghana have also recorded as having used the roots for breast tumors. The roots when pulped with salt, fruits of maniguette (*Aframomum melegueta*) and palm oil are eaten for the treatment of abdominal troubles in Côte d'Ivoire.

The composition of the essential oil of *S. jollyanum* has never been the subject of literature discussion. The plant yielded several isoquinoline alkaloids, including palmatine and columbamine (Iwu, 1993). It also contains bitter-tasting diterpenes like members of the Menispermaceae family (Gilbert et al., 1967). The plant has also been found to exhibit anti-inflammatory, anti-oxidative and antiviral properties (Moody et al., 2002; Moody et al., 2006; Nia et al., 2004; Raji et al., 2006).

This study is aimed at characterizing the essential oil constituents of *A. communis* and *S. jollyanum* and to investigate their biological activities to correlate their uses traditionally.

MATERIALS AND METHODS

Plant materials

The leaves of *A. communis* were harvested from trees growing in the plantation of the Forestry Research Institute (FRIN) Ibadan, Nigeria while the roots of *S. jollyanum* were obtained from a location within Area J4, Ijebu-Ode, Ogun State, Nigeria. All collections were done between March and May, 2004. Voucher specimen were identified by Mr. F. Usang and kept at the Herbarium Headquarters of FRIN and samples issued numbers FHI 107114 and FHI 107123 for *A. communis* and *S. jollyanum* respectively.

Extraction of the volatile oil

Air dried samples were ground and batches (500 g) were submitted to hydrodistillation for 3 h using a Clevenger-type apparatus. The resulting essential oil was kept refrigerated until analyzed.

Gas chromatography (GC) analyses

Analyses were carried out on an Orion Analytical micromat gas chromatography fitted with a thermal conductivity detector (TCD). The separation was achieved by capillary columns of different polarities, CPSil-5 (25 m x 0.25 mm i.d), equivalent to OV 101, and CPSil-19 (25 m x 0.25 mm i.d) similar to BP10, film thickness of 0.15 µm.

The essential oils were diluted with *n*-hexane at a ratio of 1:5. 0.1 µL of the diluted oil samples was injected into the GC. The column temperature was programmed from 50 ^{o}C to 230 ^{o}C at 3 ^{o}C/min. The injector and detector temperatures were maintained at 200 ^{o}C and 250 ^{o}C respectively. The carrier gas was hydrogen at a pressure of 0.5 bars and a flow rate of 1.20 mL/min.

Gas chromatography-mass spectrometry (GC-MS) analyses

The composition of the volatile constituents was established by GC/MS analyses performed on a Hewlett-Packard Gas Chromatography (GC) HP5890A, interfaced with a VG Analytical 70-250s double focusing mass spectrometer, operating at 70 eV, with an ion source temperature of 230 ^{o}C. The GC was fitted with a 25 m x 0.25 mm i.d fused silica capillary column coated with CPSil-5. Helium was used as the carrier gas at 120 mL/min. The GC operating parameters were identical with those of the GC analyses. The mass spectral data were acquired and processed by an on-line desktop computer.

The retention time of the different component from the GC analysis of the essential oils were converted to Kovat indices (KI) using Kovat formula. The mass spectra of each compound from the GC-MS analyses were compared with authentic compounds (Davies, 1960; Adams, 2001).

Toxicological assay

Brine shrimp lethality test

Sea water was collected from the ocean in Lagos State, South West Nigeria. The shrimps (*Artemia salina*) were purchased from Felimar Aquaculture Centre, Ijebu-ode, Ogun

State (produced by Coppens International bv, Helmond, Holland). Sea water (200 mL) was put in a tank or hatching chamber and shrimp eggs added. The tank or hatching chamber was a plastic bowl, partitioned in to two compartments. The partition was perforated such that the nauplii could swim through to the other side after hatching. The eggs were allowed to hatch for 48 h and mature to nauplii at room temperature. The nauplii were then harvested with a pipette after attracting the organism to one side of the vessel with a light source.

