AkiNik

American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP American Journal of Essential Oils and Natural Products

ISSN: 2321-9114
AJEONP 2017; 5(4): 14-19

Received: 15-08-2017
Accepted: 16-09-2017

Oladipupo A Lawal
Natural Products Research Unit, Department of Chemistry, Lagos State University, PMB 0001 LASU Post Office, Ojo Lagos, Nigeria

Isiaka A Ogunwande
Natural Products Research Unit, Department of Chemistry, Lagos State University, PMB 0001 LASU Post Office, Ojo Lagos, Nigeria

Rebamang A Mosa
Department of Biochemistry & Microbiology, University of Zululand, Kwa Dlangezwa, South Africa

Andy R Opoku
Department of Biochemistry & Microbiology, University of Zululand, Kwa Dlangezwa, South Africa

Correspondence:
Oladipupo A Lawal
Natural Products Research Unit, Department of Chemistry, Lagos State University, PMB 0001 LASU Post Office, Ojo Lagos, Nigeria

Essential oils of *Aframomum danielli* and *Aframomum melegueta* (Zingiberaceae): Chemical composition and antibacterial activity

Oladipupo A Lawal, Isiaka A Ogunwande, Rebamang A Mosa and Andy R Opoku

Abstract
The hydrodistilled essential oils extracted from the dry leaves and seeds of *Aframomum danielli* and seeds of *Aframomum melegueta* collected from South West, Nigeria were analyzed by GC and GC/MS. Eight and twenty-one different components representing 92.7% and 94.5% of the total oil contents were identified. The oils of *A. danielli* were found to be rich in β-pinene (25.1%) and 1,8-cineole (37.2%) for the leaf and seed oil, respectively. While, the oil seed of *A. melegueta* had 2-octyl acetate (60.4%) as the major compound. Assessement of agar-disc diffusion and broth-microdilution methods for the antibacterial activity of the oils against twelve microorganisms, revealed the oil of *A. melegueta* to be slightly more active than the oils of *A. danielli*. Among the organisms tested, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Klebsiella pneumoniae* with zones of inhibition (ZI) ranging from 13.6 mm -20.6 mm and minimum inhibitory concentrations (MIC) values of 0.2-2.5 mg/mL were the most sensitive to the oils.

Keywords: Aframomum *species, Zingiberaceae, essential oil composition, antibacterial activity, 2-octyl acetate, 1,8-cineole, α-pinene.*

Introduction
The present study is part of an extensive research into the volatile compositions and biological potentials of Nigerian medicinal plants and herbs [1]. This paper reports the chemical compositions and antimicrobial activity of essential oils of *Aframomum melegueta* and *A. danielli*. The genus *Aframomum* (family: Zingiberaceae) contains almost fifty species in West and Central Africa. The distinguishing feature of this genus is the attribute of highly pungent and aromatic seeds. All the plant parts also exude a strong aroma when pulverized [2]. The leaves of *A. melegueta* are used for the treatment of measles [3]. Extract of *A. melegueta* was found to be effective and a safe tool for reducing body fat [4], act as antiviral potentials/ anti-HIV remedy [5], and possess hepatotoxicity activity [6] and neuroprotective effects [7]. Different extracts of *A. melegueta* have shown antimicrobial [8-10], oviposition-deterrent activity [11] antifeedant [12], antioxidants [13], antidiarrheal and anti-inflammatory activity [14] and prevention of acquired immunodeficiency syndrome [15].
Previously, humulene and caryophyllene occurred in higher proportions in the volatile oil of the seed of *A. melegueta* [16]. The major components of the leaf oil were found to be myrtenyl acetate (29.06%), isolimonene (19.47%) while caryophyllene oxide (19.70%), myrtenyl acetate (14.70%), β-eudesmene (10.83%) and β-caryophyllene (10.13%) make up the composition of the stem oil [17], whereas the root essential oil comprised of myrtenyl acetate (22.70%) and pinocarvyl acetate (11.50%). However, the seed comprised mainly of α-humulene (48.78%), β-caryophyllene (32.50%). In another report, the major constituents of the leaf oil [2] were identified as sabinene (35.9%), α-pinene (15.0%) and β-caryophyllene (9.7%). The major components present in the absolute and supercritical fluid extraction product [18], respectively, were 6-paradol (35.1 and 13.3%), 6-shogaol (21.5 and 6.1%), 6-gingerdione (9.8 and 28.0%), *α*-humulene (10.5 and 7.2%) and [6]-gingerol (1.3 and 10.0%). The seed essential oil of *A. melegueta* presents a characteristic composition with β-caryophyllene (8.5%), α-humulene (31.3%) and their epoxides (respectively 17.9% and 27.7%) as main constituents [19]. Eugenol (82.2%) occurred in abundance in the oil [20]. β-Pinene (>30%) predominates in the essential oils of the leaves and seeds of *A. melegueta* [21]. GC/MS analysis of the hexane and

