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Oladipupo A. Lawal[a], Adeleke A. Kasali[a], Andy R. Opoku[b], Anthony B. Ojekale[c], Olugbenga S. Oladimeji[c] & Sena Bakare[d]

[a] Department of Chemistry, Lagos State University, PMB 001 LASU Post Office, Ojo Lagos, Nigeria

[b] Department of Biochemistry & Microbiology, University of Zululand, KwaDlangezwa 3886, South Africa

[c] Department of Biochemistry, Lagos State University, PMB 001 LASU Post Office, Ojo Lagos, Nigeria

[d] Department of Microbiology, Lagos State University, PMB 001 LASU Post Office, Ojo Lagos, Nigeria

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Oladipupo A. Lawal [1]*, Adeleke A. Kasali [1], Andy R. Opoku [2], Anthony B. Ojekale [3], Olugbenga S. Oladimeji [3] and Sena Bakare [4]

[1] Department of Chemistry, Lagos State University, PMB 001 LASU Post Office, Ojo Lagos, Nigeria
[2] Department of Biochemistry & Microbiology, University of Zululand, KwaDlangezwa 3886, South Africa
[3] Department of Biochemistry, Lagos State University, PMB 001 LASU Post Office, Ojo Lagos, Nigeria
[4] Department of Microbiology, Lagos State University, PMB 001 LASU Post Office, Ojo Lagos, Nigeria

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Abstract: The essential oil obtained by hydrodistillation from the dry leaves of *Aframomum melegueta* was analyzed by GC and GC-MS. Twenty-six components representing 92.5 % of the oil were identified. The major constituents were sabinene (35.9 %), α-pinene (15.0 %) and β-caryophyllene (9.7 %). The *in vitro* antibacterial activity of the oil was assayed against twelve microorganisms using agar-disc diffusion and broth-microdilution methods. The oil exhibited significant inhibition on the growth of *Bacillus cereus*, *Staphylococcus aureus, Streptococcus faecalis* and *Escherichia coli* with zones of inhibition and MIC values ranging from (6.3-19.3) mm and (0.2-10.0) mg/ml, respectively.

Key words: *Aframomum melegueta*, Zingiberaceae, essential oil composition, antibacterial activity, sabinene, α-pinene, β-caryophyllene.

Introduction

Aframomum melegueta (Roscoe) K. Schum (Zingiberaceae), popularly called melegueta pepper, alligator pepper or grains of paradise, is a tropical perennial and aromatic plant of about 4-6 m height, widely cultivated for its edible peppery fruits, with purple flowers and long pods containing small reddish brown aromatic and pungent seeds [1,2]. It is native to West and Central Africa, mostly, Cameroon, Central African Republic, Ivory Coast, Liberia, Nigeria and Togo [2,3]. Several biological and pharmacological activities including analgesic, antinociceptive, anti-inflammatory, anti-pyretic, antimicrobial, anti-oxidant, cytotoxic, insecticidical and repellent activities, as well as aphrodisiac, hepatoprotective and hypoglyceamic effects have been reported for this plant [4-9]. In addition, various parts of *A. melegueta* have been used as a remedy for treatments of many infectious diseases, woman's fertility, flavouring foods and stimulant for successful hunting [10,11]. Previous phytochemical studies of *A. melegueta* revealed the presence of alkaloids, flavonoids, glycosides, oleo-resins, tannins, paradol, gingerol, shogaol, zingerone, gingerdione and many sesquiterpenoids [6,9,12,13].

*Corresponding authors (Oladipupo A. Lawal)
E-mail: < jumobi.lawal@lasu.edu.ng >

Literature search on the chemical composition of essential oils of *A. melegueta* showed few reports based on different extraction methods [3-5,14-16]. The seed oils of *A. melegueta* from Nigeria obtained by hydrodistillation were reported to contain humulene (60.9 %) and caryophyllene (21.7 %), α-cardinol (15.75 %) and *cis*-calamenen -10-ol (7.76 %) as the major components [3,14]. The vacuum distillation and soxhlet had humulene (26.2 %) and α-cardinol (21.79 %) as the main compounds, respectively [5,14]. Menut *et al* [15] from Cameroon found the seed oil of *A. melegueta* to have α-humulene (31.3 %), humulene oxide II (26.4%), caryophyllene oxide (17.9 %) and β-caryophyllene (8.5 %) as the major constituents. Dongmo *et al* [4], also from Cameroon reported the leaf oil of *A. melegueta* to be rich in mono-terpenoids (57.0 %), with β-pinene (34.6 %), β-caryophyllene (27.4 %) and caryophyllene oxide (13.0 %) being the abundance compounds. while, the seed oil was dominated by sesquiterpenoids (70.7 %), with α-humulene (47.1 %) and β-caryophyllene (19.5 %) as the major components. In addition, the essential oils obtained from seeds and leaves of *Aframomum melegueta* from Central African Republic, showed the leaf oil to be rich in oxygenated mono- and sesquiterpenoids (45 %), whereas the seed oil contains about 50 % of sesquiterpene hydrocarbons [16].

