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Original Article

GC/MS and GC/FID analysis and evaluation of antimicrobial performance of Aframomum sceptrum essential oils of Benin

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ABSTRACT

This work has studied the chemical composition of essential oils of limb, leaf sheath and rhizome of *Aframomum sceptrum* (Oliv. & T. Hand.) K. Schum (Zingiberaceae) collected in southern Benin, by GC and GC / MS and were tested for their effectiveness against pathogenic microorganisms. The main compounds noted in the essential oils of *Aframomum sceptrum* also called *Aframomum masuianum* (Oliv & D. Hand) are: for limb: β-caryophyllene (33.4%), β-pinene (28.4%), α-humulene (10.6%), α-pinene (3.3%) and caryophyllene oxide (3.0%), for leaf sheath β-pinene (42.4%) and α-pinene (5,7%) and concerning the rhizomes; the results showed the following: β-pinene (15.9%), α-terpineol (l5.2%), β-caryophyllene (13.9%) and α-humulene (6.4%). The microbiological tests showed that essential oils of three organs of *A. sceptrum* have no activities related to the microorganisms studied.

Key words: *Aframomum sceptrum*, chemical composition, antimicrobial activities.

INTRODUCTION

The genus Aframomum (Zingiberaceae) is widely represented in many parts of West and Central Africa about 50 species that can be distinguished in general by their large size. They are perennials and aromatics plants [1]. *Aframomum sceptrum* (Oliv. & T. Hanb.) K. Schum is one of the species widely used in Africa, but little studied. In African traditional medicine, *Aframomum sceptrum* is particularly useful in cases of dysentery and intestinal helminthes and to fight against human trypanosomiasis [2]. In Benin, limbo and leaf sheaths are used to treat female infertility, oral and digestive candidiasis [3]. Previous work has shown some biological activities of non-volatile extracts of this plant: Standardization of liver enzyme activities in diabetic rats [4], antimicrobial and antispasmodic activities [5], and antioxidant [6, 7]. Similarly, several other compounds were able to identify in some of these non-volatile extracts [1, 8, 9]. Few studies have investigated the chemical composition and biological properties of essential oils of *Aframomum sceptrum* [10, 11, 12]. In Benin no investigations were carried out about these plant essential oils. The main interest of this study is to determine the chemical composition of three organs of *Aframomum sceptrum* essential oils from Benin and their antimicrobial activities.

MATERIALS AND METHODS

Plant materials: Limbo, leaf sheaths and rhizomes of *Aframomum sceptrum* (Oliv. & T. Hand) K. Schum were collected from Goudou (Abomey calavi) in March 2013 and were authenticated by National Herbarium of University of Benin. The material was dried in the laboratory (20-22°C) before the extractions.

The essential oils were obtained by hydro-distillation using a Clevenger-type apparatus for three hours. They were dried over anhydrous sodium sulphate and kept in glass vials at -4°C prior to analysis.

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Microorganisms tested: Three microorganisms were tested *Escherichia c*oli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and the yeast *Candida albicans* (ATCC 10231). They have been supplied by the Health Department of Water and Food of health Ministry. These strains were subjected to biochemical identification systems with APIs (Devices and Processes Identification Bio Merrieux -La-Balme-Les Grottes Cedex, France [13].

GC/MS and GC/FID: Essential oils were analyzed with a chromatograph gas model Agilent 6890 equipped with: a flame ionization detector, DB5 MS column (20m X 0,18mm; 0,18µm) with a split of 60 mL / min and helium (carrier gas) temperature program (3.2 min, 50°C to 300°C, 8°C / min). The injector was set at 280°C and the detector at 300°C. The pairing on AGILENT model 7890 equipped with a DB5 column (20m X 0,18mm; 0,18µm) used in programming temperature (50°C for 5 min) to 300°C and a gradient of 5°C / min). The carrier gas was helium (1.0 mL / min). The injections were made in split mode (1: 250). The operating temperatures of the injector and detector were 280°C and 300°C respectively. The MS working in electron impact mode at 70 eV; electron multiplier, 1800 V; ion source temperature, 230°C; mass spectra data were acquired in the scan mode in m/z range 55-550.

The compounds assayed by GC in the different essential oils were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by GC-MS by comparison of their mass spectra with those of reference substances [14, 15]. The compounds were confirmed by GC / MS by comparing their mass spectra with those of the reference compounds [16, 17].

Anti-microbial activities: They were evaluated by determining the minimum inhibitory concentrations (MIC) method reported by Yèhouénou et al. in 2010 [18] using micro-dilution in 96 well microplates. Moreover, the antibiogram activity of essential oils was studied, comparing to reference antibiotics: Tetracycline and Ampicillin for *S. aureus*; Gentamicin and colistin (Biovd F92430) for *E. coli* and nystatin for *C. albicans* was done by disk diffusion method. For microplates analysis, 12 concentrations with serial dilutions of 2 were tested; the stronger was 41.7 µl/mL, each concentration was repeated 4 times. As regards discs (6 mm diameter) using, 10µl of the crude essential oil were tested for each microorganism; assays were repeated three times.