Methanol extracts of *A. melegueta* seed yielded gingerol, zingiberone, paradol, *trans*-6-shogaol, *cis*-isoelemicin, β-bisabolene, α-guaiene, aromadendrene, *trans*-β-farnesene and geraniol [12]. The essential oil of *A. melegueta* displayed insect repellency against *Rhyzopertha dominica* [22], antimicrobial activity [2, 23], antifungal effect [24], moderate inhibition of acetyl-cholinesterase [25], antioxidant, antidiabetic and antihypertensive activity [20].

The postharvest treatment with *A. danielli* extract extended the shelf life of tomato and retained significant amount of TSS, lycopene, ascorbic acid of tomato fruits [26]. It reduces the growth of aflatoxin [27]. Extracts of the plant possessed antimicrobial [10, 28], antioxidant [29, 30] and larvicidal [31] properties. The plant has some protective effects on liver cells and ethanolic extracts of the seeds of *A. danielli* controlled three life cycle stages of the parasitic nematodes [32]. The monoterpenes in the essential oil of the plant possessed antifungal activity, inhibited food spoilage yeasts and aflatoxigenic moulds [33] and control lipid oxidation [34].

The essential oils from the seeds of *A. danielli* [19] had high content of 1,8-cineole(48.9%) while another report [35] had its content as (56.16%). The major constituents of the seed oil from Nigeria [36] were 1,8-cineole (59.8%), β-pinene (13.2%), α-terpineol (9.3%). Also, 1,8-cineole (25.5 - 34.4%), β-pinene (14.1 - 15.2%) and α-terpineol (9.9 - 12.1%) occurred as highest constituent of the rhizomes [37]. Eugenol (51.1%) and 1,8-cineole (10.9%) were also characterized in the oil of *A. danielli* [21]. In a recent study [38], the leaf, stem, rhizome and pod volatile oils were dominant in α-pinene (30.94–47.55%), while the seed oil contained a high amount of 1,8-cineole (53.44%). The bioactive compounds include phytylplastoquinone isolated from the petrol extract and plastoquinone-7 (heptaplastoquinone) from the alcohol extract which are reported to have ability to inhibit 5-lipoxygenase [39]. The oil of *A. danielli* showed antimicrobial activity against all Gram-positive and Gram-negative bacteria tested, as well as against yeasts and filamentous fungi [10, 37], displayed antioxidant [21, 38], antidiabetic and antihypertensive activity [21]. *Aframomum danielli* oils exhibited ability to reduce fumonisin B1, fumonisin B2 and ochratoxin A in kunu zaki [35] as well as ergosterol value in cocoa beans [40].

Materials and methods

Plant materials

Dried plant materials of *A. danielli* and *A. melegueta* were purchased from different markets in Lagos State, Nigeria. Botanical identification of the plant materials was carried out at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria, where voucher specimens FHI 107665 (*A. danielli*) and FHI 107666 (*A. melegueta*) were deposited.

Distillation of the essential oils

Aliquots of 150 g (seeds) and 300 g (leaves) of each of the pulverized samples of *A. danielli* and 150 g (seeds) of *A. melegueta* were carefully introduced into a 5 L flask and distilled water was added until it covered the sample completely. Essential oils were obtained by separate hydrodistillation which was carried out in an all glass Clevenger-type distillation unit designed according to the established specification [41]. The distillation time was 3 h and conducted at normal pressure. The volatile oils distilled over water and were collected in the receiver arm of the apparatus into separate clean and previously weighed sample bottles. The oils were kept under refrigeration (4ºC) until the moment of analyses.

Analysis of the essential oils

Gas Chromatography (GC) analysis

GC analyses of the oils were carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and HP-5MS 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

GC-MS analyses of the oils were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a HP5-MS capillary column (30m X 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from 70- 240 ºC at the rate of 5 ºC/min. The ion source was set at 240 ºC and electron ionization at 70eV. Helium was used as the carrier gas at a flow rate of 1 ml/min. Scanning range was 35 to 425 amu. 1.0 µl of diluted oil in hexane was injected into the GC/MS.

