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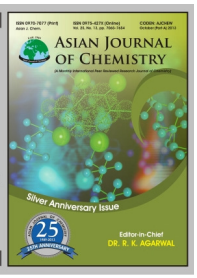

Chemical Study of Extracts of *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt (Zingiberaceae) from Benin

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The chemical composition of essential oils, fatty acids and unsaponifiable of the limbs, foliars sheaths and rhizomes of *Siphonochilus aethiopicus* were studied by GC/MS. In petroleum ether extracts, the lipidic fractions are marked by significant rates (7.41 to 23.45 %) of fatty acids in C_{18}. The majority of unsaponifiable compounds from the limbs, foliars sheaths and rhizomes of *S. aethiopicus* are essentially consist of terpenoïds accompanied by heneicosane (6.2 to 21.6 %). Otherwise, *S. aethiopicus* contains varied rates of catechic saponins, tanins and leucoanthocyanes in its organs.

Key Words: Fatty acids, Insaponifiables, Leucoanthocyanes, Terpenoïds.

INTRODUCTION

Since the ancient period, the volatile extracts of the aromatic plants were sought for their biological properties. These plants were frequently used in traditional medicine for therapeutic purposes especially to relieve the cutaneous affections, respiratory disorders, digestive and cardiovascular diseases etc. Other biological properties of plants (antiradical and antiinflammatory power) generated interesting applications in chemical industry, in cosmetic and in pharmaceutical industry. *S. aethiopicus* (Zingiberaceae) is a fragrant aromatic plant of tropical Africa savannas. It is used as spices in the dishes flavouring by the Igede people of Nigeria[1]. In Benin, this plant is one of the endangered botanical species. It grows spontaneously state and appears as a plant less referred in the literature.

Some work has highlighted the biological activities of *S. aethiopicus* extracts. Makhuvha *et al.*[2] used a set "starch gel - horizontal electrophoresis" to study the genetic diversity of *S. aethiopicus* between a spontaneous natural population and individuals cloned by the same species, intended for the marketing. The cyclooxygenase inhibitory activity of ethanol and aqueous extracts of *S. aethiopicus* was tested by Lyndy *et al.*[3]. Lindsey *et al.*[4] showed that *S. aethiopicus* calms the menstrual pains by cyclooxygenase inhibition of prostaglandin biosynthesis. Zschocke *et al.*[5] reported the importance of the substitution strategy of the parts of medicinal plants for their preservation over a long period in South Africa. More recently, researchers[6] have isolated and identified in 2002 through Nuclear Magnetic Resonance (NMR) high-field two furanoterpenoïc compounds: 4aαH-3,5α,8aβ-trimethyl-4,4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one et 2-hydroxy-4aα*H*-3,5α,8aβ-trimethyl-4,4a,9-tetrahydronaphtho[2,3-b]-furan-8-one. They were also interested in the changes of chemical composition and biological activity due to *S. aethiopicus* storage[7]. Igoli and Obanu[1] have isolated volatile components from the matrix of fresh and roasted samples of *S. aethiopicus* by solvent extraction and vacuum distilled. They made a quantitative determination of the volatiles components in the distillate by GC/FID and organoleptic examination by GC/Olfactometry. They have identified sesquiterpenes as major components of fresh wild *S. aethiopicus*[1]. Other researchers[8-10] relating *S. aethiopicus* biological activities have been reported in the literature.

Due to the importance which dresses this plant in traditional medicine of Africa in the South of Sahara and in Benin in particular, it is timely to investigate the chemical constituents of its cellular reserves. For that purpose, the present work

mainly focused on the determination by GC/MS of fatty acids obtained after saponification and unsaponifiable constituents contained in limbs, leaf sheaths and rhizomes of *S. aethiopicus* from Benin.

EXPERIMENTAL

Limbs, leaf sheaths and rhizomes of *S. aethiopicus* were collected in september 2007 in a wild state in Manigri (Northern Benin). In the laboratory, the rhizomes were washed to remove the sand and all plant material was kept between 18 and 22 ºC. To extract fatty acids, limbs, leaf sheaths and rhizomes of *S. aethiopicus*, become dry, were transformed into powder to a crusher IKA WERK type MF 10 BASIC. The powders obtained were sieved to particle sizes 0.335 and 0.600 mm. A voucher specimen of each part of *S. aethiopicus* has been deposited in Abomey-Calavi University National Herbarium.

Extraction of unsaponifiable compounds and fatty acids (FA): 15 g of powder (limbs, leaf sheaths and rhizomes) were treated twice successively with 100 mL of petroleum ether (40-65 ºC) under magnetic stirring at room temperature for 1 h. After filtration and evaporation of the solvent under reduced pressure, the extract was dried then weighed. The extraction yield was established by calculating the average of three replicates.