Although previous publications have reported the seed oils of *A. melegueta* from Nigeria [3,5,14]. However, to the best of our knowledge, no study has reported the chemical composition of the leaf oil of *A. melegueta* from Nigeria. This paper reports the chemical composition and antibacterial activity of essential oil from the leaves of *Aframomum melegueta*.

Experimental

Plant material

Fresh plant materials of *Aframomum melegueta* were purchased from Ikotun central market, Alimosho Local Government Area, Lagos State, Nigeria. Identification of the plant material was carried out at the herbarium of Forestry Research Institute of Nigeria, Ibadan by Mr. K. T. Odewo. A voucher specimen (FHI 107665) was deposited at the Institute Herbarium.

Oil isolation

The air-dry and powdered leaves (500 g) of *A. melegueta* were hydrodistillated in all Clevenger-type apparatus for 3 h in accordance with the British Pharmacopoeia specification [17]. The distillate isolated was preserved in a sealed sample tube and stored under refrigeration until analysis.

Gas chromatography

GC analysis was carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and DB-5 column (30 m x 0.25 mm id), film thickness was 0.25 μm and the split ratio was 1:25. The oven temperature was programmed from 50°C (after 2 min) to 240°C at 5°C/min and the final temperature was held for 10 min. Injection and detector temperatures were 200°C and 240°C, respectively. Hydrogen was the carrier gas. An aliquot (0.5 μL of the diluted oil) was injected into the GC. Peaks were measured by electronic integration. A homologous series of *n*-alkanes were run under the same conditions for determination of retention indices.

Gas chromatography-Mass spectrometry

GC-MS analysis of the oil was performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a DB-5 column (30 m x 0.25 mm id, film thickness 0.25 μm). The oven temperature was programmed from 70- 240°C at the rate of 5°C/min. The ion source was set at 240°C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 ml/min. Scanning range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 μL) was injected into the GC-MS.

The components of the oils were identified based on the comparison of their retention indices and mass spectra with those standards, Wiley 275 library mass spectra databased of the GC-MS system and published data [18,19].

Antibacterial assay

The essential oil was tested against 8 reference bacterial strains and 4 local isolates (two clinical and two environmental strains) obtained from the Department of Biochemistry & Microbiology,

University of Fort Hare, South Africa. Gram-positive bacteria: *Bacillus cereus* (ATCC 10702), *Staphylococcus aureus* (ATCC 6538) and *Streptococcus faecalis* (ATCC 29212). Gram-negative strains: *Enterobacter cloacae* (ATCC 13047), *Escherichia coli* (ATCC 8739), *Kiebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC19582), *Serratia marcescena* (ATCC 9986), *Acinetobacter calcaoceticus*, *Enterococcus faecailis*, *Micrococcus Kristinae* and *Shigella flexineri*. The stock cultures were maintained at 4°C in Müeller-Hinton agar (Oxoid, Germany).

Agar disk diffusion

The *A. melegueta* oil was tested for antibacterial activity by the agar disc diffusion method according to Vijoen *et al*. [20]. The microorganisms were grown overnight at 37°C in 20 mL of Müeller-Hinton broth (MHB). The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 5 standard (1.0×10^8) CFU/mL. 90 mm Petri dishes (Merck, South Africa) containing 12 mL of sterilized Müeller-Hinton agar were inoculated with the microbial suspensions. Sterile Whatman No.1 (6 mm) discs papers were individually placed on the surface of the seeded agar plates and 10 µL of essential oil in dimethylsulfoxide (DMSO) was applied to the filter paper disk. The plates were incubated at 37°C for 24 h and the diameter of the resulting zones of inhibition was measured. All tests were performed in triplicates. Ciprofloxacin and nalidixic acid were used as positive controls, while hexane and DMSO served as negative controls.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the oil was determined using 96-well microtitre dilution method as described by Eloff [21]. Bacterial cultures were incubated in Müller-Hinton broth overnight at 37°C and a 1:1 dilution of each culture in fresh MHB was prepared prior to use in the micro dilution assay. Sterile water (100 µL) was pipetted into all wells of the microtitre plate, before transferring 100 µL of essential oil in DMSO. Serial dilutions were made to obtain concentrations ranging from 10 mg/mL to 0.078 mg/mL. One hundred µL of bacterial culture of an approximate inoculum size of 1.0×10^8 CFU/mL was added to all well and incubated at 37°C for 24 h. After incubation, 40 µL of 0.2 mg/mL *p*-iodonitotetrazolium violet (INT) solution was added to each well and incubated at 37°C. Plates were examined after about 30-60 min. of incubation. Microbial growth is indicated by the presence of a reddish colour which is produced when INT, a dehydrogenase activity detecting reagent, is reduced by metabolically active microorganism to the corresponding intensely coloured formazan. MIC is defined as the lowest concentration that produces an almost complete inhibition of visible micro-organism growth in liquid medium. Solvent controls (DMSO and hexane) and the standard antibiotics ciprofloxacin and nalidixic acid were included in the assay.