Identification of constituents of the essential oils

The constituents of the essential oils were identified by comparison of their relative GC retention indices with standards from literature, retention indices on HP-5 MS column, peak enrichment on co-injection with authentic standard wherever possible and comparison of mass spectra with literature data [42, 43].

Antibacterial assay

The antibacterial activity of the oils was tested against twelve microbes obtained from Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. The microbes including *Bacillus subtilis* (ATCC 10702), *Staphylococcus aureus* (ATCC 6538), *S. faecalis* (ATCC 29212), *Escherichia coli* (ATCC 9739), *Enterobacter cloacae* (ATCC 13047), *Kliebsiella pneumonia* (ATCC 19582), *Serratia marcescena* (ATCC 9986), *Acinetobacter calcaoceticus*, *Enterococcus faecailis*, *Micrococcus kristinae*, and *Shigella flexineri*. The stock cultures were maintained at 4 ºC in Müeller-Hinton agar (Oxoid, Germany).

Agar disc diffusion

The essential oils were tested for antibacterial activity by the agar disc diffusion method according to an established method [44]. The microorganisms were grown overnight at 37ºC 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. Petri dishes (90 mm, 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 40 mg/mL essential oil in dimethylsulfoxide solution (DMSO) was applied to the filter paper disk. The plates were incubated at

37ºC for 24 h and the diameter of the resulting zones of inhibition (ZI) was measured. All tests were performed in triplicates. Ciprofloxacin (25 µg/mL) and nalidixic acid (50 µg/mL) were used as positive controls, while hexane and DMSO solution served as negative controls.

Minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations (MIC) of the samples were determined using 96-well microtitre dilution method as described previously [45]. Bacterial cultures were incubated in Müller-Hinton broth overnight at 37ºC 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 each sample in DMSO. Serial dilutions were made to obtain concentrations ranging from 10 mg/mL to 0.078 mg/mL. Then, 100 µL of bacterial culture of an approximate inoculum size of 1.0 X 10^8 CFU/mL was added to all well and incubated at 37ºC 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 37ºC. Plates were examined after about 30-60 min. of incubation. 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 & Discussion

Chemical constituents of the essential oils

The yields of essential oils were 0.21% (v/w, *A. melegueta* seeds), 0.12% (v/w, *A. danielli* leaf) and 0.45% (v/w, *A. danielli* seeds), calculated on a dry weight basis. Oil samples were light yellow in colouration. The identity and percentages of the chemical constituents present in the oil and their retention indices on HP-5MS column could be seen in Table 1. The main classes of compounds present in the seed oil of *A. melegueta* were the acetate (60.4%), oxygenated monoterpenes (16.5%) and sesquiterpene hydrocarbons (10.2%) with 2-octyl acetate (60.4%) and linalyl acetate (16.5%) being the most abundant compounds of the essential oil. The present compositional pattern was found to be different from previous reports on the essential oil of *A. melegueta*. Except for the presence of *α*-humulene and β-caryophyllene, all other compounds were not reported previously as constituents of the oil of *A. melegueta*. In addition, some compounds such as sabinene and α-pinene [2], as well as myrtenyl acetate, isolimonene, pinocarvyl acetate and β-eudesmene [17] previously identified in the oil samples were conspicuously absent in the present study. Moreover, 6-paradol, 6-shogaol, 6-gingerdione [18] along with eugenol [20] were not identified in the present oil sample. Monoterpene hydrocarbons (53.0%), oxygentaed monoterpenes (19.2%) and oxygenated sesquiterpenes were the classess of compounds present in the leaf of *A. danielli*. However, the seed oil contained larger quantity og oxygenated monoterpenes (83.5%). The main constituents of the leaf oil were β-pinene (25.1%), limonene (13.8%) and α-pinene (10.9%). On the other hand, 1,8-cineole (37.2%) and linalool (31.3%) were the major compounds of the seed oil. The high contents of 1,8-cineole and β-pinene makes the composition of the present oil samples similar to previous reports [19, 35-38]. However, α-pinene, α-terpineol and euegnol previously defined in the essential oils of *A. danielli* [21, 37, 38] were not identified in the present study. The variation in the chemical constituents between the studied oil samples and previous analyses may be attributed to factors such as the age and nature of the plant, handling conditions and chemotype. Other notable factors include period of collection of the plant as well as variation in the environmental and climatic conditions between the different country of analyses.