The essential oils were prepared in sea water into vials at 1000, 100, and 10 µg/mL (each test in triplicates). The essential oils had been previously dissolved in 2 mL of Dimethylsulfoxide (DMSO) since they are not soluble in water and 0.5 mL each of the dose level was introduced in a test-tube to which 4 mL of sea water added. 10 shrimps per test tube were added for each concentration and made up to 5 ml seawater to make 1000-10 µg/mL of final concentration of extract. After 24 h, the number of deaths over the number of total shrimps (survivors) was counted and recorded (McLaughlin et al., 1973).

The data were analyzed statistically (Finner Computer Programme) to determine LC_{50} values. A control was in place without the test solutions (Finney, 1971).

Antibacterial assay

An agar diffusion technique was adopted using three Gram-positive and three Gram-positive bacteria. The six microorganisms were clinical isolates collected from the Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria. The tested organisms were *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis*.

The disc plate technique was adopted for the assay using agar from Antec Diagnostic Product, U.K. The nutrient agar plates were swabbed with the test microorganisms and the activity was measured as zones of inhibition [diameter (in mm) of the clear zone of inhibited microbial growth around the paper disk].

Each essential oil concentration (1000, 100, 10 µg/mL prepared in DMSO, as well as the standard antimicrobial agent gentamycin (10 µg/mL) from Nicholas Laboratories Ltd., U.K.) were impregnated onto paper discs (6 mm in diameter), which were then placed on the plates. The solvents, DMSO and *n*-hexane were used as controls. The plates were left at room temperature for 2 h to allow for diffusion and then incubated at 37 ºC for 24 h. The essential oils were considered to be active if, after 24 h, a clear zone extended around the impregnated disc in which no growth was observed. The antimicrobial activities of the essential oils were reported as the diameter of the zone of growth inhibition recorded in mm. The assays were performed in triplicates.

RESULTS

The compositions of the investigated oils are reported in Table 1. The two oils were complex mixtures of terpenoids, fatty acids and aliphatic compounds. In the essential oil of *A. communis*, the monoterpenoid contents were 58.8%, while 14.8% sesquiterpenes, 6.3% fatty acids and 10.7% aliphatic compounds accounted for the remaining percentage composition. The main compounds found in significant quantity were α-pinene (14.1%), 6-methyl-5-heptene-2-one (7.0%), α-phellandrene (8.9%), α-ionone (6.1%), E-nerolidol (8.3%) and 1,8-cineole. A total of 19 compounds (Table 1) were identified form the root oil of *S. jollyanum* consisting of monoterpenoid (33.5%), sesquiterpenoid (56.3% while 10.2% of the total oil constituents remain unidentified. The major constituents of the oil were 1,8-cineole (6.3%) and β-pinene (5.3%).

The essential oil of *A. communis* showed very weak activity to only *P. mirabilis* as compared to the standard drug Gentamicin used. It showed activity at 1000 ppm (8.0 mm) and 100 ppm (6.5 mm). However, the oil of *S. jollyanum* was susceptible to the strains of *B. subtilis* and *P. aeruginosa* (Table 2).

Toxicity of the two essential oils to brine shrimp (Table 3) reveals that *A. communis* exerted LC_{50} of 24.55 ppm while *S. jollyanum* displayed LC_{50} of 84.87 ppm.

Table 1: Essential Oil Composition of *Artocarpus communis* (Leaf) and *Spenocentrum jollyanum* (root).