0.5 g of the extract was saponified with 25 mL of ethanolic solution of potassium hydroxide (2 N) by refluxing for 1.5 h. After cooling, it was added 50 mL of distilled water and the unsaponifiable matter were extracted with 3 × 50 mL of cyclohexane. The soap solution produced was acidified (H_2SO_4; 98 %) until the precipitation of fatty acid (pH = 5 to 6). These fatty acid released were extracted with 3 × 50 mL of diethyl ether[11-13].

The fatty acids were transformed into their methylic esters by addition of a methanolic solution at 10 % of BF_3[14] and the methylic esters were extracted by the cyclohexane for analysis by GC/MS. The fatty acid yields and unsaponifiables matter relative to the mass of plant material used or to that of the petroleum ether crude extract are reported in Table-2.

GC/FID: The extracts were analyzed on a Hewlett-Packard gas chromatograph Model 6890, equiped with a DB5 MS column (30 m × 0.25 mm, 0.25 µm), programming from 50 ºC (5 min) to 300 ºC at 5 ºC/min, 5 min hold. Hydrogen as carrier gas (1.0 mL/min); injection in split mode (1:60); injector and detector temperature: 280 and 300 ºC, respectively. Each extract is diluted in hexane: 1/30.

GC/MS: The extracts compositions were analyzed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packad MS model 5871, equipped with a DB5 MS column (30 m × 0.25 mm, 0.25 µm), programming from 50 ºC (5 min) to 300 ºC at 5 ºC/min, 5 min hold. Helium as carrier gas (1.0 mL/min); injection in split mode (1:30); injector and detector temperature, 250 and 280 ºC, respectively. The MS working in electron impact mode at 70 eV; electronmultiplier: 2500 eV; ion source temperature: 180 ºC; mass spectra data were acquired in the scan mode in m/z range 33-450.

The compounds determined in various essential oils by CG/FID were identified by comparison between their indices of retention and those of reference components in the literature. These compounds identified by GC/FID are confirmed by GC/SM by comparing their spectra of mass with those of reference substances[15-23].

Identification of saponins, tannins catechetical, leucoanthocyanes, oses and holosides, mucilages: The phytochemical screening was made according to the standard technical described by Paris and Moyse[24], Bouquet[25] and Debray *et al.*[26].

Saponins: A decoction was prepared during 0.5 h from 2 g of plant powder and 100 mL of distilled water. After

TABLE-1
FATTY ACID AND UNSAPONIFIABLES YIELDS OF LIMBS, LEAF SHEATHS AND RHIZOMES OF *S. aethiopicus*

	Fatty acid yield of the plant material			Fatty acid yield of petroleum ether extracted		
	L (%)	Ls (%)	Rz (%)	L (%)	Ls (%)	Rz (%)
FA	0.3	0.1	0.8	6.2	25.6	27.8
Un	2.3	0.2	0.4	56.0	51.2	13.9

L = Limbs; Ls = leaf sheaths; Rz = rhizomes; FA = fatty acids; Un = unsaponifiables.

TABLE-2
FATTY ACID COMPOSITION OF THE LIMBS, LEAF SHEATHS AND RHIZOMES EXTRACTS OF *S. aethiopicus*

Fatty acid	KI	R_t (min)	L (%)	Ls (%)	Rz (%)
Capric acid ($C_{10:0}$)	1522	27.4	2.5	–	–
Myristic acid ($C_{14:0}$)	1723	31.9	1.7	–	0.4
Palmitic acid ($C_{16:0}$)	1925	36.0	13.0	25.8	13.3
Margaric acid ($C_{17:0}$)	2025	37.8	–	1.5	11.0
Linoleic acid ($C_{18:2\ (9,12)}$)	2091	39.1	7.1	20.4	15.7
Oleic acid ($C_{18:1}$)	2098	39.3	11.5	23.5	14.9
Stearic acid ($C_{18:0}$)	2125	39.7	–	7.4	2.7
Arachidic acid ($C_{20:0}$)	2326	43.2	–	1.3	0.8
Behenic acid ($C_{22:0}$)	2976	46.4	–	–	0.9
FA saturated	–	–	17.2	36.9	29.1
FA unsaturated	–	–	18.6	43.9	30.6
Total	–	–	35.8	79.9	59.7

KI = Kovats Indice, R_t = retention time, L = Limbs, Ls = leaf sheaths, Rz = Rhizomes.

filteration the obtained mixture, the filtrate was divided into 10 different volumes (1, 2, 3 and 10 mL) in 10 calibrated tubes (internal diameter: 1.3 cm). The content of each tube was adjusted to 10 mL with distilled water. After shaking each tube in an horizontal position for 15 s, followed by a rest of 15 min in an upright position, the height of the foam supernatant was measured in cm. When this height is close to 1 cm in the Xth tube, the foam index (I) is calculated by the following formula: I = foam height (in cm) in the Xth tube × 5/0. 0X. The presence of saponins in the plant is confirmed when the value of the foam index is greater than 100.