Antimicrobial activity of the essential oils

The results of the antibacterial activity of the essential oils could be seen in Table 2 and Table 3. The results indicated that the essential oils of *A. danielli* (leaf), *A. danielli* (seed) and *A. melegueta* (seed) displayed the strongest inhibitions to the growth of *E. coli* with ZI of 19.6 ± 2.1 mm, 19.3 ± 2.1 mm and 20.3 ± 2.1 mm with MIC of 0.6 mg/mL, 0.3 mg/mL and 0.3 mg/mL respectively. Only *A. melegueta* (seed) was the most active against *S. aureus* with ZI 20.6 ± 2.1 mm as well as MIC of 0.2 mg/mL. Both samples of *A. danielli* (leaf) and *A. danielli* (seed) showed stronger inhibition against *S. aureus* with ZI of 15.0 ± 1.0 mm and 19.0 ± 1.0 mm respectively while the MIC were 0.6 mg/mL and 0.3 mg/mL respectively. While *A. danielli* (leaf) exhibited strong activity towards *B. cereus* (ZI 11.6 ± 1.5 mm, MIC 2.5 mg/mL), both *A. danielli* (seed, ZI 12.6 ± 1.5 mm, MIC 1.3 mg/mL) and *A. melegueta* (seed, ZI 12.6 ± 1.5 mm, MIC 1.3 mg/mL) displayed stronger inhibition of the organism. This same trend was shown by the essential oils towards the growth of *S. faecalis* with *A. danielli* (seed, ZI 14.6 ± 1.5 mm, MIC 1.3 mg/mL) and *A. melegueta* (seed, ZI 13.6 ± 1.5 mm, MIC 1.3 mg/mL) displaying stronger inhibition than *A. danielli* (leaf) having ZI of 14.6 ± 1.5 mm and MIC of 2.5 mg/mL. All the essential oils showed moderate inhibition to the growth of *P. aeruginosa* and *S. marcescens* with MIC of 2.5 mg/mL. But, the oil of *A. melegueta* (seed) inhibited the growth of *K. pneumoniae* (ZI 15.0 ± 1.0 mm, MIC 1.3 mg/mL) much more than *A.danielli* (leaf) having ZI of 14.6 ± 1.2 mm and MIC of 2.5 mg/mL as well as *A. danielli* (seed) with ZI of 14.3 ± 1.5 mm and MIC of 2.5 mg/mL. Generally, all the studied oil samples exhibited weak activities against *E. cloacae, A. calcaoceticus, E. faecailis, M. kristinae*, and *S. flexineri.* The result shows that the studied oil samples possess some levels of antimicrobial potentials and are in agreement with previous findings on oils of *A. danielli* [10, 37] and *A. melegueta* [2, 23].

Table 1: Chemical composition of essential oils from *A. danielli* and *A. Melegueta*

Compounds[a]	RI(Cal.)	RI (Lit.)	Percent composition		
			A.dl	***A.ds***	***A.ms***
α-pinene	937	937	10.9	-	-
camphene	951	951	0.6	-	-
β-pinene	981	981	25.1	-	-
α-phellandrene	1005	1005	2.5	-	-
(*E*,*E*)-2,4-heptadienal	1011	1011	3.8	-	-
limonene	1028	1028	13.8	1.3	-
1,8-cineole	1033	1033	-	37.2	-
trans-(β)-ocimene	1043	1043	-	6.3	-
(Z)-linalool oxide (furanoid)	1072	1072	-	2.1	-
(*E*)-linalool oxide (furanoid)	1089	1089	-	1.9	-
linalool	1100	1100	-	31.3	-
fenchyl alcohol	1106	1106	1.6	-	-
2-octyl acetate	1147	1147	-	-	60.4
(*E*)-pinocarveol	1151	1151	5.0	-	-
terpinen-4-o1	1177	1177	1.4	7.2	-
α-terpineol	1189	1189	2.3	3.8	-
myrtenal	1205	1205	3.8	-	-
linalyl acetate	1263	1263	-	-	16.5
bornyl acetate	1289	1289	1.3	-	-
myrtenyl acetate	1335	1335	3.8	-	-
β-elemene	1389	1389	0.5	-	-
β-caryophyllene	1426	1426	4.1	-	2.4
α-humulene	1461	1461	-	-	6.0
germacrene D	1483	1483	1.1	-	-
β-selinene	1489	1489	-	-	1.8
δ-cadinene	1518	1518	0.3	-	-
elemol	1548	1548	1.8	-	-
(*E*)-nerolidol	1565	1565	-	-	1.1
caryophyllene oxide	1586	1586	7.4	3.2	3.5
selina-1,3,7(II) trine-8-one	1606	1606	1.9	-	-
zingerone	1643	1643	-	-	1.0
incensole	2146	2146	1.5	-	-
Total			94.6	94.3	94.7
Monoterpene hydrocarbons			53.0	7.6	-
Oxygenated monoterpenes			19.2	83.5	16.5
Sesquiterpene hydrocarbons			6.0	-	10.2
Oxygenated sesquiterpenes			12.6	3.2	7.6
Others			3.8	-	60.4