RESULTS AND DISCUSSION

Aframomum sceptrum (harvested in Benin) essential oil yields were: 0.1% for limbo (colorless), 0.06% for the leaf sheath (colorless) and 0.03% for rhizomes (yellow). These yields are quite different compared to those studied in Côte d'Ivoire on limbo (0.28%) [12] and rhizomes (0.17%) [10]. This variation could be justified by the soil nature and vegetative period during which the species were collected. GC and GC / MS analysis identified 93.6%, 91.7% and 94.3% of chemical compounds respectively for limbo, leaf sheath and rhizomes. These compounds are grouped in 35.3%; 66.6% and 36.1% of hydrogenated monoterpenes; 5.1%; 10.2% and 18.2% of oxygenated monoterpenes and 48.0; 10.1 and 33.0% of hydrogenated sesquiterpenes and 5.2; 4.8 and 6.7% of oxygenated sesquiterpenes respectively for: limbo, leaf sheaths and rhizomes (Table 1).

Limbo essential oil is mainly rich in: β-caryophyllene (33.3%), β-pinene (28.4%), α-humulene (10.6%). This composition has some similarity with limbo essential oil of Côte d'Ivoire [12] which major compounds are: β-caryophyllene (31.3%), β-pinene (15.1%), α-humulene (10.1 %). While β-pinene (42.2%), α-pinene (5.7%), β-caryophyllene (3.9%), caryophyllene oxide (3.3%) make up the majority of the compounds of the leaf sheath essential oil; rhizomes essential oil is rich in β-pinene (15.9%), α-terpineol (l5. 2%), β-caryophyllene (13.9%) and α-humulene (6.6%). This composition is close to that of rhizomes studied in Côte d'Ivoire in 2011 by Sheikh-Ali et al. [10] as the major compound with β-pinene (12.7%).

As regards to antimicrobial properties, Nguikwie et al. (2013) worked on essential oils of three Aframomum species of Cameroon; they showed activities against bacteria such as *E. coli*[19].However, in the study carried out, no essential oil showed activity against the three microorganisms with the use of the tests microplate or the diffusion susceptibility tests. Minimum inhibitory concentrations (MIC) and zones of inhibition have not therefore been determined (Table 2). Several essential oils of the same genus: *A. dalzielii*, A. and *A. letestuianum* pruinosum, *A. corrorima*, *A. melegueta* are potential sources of (E) - (R)-nerolidol [19, 20, 21]. This compound could be based on the antimicrobial activity of those species [19, 22]; while no trace of nerolidol was observed in essential oils of *A. sceptrum* in this study as those of Côte d'Ivoire. Only Sheik (2011) [10] work on the essential oil of rhizomes of *A. sceptrum* showed its low activity on *C. albicans*, *E.*

coli and *S. aureus*, but a very effective activity on *Trypanosoma brucei* brucei and *Trichomonas vaginalis* unlike the present study. This could be justified by the wealth of oils oxygenated compounds (41.3%) of oils from rhizomes of Côte d'Ivoire. Benin unlike that of 24.1% for 69.1% of hydrogenated compounds.

Previous work [23, 24] showed that oxygenated compounds exhibit antimicrobial activities that more hydrogenated because of their ability to promote the formation of hydrogen bonds and the solubility of the oil in water. Furthermore, although pinenes are known for their antimicrobial activities [25, 26] and that the three essential oils containing (31.7%, 47.9% and 19% against 15.5% in Côte d'Ivoire rhizomes); they didn't show any activity on the three microorganisms. Combination of certain compounds (1,8 cineole, linalool) known for their antimicrobial action, which are present in small quantity in Côte d'Ivoire rhizomes and absent in that of Benin could also justify this difference of activity. Synergism between hydrogenated and oxygenated compounds or aromatic compounds failed in Benin *Aframomum sceptrum* essential oil. This explains why it does not have the same antimicrobial spectrum like essential oil extracted in Côte d'Ivoire. Previous work showed synergies between major compounds which in this study are hydrogenated compounds and minor ones: oxygenated and aromatic compounds [24].

CONCLUSION

The present study identified for the first time in our country, the chemical composition of essential oils extracted from limbo, leaf sheath and rhizome of Benin *Aframomum sceptrum*. Although this aromatic plant is used in the treatment of some candidiasis, essential oils derived from *A. sceptrum* had no effect on *E. coli*, *S. aureus* and *C. albicans*.