Results and discussion

Hydrodistillation of the leaves of *A. melegueta* afforded pale yellow oil with the yield 0.91 %. Twenty-six components, representing 94.5 % of the oil were identified. Table 1 list the constituents identified in order of their elution on the apolar DB-5 column, together with their percentage compositions. The leaf oil of *A. melegueta* was mainly composed of monoterpenoids (77.6 %), and the total amount of monoterpene hydrocarbons (64.7 %) was relatively higher than the oxygenated monoterpenes (12.9 %). While, sesquiterpenoids accounted for 16.9 % of the total oil. The major constituents of the oil were sabinene (35.9 %), α-pinene (15.0%), β-caryophyllene (9.7 %), caryophyllene oxide (4.2 %), terpinen-4-o1 (4.1 %) and γ-terpinene (4.0 %).

Concerning the major constituents found in previously analyzed leaf oils of *A. melegueta* [4,16] with the present study, large diversity was observed, which suggest the existence of chemical variety within the *A. melegueta* species. Although, all the oils were dominated by monoterpenoids, but compositional constituents differs. The species from Cameroon was characterized by β-pinene (34.6 %), β-caryophyllene (27.4 %), caryophyllene oxide (13.0 %), bornyl acetate (7.2 %) and α-pinene (6.2 %), and the Nigerian species was

Table 1. Chemical composition of *Aframomum melegueta* essential oil

Compound	RI[a]	% Composition
α-Pinene	936	15.0
Sabinene	971	35.9
Myrcene	985	1.9
α-Terpinene	1017	2.4
Limonene	1028	1.8
trans-(β)-ocimene	1043	0.5
γ-Terpinene	1056	4.0
trans-Sabinene hydrate	1066	2.1
Terpinolene	1083	1.0
ρ-Cymenene	1086	2.2
cis-Sabinene hydrate	1091	2.3
trans-Pinocarveol	1142	0.6
Terpinen-1-o1	1172	0.5
Terpinen-4-o1	1176	4.1
α-Terpineol	1181	0.3
Myrtenol	1199	0.6
Sabinyl acetate	1279	0.9
Myrtenyl acetate	1329	1.5
α-Copaene	1363	0.2
β-Bourbonene	1392	0.2
β-Caryophyllene	1421	9.7
γ-Selinene	1485	1.6
epi-α-Selinene	1511	0.2
Caryophyllene oxide	1576	4.2
Viridiflorol	1587	0.6
Humulene epoxide 11	1601	0.2
Monoterpene hydrocarbons		64.7
Oxygenated monoterpenes		12.9
Sesquiterpene hydrocarbons		11.9
Oxygenated sesquiterpenes		5.0
Total identified		94.5

[a]RI = Retention Indices relative to C_9-C_{24} *n*-alkanes on the DB-5 column

subjugated by sabinene (35.9 %), α-pinene (15.0 %), β-caryophyllene (9.7%), caryophyllene oxide (4.2 %), terpinen-4-o1 (4.0%) and γ-terpinene (4.0 %). Interestingly, β-pinene, which was found to be a major component of the Cameroonian species, along with bornyl acetate, terpinen-4-ol acetate and caryophylladienol were not detected in our study. In addition, sabinene, γ-terpinene and other constituents in relatively smaller amount such as p-cymenene, *trans*-sabinene hydrate, myrcene and β-selinene were been identified for the first from the leaf oils of *A. melegueta*. Futhermore, reported studies on the chemical composition of essential oils from leaves of *Aframomum* species showed that pinenes, limonene, 1,8-cineole, β-caryophyllene, caryophyllene oxide, α-humulene, *cis*-pinocamphone and thymol were found to be prevalent [4,16,22-30]. On the other hand, the compositional profile of the present study predominated by sabinene, has not been reported as the main constituent in any *Aframomum* species. The qualitative differences

in the chemical composition of the essential oils of *A. melegueta* from the pervious reports [4,16] and the present study may be attributable to climactic and environmental conditions, nutritional status of each plant, and other factors, which can influence essential oil composition [31,32].