Elution order on HP-5MS column relative to C_9-C_{24} *n*-alkanes; RI (Cal.) Retention indices on HP-5MS column; RI (Lit.) Literature retention indices; -, Not identified; *A.dl*, *A. danielli* (leaf); *A.ds*, *A. danielli* (seed); *A.ms*, *A. melegueta* (seed).

Table 2: Antibacterial activity of essential oils of *A. danielli* and *A. Melegueta*

Microorganisms[b]	Zones of inhibition (mm)[a]				
	A. daniellii		***A. melegueta***	**Ciprofloxacin**	**Nalidixic acid**
	Leaf	**Seed**	**Seed**		
B. cereus	11.6 ± 1.5	12.6 ± 1.5	12.6 ± 1.5	16.0 ± 3.0	12.7 ± 1.5
S. aureus	15.0 ± 1.0	19.0 ± 1.0	20.6 ± 2.1	19.0 ± 1.0	19.3 ± 1.5
S. faecalis	14.6 ± 1.5	13.6 ± 2.1	15.6 ± 1.5	13.7 ± 1.5	15.0 ± 1.0
E. cloacae	8.0 ± 0.0	7.0 ± 1.0	8.3 ± 0.6	13.0 ± 1.0	10.3 ± 0.6
E. coli	19.6 ± 2.1	19.3 ± 2.1	20.3 ± 2.1	17.3 ± 4.0	18.3 ± 1.2
K. pneumoniae	14.6 ± 1.2	14.3 ± 1.5	15.0 ± 1.0	12.0 ± 1.0	12.3 ± 0.6
P. aeruginosa	12.0 ± 1.0	11.6 ± 0.6	12.6 ± 1.5	13.7 ± 1.5	ND
S. marcescens	11.0 ± 0.0	10.3 ± 0.6	11.3 ± 0.6	12.3 ± 1.2	11.7 ± 1.5
A. calcaoceticus[‡]	11.3 ± 0.6	10.0 ± 0.0	9.6 ± 0.6	14.0 ± 1.0	11.0 ± 0.0
E. faecailis[‡]	6.3 ± 0.6	8.6 ± 0.6	10.0 ± 0.0	16.3 ± 1.5	12.6 ± 2.1
M. kristinae[§]	6.6 ± 1.2	9.3 ± 0.6	11.6 ± 0.6	14.0 ± 1.0	12.3 ± 1.2
S. flexineri[§]	7.6 ± 0.6	10.6 ± 0.6	14.0 ± 1.0	14.3 ± 0.6	13.0 ± 0.0

[a]ZI -Inhibition zones diameter (mm) including diameter of sterile disc (6 mm), values are given as Mean ± SE (n = 3); [b]ATCC = American Type Culture Collection, USA; [‡]- Clinical isolates; [§] - Environmental strains; ND Not determined.