S/N	Compound	RI*	% (1)	% (2)
1.	trans-2-hexenal	854	0.4	-
2.	α-pinene	943	14.1	11.2
3.	camphene	956	2.4	0.5
4.	6-methyl-5-heptene-2-one	971	7.0	-
5.	3-octanone	974	1.8	-
6.	B-pinene	981	2.8	5.3
7.	2-pentylfuran	987	2.3	-
8.	α-phellandrene	1007	8.9	-
9.	d3-carene	1017	-	2.3
10.	p-cymene	1021	0.6	3.8
11.	2,6,6-trimethylcyclohexanone	1024	0.4	-
12.	1,8-cineol	1030	5.5	6.3
13.	citronellol	1041	0.3	-
14.	*E*-β-ocimene	1044	0.9	-
15.	γ-terpinene	1057	3.2	4.1
16.	terpinolene	1086	1.8	-
17.	*n*-nonanal	1089	0.8	-
18.	4,8-dimethyl-1,3,7-nonatriene	1093	0.4	-
19.	*n*-nonanol	1143	0.3	-
20.	thymohydrochinon	1165	0.8	-
21.	2-decanone	1176	0.4	-
22.	safranal	1182	0.9	-
23.	*n*-decanal	1189	0.3	-
24.	β-cyclocitral	1202	0.9	-
25.	geranial	1252	0.6	-
26.	dihyroedulan	1290	0.6	-
27.	α-ylangene	1379	-	1.8
28.	isocaryophyllene	1404	-	7.7
29.	α-ionone	1410	6.1	-
30.	*E*-β-isocaryophyllene	1422	4.6	4.6
31.	geranylacetone	1430	2.4	-
32.	aromadendrene	1437	-	0.6
33.	selina-4(15),6-diene	1443	-	4.6
34.	β-ionone	1467	2.5	-
35.	γ-humulene	1485	1.9	0.9
36.	epi-zonarene	1497	-	0.9
37.	δ-amorphene	1510	-	3.9
38.	*E*-nerolidol	1549	8.3	-
39.	guaia-6,9-diene-4α-ol	1566	-	0.7
40.	globulol	1575	-	0.9
41.	5-guaiene-11-ol	1614	-	3.6
42.	α-eudesmol	1650	-	26.1
43.	*n*-hexadecanoic acid	1937	6.3	-
	TOTAL		**90.6%**	**89.8%**

RI* = Retention Indices, % (1) *Artocarpus communis*, % (2) *Spenocentrum jollyanum* (Adams, 2001)

Table 2: Antibacteria activities of the essential oils.

Organism	Concentration of essential oil (µg/mL)	Inhibition zone (mm)		Gentamicin (10 µg/mL)
		S. jollyanum	*A. communis*	
Salmonella typhi	1000	-	-	18.0
	100	-	-	
	10	-	-	
Proteus mirabilis	1000	-	8.0	19.0
	100	-	6.5	
	10	-	-	
Bacillus cereus	1000	-	-	19.0
	100	-	-	
	10	-	-	
Bacillus subtilis	1000	10.0	-	18.0
	100	8.5	-	
	10	8.0	-	
Pseudomonas aeruginosa	1000	9.0	-	15.0
	100	8.5	-	
	10	7.5	-	
Staphylococcus aureus	1000	-	-	-
	100	-	-	
	10	-	-	

Table 3: Brine Shrimp lethality test.

Essential Oil	LC_{50} (ppm)
A. communis	24.55
S. jollyanum	84.87

DISCUSSION

The moderate antimicrobial activity displayed by the essential oil supports the use of the root as a stomachic for abdominal disorders, purgatives and for ulcers. The phytochemical extracts of the roots of *S. jollyanum* have also demonstrated antiviral (Moody et al., 2002), antioxidant and anti-inflammatory activities (Moody et al., 2006; Nia et al., 2004).

This result of toxicity suggests that the oils can be regarded as being moderately toxic. Sharififar et al. (2009) evaluated the toxicity of four commonly used species of plants and classified them as being considerable toxic with LC_{50} values ranging from 0.07 to 533.6 ppm. The leaves of *Tetrapleura tetraptera* essential oil exhibited moderate toxicity to brine shrimps at a concentration of 117.5 ppm (Aboaba et al., 2009). Toxicity to brine shrimps has been correlated with cytotoxic, pesticidal, antimicrobial and anti-tumor properties (McLaughlin et al., 1993).

To the best of our knowledge, there are no literature reports on the oils of these West African Plants. This paper represents the first attempt for an accurate characterization, antibacterial and toxicological assays of the oils of *A. communis* and *S. jollyanum*.

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