Lastly, according to the classification of the constituents from the essential oils of several species of the genus *Aframomum* that has been previously reported [33]; six different chemotypes had been distinguished from the essential oils of *Aframomum* species. Type I, group having monoterpenes, with roughly equal amounts of hydrocarbons and oxygen-containing compounds (*A. angustifolium* and *A. korarima*); type II, group rich in oxygen-containing monoterpenes, with 1,8-cineole as major constituent (*A. alboviolaceum* and *A. masuianum*); type III, those characterized by oxygen-containing monoterpenes, with major constituents different from 1,8-cineole (*A. danielli* and *A. exscapum*); type IV, group with higher sesquiterpene contents (*A. polyanthum* and *A. chlamydanthum*); type V, those containing aliphatic components (*A. sulcatum* and *A. alboviolaceum*) and type VI, those characterized by high monoterpene hydrocarbon content (*A. citratum* and *A. kayserianum*). From our results, the chemical composition of the leaf oil of *Aframomum melegueta* from Nigeria, with a 64.7 % monoterpene hydrocarbon compounds belongs to the type VI.

The data from the antibacterial activity of *A. melegueta* essential oil (Table 2) revealed *S. aureus* to be the most susceptible bacteria with the largest inhibition zone of 19.3 mm, while, *S. marcescena* (9.7 mm), *S. flexineri* (9.3 mm), *M. Kristinae* (8.7 mm), *E. faecailis* (8.3 mm) and *E. cloacae* (6.3 mm) exhibited the smallest zones of inhibition. In addition, the MIC values showed *S. aureus* having the lowest MIC value (0.2 mg/mL) and the highest MIC of 10.0 mg/mL was against *E. cloacae*, *E. faecailis* and *M. Kristinae*, respectively. When compared with standard antibiotics (ciprofloxacin and nalidixic acid), the oil showed a weak to moderate range of inhibition zones (6.3 ± 0.6 to 19.3 ± 1.5) mm against the standard antibiotics (12.0 ± 1.0 to 19.3 ± 1.5) mm.

Comparing this result with the literature data

Table 2. Antibacterial activity of essential oil from leaves of *A. melegueta*

Microorganisms	*A. melegueta*		Ciprofloxacin		Nalidixic acid	
	IZ[a]	MIC[b]	IZ[a]	MIC[b]	IZ	MIC
B. cereus	16.0 ± 1.5	1.3	16.0 ± 3.0	0.6	12.7 ± 1.5	1.3
S. aureus	19.3 ± 1.5	0.2	19.0 ± 1.0	0.2	19.3 ± 1.5	0.3
S. faecalis	15.7 ± 0.6	1.3	13.7 ± 1.5	2.5	15.0 ± 1.0	1.3
E. cloacae	6.3 ± 0.6	10.0	13.0 ± 1.0	5.0	10.3 ± 0.6	10.0
E. coli	17.3 ± 1.2	0.6	17.3 ± 4.0	0.3	18.3 ± 1.2	0.3
K. pneumoniae	11.7 ± 1.5	2.5	12.0 ± 1.0	5.0	12.3 ± 0.6	2.5
P. aeruginosa	12.0 ± 1.0	2.5	13.7 ± 1.5	1.3	ND	1.3
S. marcescens	9.7 ± 0.6	5.0	12.3 ± 1.2	10.0	11.7 ± 1.5	5.0
A. calcaoceticus ‡	11.0 ± 1.0	5.0	14.0 ± 1.0	5.0	11.0 ± 0.0	10.0
E. faecailis ‡	8.3 ± 0.6	10.0	16.3 ± 1.5	0.6	12.6 ± 2.1	2.5
M. kristinae §	8.7 ± 1.2	10.0	14.0 ± 1.0	2.5	12.3 ± 1.2	5.0
S. flexineri §	9.3 ± 1.2	5.0	14.3 ± 0.6	1.3	13.0 ± 0.0	2.5

[a]IZ -Inhibition zones diameter (mm) including diameter of sterile disc (6 mm)
Values are given as Mean ± SE (n = 3)
[b]MIC - minimum inhibitory concentration values are given as mg/ml
ATCC = American Type Culture Collection
‡ - Clinical isolates; § - Environmental strains

on antibacterial activity of essential oils of *Aframomum* species [22,26,34,35], it appears that *A. melegueta* oil exhibited prominent activity which could be attributed to the presence of some major components such as sabinene, α-pinene and β-caryophyllene along with other components in lower amount such as, β-pinene and linalool, which were already known to exhibit antimicrobial and bacteriostatic activities [36,37].

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