Table 3: Minimum inhibitory concentration of *A. daniellii* and *A. melegueta* essential oils[a]

Microorganisms	*A. daniellii*		*A. melegueta*	Ciprofloxacin	Nalidixic acid
	Leaf	Seed	Seed		
B. cereus	2.5	1.3	1.3	0.6	1.3
S. aureus	1.3	0.6	0.2	0.2	0.3
S. faecalis	2.5	1.3	1.3	2.5	1.3
E. cloacae	> 10	> 10	> 10	5	10
E. coli	0.6	0.3	0.3	0.3	0.3
K. pneumoniae	2.5	2.5	1.3	5	2.5
P. aeruginosa	2.5	2.5	2.5	1.3	1.3
S. marcescens	2.5	2.5	2.5	10	5
A. calcaoceticus‡	5	10	10	5	10
E. faecailis‡	> 10	10	5	0.6	2.5
M. kristinae§	> 10	10	5	2.5	5
S. flexineri§	> 10	5	5	1.3	2.5

[a]MIC - minimum inhibitory concentration values are given as mg/mL; values are given as Mean ± SE (n = 3); [b]ATCC = American Type Culture Collection, USA; ‡- Clinical isolates; § - Environmental strains.

Conclusions

The present study is a contribution to the understanding of the chemical variations and biological activity of the essential oils of *A. danielli* and *A. melegueta* grown in Nigeria. The results indicated variations in the compositional pattern of the essential oils and validity of the antibacterial potentials of the essential oils, (particularly against *E. coli* and the Gram-positive bacteria), which verifies the claims of their uses in folk medicine.

Acknowledgments

Dr. Ogunwande, IA and Dr. Lawal, OA are grateful to TETFund Nigeria for financial support.

References

1. Lawal OA, Ogunwande IA, Jinadu BT, Bejide OT, Essien EE. Chemical composition and biological activities of essential oils from three medicinal plants of Nigeria. In: Advances in Chemistry Research. Nova Science Publisher Inc, New York. 2017, 95.
2. Lawal OA, Kasali AA, Opoku RA, Ojekale AB, Oladimeji OS Bakare S. Chemical composition and antibacterial activity of essential oil from the leaves of *Aframomum melegueta* (Roscoe) K. Schum from Nigeria. Journal of Essential Oil-Bearing Plants. 2015; 18(1):222-229.
3. Oladunmoye MK, Kehinde FY. Ethnobotanical survey of medicinal plants used in treating viral infections among Yoruba tribe of South Western Nigeria. African Journal of Microbiology Research. 2011; 5(19):2991-3004.
4. Sugita J, Yoneshiro T, Sugishima Y, Ikemoto T, Uchiwa H, Suzuki I, Saito M. Daily ingestion of grains of paradise (*Aframomum melegueta*) extract increases whole-body energy expenditure and decreases visceral fat in humans. Journal of Nutritional Science and Vitaminology (Tokyo). 2014; 60(1):22-27.
5. Esimone CO, Omabuwajo OR, Amadi C, Adikwa MU, Edrada R, Proksch P *et al.* Antiviral potentials of Nigerians *Aframomum melagueta* Roscoe and *Piper guineese* Schum. and Thonn. Nigerian Journal of Natural Products and Medicine. 2010; 10:51-54.
6. Nwaehujor CO, Eban LK, Ode JO, Ejiofor CE, Igile GO. Hepatotoxicity of methanol seed extract of *Aframomum melegueta* [Roscoe] K. Schum. (Grains of paradise) in sprague-Dawley Rats. American Journal of Biomedical Research. 2014; 2(4):61-66.
7. Kumar A, Kennedy-Boone D, Weisz HA, Capra BA, Uchida T, Jennings K *et al.* Neuroprotective effects of *Aframomum melegueta* extract after experimental traumatic brain injury. Natural Product Chemistry and Research. 2015; 3:167. doi: 10.4172/2329-6836.1000167.
8. Doherty FV, Olaniran OO, Kanife UC. Antimicrobial activities of *Aframomum melegueta* (Alligator Pepper). International Journal of Biology. 2010; 2(2):126-131.
9. Odetunde SK, Adekola IT, Avungbeto MO, Lawal A. Antimicrobial effect and phytochemical analysis of *Aframomum melegueta* on some selected bacteria and fungi. European Journal of Biotechnology and Bioscience. 2015; 3(4):15-19.
10. Njimoh DL, Assob JCN, Mokake SE, Nyhalah DJ, Yinda CK, Sandjon B. Antimicrobial activities of a plethora of medicinal plant extracts and hydrolates against human pathogens and their potential to reverse antibiotic resistance. International Journal of Microbiology, Volume 2015 Article ID 547156, 15 pages. 2015; http://dx.doi.org/10.1155/2015/547156.
11. Adesina JM, Jose AR, Rajashaker Y, Afolabi LA. Entomotoxicity of *Xylopia aethiopica* and *Aframomum melegueta* in suppressing oviposition and adult emergence of *Callasobruchus maculatus* (Fabricus) (Coleoptera: Chrysomelidae) infesting stored Cowpea seeds. Jordan Journal of Biological Sciences. 2015; 8(4): 263-268.
12. Ntonifor NN, Mueller-Harvey I, van Emden HF, Brown RH. Antifeedant activities of crude seed extracts of tropical African species against *Spodoptera littoralis* (Lepidoptera: Noctuidae). International Journal of Tropical Insect Science 2006; 26(2):78-85.
13. Adegoke GO, Makinde O, Falade KO, Uzopeters PI. Extraction and characterization of antioxidants from *Aframomum melegueta* and *Xylopia aethiopica*. European Food Research and Technology. 2003; 216:526-528.
14. Umuoko S, Ashorobi RB. Further evaluation of the anti-inflammatory activity of *Aframomum melegueta* seed extract and its possible mechanism of action. Nigerian Journal of Health and Biomedical Science. 2005; 4:35-39.
15. Vidjin E, Gbodossou A. Water-soluble antiviral product containing *Aframomum melegueta*, for the treatment and prevention of acquired immunodeficiency syndrome (aids). US Patent 20120052140 A1; 2012.
16. Ajaiyeoba EO, Ekundayo O. Essential oil constituents of *Aframomum melegueta* (Roscoe) K. Schum. seeds (alligator pepper) from Nigeria. Flavour and Fragrance Journal. 1999; 14(2):109-111.
17. Owokotomo IA, Ekundayo O, Oguntuase BJ. Chemical constituents of the leaf, stem, root and seed essential oils of *Aframomum melegueta* (K. Schum) from South West