Catechin tannins: An aqueous infusion was prepared from 5 g of plant powder and 100 mL of boiling distilled water. After leaving for 15 min, the mixture was filtered and infused obtained is completed to 100 mL with hot water. It was added to 30 mL of the infused (5 %), 15 mL of Stiasny reagent [formol (40 %) + HCl (97 %)]. After 15 min in a water bath at 90º, a red precipitate, soluble in isoamylic alcohol, appeared indicating the presence of catechin tannins.

Leucoanthocyanes: They were identified by introducing into a test tube 5 mL of infused (5 %) and 5 mL of hydrochloric alcohol (ethanol 95º + distilled water + hydrochloric acid 37 % of equal volumes). The mixture was completed with 1 mL of isoamylic alcohol and then, heated to 90º through a water bath. After fifteen minutes, it had developed a red-cerise tint (or purple) indicating the presence of leucoanthocyanes.

Oses and holosides: 5 mL of decoction (10 %) were evaporated to dryness and the residue obtained was treated with two to three drops of concentrated H_2SO_4, then, with three drops of ethanol saturated with thymol. A red colouring was observed indicating the presence of monosaccharides and holosides.

Mucilages: 1 mL of decoction realized previously was treated with 5 mL of absolute ethanol and the presence of mucilages were noticed by the appearance of a flaky precipitate.

RESULTS AND DISCUSSION

Fatty acids (FA) identified (Table-1) represent 0.1-0.8 % of the mass of plant material and 6.2-27.8 % of the crude extract to petroleum ether. Fatty acids yields, obtained from the extracts of leaf sheaths (25.6 %) and rhizomes (27.8 %) of *S. aethiopicus*, are superior to that determined in the flowering tops (13.1 %) of *Vetiveria nigritana* (Benth.) by Champagnat *et al.*[27]. Moreover, Champagnat *et al*. obtained 64.95 % of FA[27] whereas in the rhizomes of *S. aethiopicus*, the proportion of FA is 27.8 %. Only the crude extracts from the rhizomes contain less than 15.0 % of unsaponifiable compounds (13.9 %). In limbs and leaf sheaths, this value is estimated at more than 50 % (51.2 and 56 %).

According to the results (Table-2); 35.8 in 79.9 % of the fatty acid were identified in limbs, leaf sheaths and rhizomes

TABLE-3
UNSAPONIFIABLE COMPOSITION OF THE LIMBS, LEAF SHEATHS AND RHIZOMES EXTRACTS OF *Siphonochilus aethiopicus*

Compounds	KI_{exp}	KI_{th}	R_t (min)	L (%)	Ls (%)	Rz (%)
Sabinene	974	969	11.6	0.1	0.2	–
β-Pinene	978	974	11.9	0.1	5.1	3.8
Bicyclohexane	1304	–	21.8	1.2	0.8	–
β-Elemene	1386	1389	23.9	0.3	1.0	–
Cyperene	1408	1398	24.6	8.0	0.2	9.9
β-Caryophyllene	1419	1417	24.7	7.3	2.2	–
(Z)-β-Farnesene	1434	1440	24.9	–	0.5	1.1
α-Humulene	1455	1452	25.6	3.3	3.2	–
Allo-aromadendrene	1459	1458	25.8	0.9	0.3	–
9-Epi-(e)-caryryophyllene	1466	1464	26.0	3.0	–	3.0
Germacrene-D	1479	1484	26.3	1.9	1.4	–
β-Selinene	1488	1489	26.6	–	0.2	5.8
Valencene	1493	1496	26.7	0.7	0.7	1.2
Aciphyllene	1496	1501	26.8	1.0	–	1.5
Germacrene-A	1510	1508	27.0	0.7	1.0	8.3
γ-Cadinene	1514	1513	27.1	–	0.4	–
δ-Cadinene	1518	1522	27.2	2.1	0.5	–
7-Epi-α-selinene	1523	1520	27.4	1.9	15.1	6.2
Germacrene-B	1556	1559	28.1	2.1	0.8	–
Caryophyllene oxide	1585	1582	28.9	25.7	2.5	2.8
Humulene epoxide II	1606	1608	29.5	4.8	0.7	3.0
Epi-α-cadinol	1639	1638	30.0	1.8	0.6	2.5
Epi-α-muurolol	1641	1640	30.1	–	1.0	1.9
α-Cadinol	1654	1652	30.3	2.5	1.9	3.2
Selin-11-en-4-α-ol	1660	1658	30.4	2.1	0.3	17.7
Intermedeol	1670	1665	30.8	2.8	27.8	13.7
2,2,4,5,7,7-Hexamethylocta-3,5-diene	1806	–	33.6	5.8	–	7.0
6,10,14-Trimethylpentadecan-2-one	1840	–	34.3	3.8	0.6	–
Heneicosane	2112	2100	39.5	14.6	21.6	6.2
Hexatriacontane	2989	–	49.8	–	0.8	0.1
Total	–	–	–	98.5	91.4	98.9