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Table 1: Chemical composition of essential oils extracted from limbo, leaf sheaths and rhizomes of *Aframomum sceptrum* (Oliv. & T. Hanb) K. Schum

Compounds identified	KI	Percentage (%)		
		L	LS	R
heptan-2-ol	901	-	-	0.3
α-thujene	925	0.1	0.3	0.1
α-pinene	933	**3.3**	**5.7**	**3.1**
camphene	949	0.2	0.4	0.4
Sabinene	973	0.7	**3.1**	0.6
β-pinene	978	**28.4**	**42.2**	**15.9**
myrcene	989	0.4	**1.0**	0.8
δ-2-carene	998	-	0.1	-
mentha-1(7).8-diene	1004	-	-	0.1
α-phellandrene	1006	-	**3.4**	0.4
α-terpinene	1009	-	-	0.1
δ-3-carene	1011		0.2	-
p-cymene	1025	-	**1.6**	0.6
limonene	1027	0.8	1.8	2.5
β-phellandrene	1031	1.2	**2.9**	10.3
eucalyptol	1033	-	0.147	-
(Z)-β-ocimene	1039	-	t	-
(E)-β-ocimene	1047	0.1	0.2	0.4
ϑ-terpinene	1059	0.1	**2.9**	0.3
terpinolene	1085	-	0.8	0.2
linalool	1099	t	0.1	**2.6**
fenchyl alcool	1117	-	0.1	0.1
cis-p-menth-2-en-1-ol	1126	-	0.1	0.2
trans-pinocarveol	1143	-	0.3	0.3
trans-p-menth-2-en-1-ol	1144	-	-	0.1
camphor	1143	-	-	0.1
camphene hydrate	1148	-	-	0.1
pinocarvone	1162	0.1	0.1	0.1
Isoborneol	1163	-	-	0.1
borneol	1174	0.2	1.0	**2.4**
terpinen-4-ol	1182	0.2	**2.1**	**1.3**
cryptone	1190	0.2	0.5	1.7
α-terpineol	1194	-	**2.8**	**5.2**
myrtenol	1197	0.7	-	-
trans-piperitol	1209	-	-	0.1
bornyl acetate	1286	t	0.1	0.4
cis-pinocarvyl acetate	1310	0.3	-	-
myrtenyl acetate	1324	**3.4**	**2.9**	**3.4**
δ-elemene	1337	0.1	0.2	0.3

α-copaene	1379	0.1	0.1	0.4
β-bourbonene	1387	0.3	-	0.7
β-elemene	1391	0.8	0.7	**2.6**
cyperene	1408	-	0.9	0.4
α-*cis*-bergamotene	1416	0.1	0.2	0.7
β-caryophyllene	1426	**33.3**	**3.8**	**13.9**
8-elemene	1431	0.4	0.1	0.6
β-sesquifenchene	1447	-	0.1	0.4
α-humulene	1461	**10.6**	**1.7**	**6.4**
alloaromadendrene	1461	0.2	0.1	0.4
germacrene-D	1485	**1.3**	**1.2**	**3.7**
β-selinene	1494	-	-	0.3
α-selinene	1498	-	0.1	-
bicyclogermacrene	1500	0.4	0.4	0.9
β-bisabolene	1506	-	-	0.1
8-cadinene	1517	-	-	0.1
zonarene	1521		0.2	-
δ-cadinene	1525	0.1	-	0.3
elemol	1552	-	0.3	0.2
germacrene-B	1565	0.3	0.3	0.8
spathulenol	1582	0.2	0.2	0.6
caryophyllene oxide	1589	**3.0**	**3.3**	**3.2**
humulene epoxide II	1616	0.6	0.5	0.8
isospathulenol	1633	-	-	0.7
caryophylla -4(12).8(13)-dien-5-β-ol	1644	1.1	-	-
epi-α-muurolol	1649	-		0.3
α-cadinol	1661	0.3	0.4	0.9
aristolone	1704	-	0.1	-
NI	2362	-	1.3	-
Monoterpene Hydrogenateds		**35.3**	**66.6**	**36.1**
Oxygenated monoterpenes		**5.1**	**10.3**	**18.2**
Sesquiterpene hydrogenateds		**48.0**	**10.1**	**33.0**
Oxygenated sesquiterpenes		**5.2**	**4.8**	**6.7**
Oxygenated aliphatic compound				**0.3**
Total		**93.6**	**91.8**	**94.3**
L= Limbo ; Ls = Leaf sheaths ; R = Rhizomes ; KI = Kovats Indices				

Table 2: *In vitro* Anti-Microbial Activity of *Aframomum sceptrum* (Oliv. & T. Hanb) K. Schum essential oils.

	MIC (in µl/mL)			**Zones of inhibition** (in mm)		
	L	**Ls**	**R**	**L**	**Ls**	**R**
Yeats						
Candida albicans ATCC 10231	> 41.7	> 41.7	> 41.7	NI	NI	NI
Gram-Negative bacteria						
*Escherichia c*oli ATCC 25922	> 41.7	> 41.7	> 41.7	NI	NI	NI
Gram-positif bacteria						
Staphylococcus aureus ATCC 25923	> 41.7	> 41.7	> 41.7	NI	NI	NI

L= Limbo**; Ls =** Leaf sheaths; **R =** Rhizomes; **MIC** = Minimum Inhibitory Concentrations, **MBC** = Minimum Bactericidal Concentrations; **NI** = No Inhibition

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