Nigeria. International Research Journal of Pure and Applied Chemistry. 2014; 4(4):395-401.
18. Fernandez X, Pintaric C, Lizzani-Cuvelier L, Loiseau AM, Morello A, Pelleri P. Chemical composition of absolute and supercritical carbon dioxide extract of *Aframomum melegueta* Flavour and Fragrance Journal, 2006; 21(1):162-165.
19. Menut C, Lamaty G, Amvam Zollo PH, Atogho BM, Abondo R, Bessière JM. Aromatic plants of tropical central Africa. V. volatile components of three Zingiberaceae from Cameroon: *Aframomum melegueta* (roscoe) K. Schum., *A. daniellii* (hook. f.) K. Schum. and *A. sulcatum* (oliv. and hanb.) K. Schum. Flavour and Fragrance Journal 1991; 6(3):183-186.
20. Lamaty G, Menut C, Koudou J, Régnier P, Bessière JM. Aromatic plants of tropical central Africa. XI. Essential oils of leaf and seed of *Aframomum melegueta* (Roscoe) K. Schum. from Central African Republic, Journal of Essential Oil Research. 1993; 5(1):81-83.
21. Adefegha SA, Olasehinde TA, Oboh G. Essential oil composition, antioxidant, antidiabetic and antihypertensive properties of two *Afromomum* species. Journal of Oleo Science. 2017; 66(1):51-63.
22. Ukeh DA. Bioactivities of essential oils of *Aframomum melegueta* and *Zingiber officinale* both (Zingiberaceae) against *Rhyzopertha dominica* (Fabricius). Journal of Entomology. 2008; 5:193-199.
23. Uzeh RE, Oguntosin DO. Efficacy of essential oils from some African spices against two strains of *Bacillus cereus* isolated from vegetable salad. Journal of Food Research. 2013; 2(6):48-54.
24. Héritier VNV, Paulin MK, Nadège NK, Michel MP, Jude-Thaddée MN. *In vitro* antifungal activity of essential oils extracted from some plants of *Tangawisi*® products on *Candida albicans.* Journal of Pharmacognosy and Phytochemistry. 2017; 6(3):01-05.
25. Owokotomo IA, Ekundayo O, Abayomi TG, Chukwuka AV. *In-vitro* anti-cholinesterase activity of essential oil from four tropical medicinal plants. Toxicology Reports. 2015; 2:850-857.
26. Babarinde GO, Adegoke GO, Akinoso R. Effect of *Aframomum danielli* extract on some chemical and antioxidant components of Roma tomato variety during storage. American Journal of Food Technology. 2014; 9: 28-38.
27. Adegoke GO, Adekoyeni OO, Akinoso R, Olapade AA. Reduction in growth and aflatoxin production in orange juice inoculated with *Aspergillus flavus* using *Aframomum danielli*. African Journal of Agricultural Research. 2015; 10(14):1728-1734.
28. Fasoriyo SB, Adegoke GO, Ashaye VA, Obatolu VA, Owolade OF. Antimicrobial characteristics of *Aframomum danielli* spice on *Escherichia coli* and *Staphylococcus aureus*. Moor Journal of Agricultural Research. 2001; 2:159-161.
29. Fasoriyo SB, Adegoke GO, Obatolu VA, Ashaye, Aroyeun SO. The antioxidant property of *Aframomum danielli* spice in oils. The Journal of Food Technology in Africa. 2001; 6:135-137.
30. Afolabi MO, Adegoke GO. Antioxidative and flavouring effects of *Aframomum danielli* on biscuits. African Journal of Food Science. 2014; 8(4):200-203.