KI = Kovats Indice, R_t = Retention time, L = Limbs, Ls = leaf sheaths, Rz = Rhizomes.

of *S. aethiopicus*. Almost equivalent proportions in saturated and unsaturated fatty acid, in limbs (17.17 and 18.6 %) and rhizomes (29.1 and 30.6 %) were also observed. In leaf sheaths, the unsaturated fatty acids were the most dominant (43.9 %). However, 17.2 to 36.9 % were constitued by saturated fatty acids. The most abundant are palmitic acid (13.0-25.8 %), margaric acid (11.0 %) and stearic acid (7.4 %). Linoleic aid (7.1-20.4 %) and oleic acid (11.5-23.5 %) are the only unsaturated fatty acids identified in the plant organs.

It is important to note the appearance in the rhizomes a long chain fatty acid in small proportion [behenic acid (0.9 %)] and that of a saturated fatty acid having an odd number of carbon atoms [margaric acid (11.0 %)]. The relatively low proportions of these last ones are evidence that the C_{20} fatty acid are uncommon in the plant kingdom[23,28]. Also, a total absence or low proportion of C_{10} fatty acid was reported in the organs of *S. aethiopicus*[28]. Indeed, capric acid (2.5 %), only acid C_{10}, appeared in the limbs of *S. aethiopicus* but its presence was not noticed in the leaf sheaths and rhizomes.

GC/MS analysis revealed 19 to 27 constituents corresponding to 91.4 to 98.9 % of the total weight of cyclohexane extracts from the limbs, leaf sheaths and rhizomes of *S. aethiopicus.* The oxygenated compounds, essentially sesquiterpenoics, are around 35.4 to 44.8 %. The main unsaponifiables (> 5 %) characteristics of the plant parts are β-pinene (5.1 %); β-selinene (5.8 %); 2,2,4,5,7,7-hexamethylocta-3,5-diene (5.8-7.0 %); heneicosane (6.2-21.6 %); β-caryophyllene (7.3 %); cyperene (8.0-9.9 %); germacrene-A (8.3 %); 7-epi-α-selinene (6.2-15.1 %); intermedeol (13.7-27.8 %), selin-11-en-4-α-ol (17.7 %), caryophyllene oxide (25.7 %). Only two saturated hydrocarbons have emerged: heneicosane, most abundant in leaf sheaths (21.6 %) and hexatriacontane (< 1.0 %) in limbs, leaf sheaths and rhizomes of *S. aethiopicus* (Table-3).

The characterization tests have shown the presence of saponins, catechic tannins, leucoanthocyanes, oses, holosides and mucilages (Table-4). Except the saponins which strong proportion was noticed in all *S. aethiopicus* organs, rhizomes also, were a potential reserve of catechins tannins, leucoanthocyanes and mucilages. Similarly, it was noticed a high presence of monosaccharids and holosides in the leaf sheaths.

TABLE-4
CHEMICAL FAMILIES IDENTIFIED
IN *Siphonochilus aethiopicus* ORGANS

Chemical families	*S. aethiopicus* organs treated		
	Limbs	Leaf sheaths	Rhizomes
Saponins (IF)	+++ (183.33)	+++ (225.00)	+++ (166.67)
Catechins tannins	++	+	+++
Leucoanthocyans	++	+	+++
Oses and holosides	0	+++	0
Mucilages	++	++	+++

IF = Index foam, abundant: +++, average: ++, very little: +, negative test: 0.

Conclusion

This work has emerged some of the potential of chemical compounds in *S. aethiopicus*, a plant species rich in hydrocarbon compounds. Three fatty acids, commonly found in plant cells, were noted in the parts of the plant *i.e.*, palmitic acid, oleic acid and linoleic acid. The unsaponifiable compounds of its organs were formed mainly by hydrocarbons and some oxygenated sesquiterpenes. The most importants were caryophyllene oxide (25.7 %) in limbs and intermedeol (13.7 to 27.8 %) in the rhizomes. The characterization tests revealed that *S. aethiopicus* organs were important sources of saponins. Catechic tannins, mucilages and leucoanthocyanes were particularly abundant in rhizomes.

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