31. Ajayi IA, Ademola IO, Okotie SV. Larvicidal effects of *Aframomum danieli* seed extracts against gastrointestinal nematode of sheep: *in vitro* studies. African Journal of Traditional, Complementary and Alternative Medicines. 2008; 5(3):244-246.
32. Kollins EN, Wabo PJ, Payne VK, Jeannette Y, Marie-Claire K. *In vitro* comparative effect of aqueous (Cold and Hot Water) and ethanolic extracts of the seeds of *Aframomum danielli* (Zingiberaceae) on three life cycle stages of the parasitic nematode *Heligmosomoides bakeri* (Nematoda; Heligmosomatidae), parasite of the laboratory mice (Mus Musculus). Medicinal and Aromatic Plants. 2012; 1(1):11-20.
33. Adegoke GO, Iwahashi H, Komatsu YK, Obuchi K, Iwahashi Y. Inhibition of food spoilage yeasts and aflatoxigenic moulds by monoterpenes of the spice *Aframomum danielli*. Flavour and Fragrance Journal. 2000; 15(3):147-150.
34. Adegoke GO, Fasoyiro SB, Skura S. Control of microbial growth, browning and lipid oxidation by the spice *Aframomum danielli*. European Food Research and Technology. 2000; 211(5):342-345·
35. Olosunde OO, Adegoke GO, Abiodun OA. Composition of sorghum-millet flour, *Aframomum danielli* essential oil and their effect on mycotoxins in kunu zaki. African Journal of Food science. 2015; 9(7):411-416.
36. Adegoke GO, Rao LJM, Shankaracharya NB. A comparison of the essential oils of *Aframomum daniellii* (Hook. F.) K. Schum. and *Amomum subulatum* Roxb. Flavour and Fragrance Journal. 1998; 13(5):349-352·
37. Martins AP, Salgueiro L, Gonçalves MJ, da Cunha AP, Vila R, Cañigueral S *et al.* Essential oil composition and antimicrobial activity of three Zingiberaceae from S.Tomé e Príncipe. Planta Medica. 2001; 67(6):580-584.
38. Essien EE, Thomas PS, Oriakhi K, Choudhary MI. Characterization and antioxidant activity of volatile constituents from different parts of *Aframomum danielli* (Hook) K. Schum. Medicines. 2017; 4:2935.
39. Odukoya OA, Houghton PJ, Raman A. Lipoxygenase inhibitors in the seeds of *Aframomum danielli* K. Schum (Zingiberaceae). Phytomedicine. 1999; 6(4):251-256.
40. Aroyeun SO, Adegoke GO, Varga J, Teren J. Reduction of aflatoxin B_1 and Ochratoxin A in cocoa beans infected with Aspergillus via ergosterol Value. World Review of Science, Technology and Sustainable Development. 2009; 6(1):75-89.
41. British Pharmacopoeia II. P.A. 109, H. M. Stationary Office, London, 1980.
42. National Institute of Science and Technology. Chemistry Web Book. Data from NIST Standard Reference Database, 2011, 69.
43. Joulain D, Koenig WA. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons, E. B. Verlag, Hamburg. 2008, 254.
44. Viljoen A, Vuuren SV, Ernst E, Klepser M, Demirci B, van Wyk B. *Osmitopsis asteriscoides* (Asteraceae)-the antimicrobial and essential oil composition of a Cape-Dutch remedy. Journal of Ethnopharmacology. 2003; 88(2-3):137-143.
45. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica. 1998; 64(5):711-713.