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CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM *COSTUS LUCANUSIANUS* J. BRAUN & K. SCHUM.

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ABSTRACT

The essential oil of different organs of *Costus lucanusianus* J. Braun & K. Schum (Family Costaceae) obtained by hydrodistillation were analysed by Gas chromatography-mass spectrometry and its antimicrobial activity evaluated. Thirty-eight constituents were identified, representing an average of 73.64-99.95% of the total oil composition. The main constituents of the inflorescence were heptacosane (30.23%), phytol (17.41%) and the leaf oil mainly consisted of 11-Octadecenoic acid, methyl ester (41.00%), squalene (16.40%) and hexadecanoic acid, methyl ester (10.19%) while the stem consisted majorly of 4-(1,3,3-trimethyl-bicyclo[4.1.0] hept-2-yl)-but-3-en-2-one (28.10%), 3',8,8'-trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone (22.06%). The rhizome oil contained mainly octadecane, 3-ethyl-5-(2-ethylbutyl) – (18.23%). The antimicrobial results showed that the oils exhibited varying degrees of activity against tested microorganisms at 12.5-100 µg/mL, except for the stem oil, which showed no antifungal activity. The antimicrobial activity of the essential oils showed *in vitro* in this study could justify the use of the plant in the treatment of infectious diseases in traditional medicine.

KEYWORDS: *Costus lucanusianus* J. Braun & K. Schum, Essential oils, Gas chromatography-mass spectrometry, Antimicrobial activity, Costaceae.

1. INTRODUCTION

The seven genera in Costaceae contain about 143 known species.[1] *Costus* genus which contains the largest number of species, contains 25 species in tropical Africa and about 5 species in South-eastern Asia.[2] Literature reports on the phytochemistry of these species reveal the presence of a wide variety of steroidal saponins and sapogenins (diosgenin)[3,4], sesquiterpenoids[5], Bis-(2-ethylhexyl) phthalate[6-8], triterpenes, fatty acids and oxo-acids from the rhizome extract of *C. speciosus*.[9-12] Essential oils from the stem, leaf and rhizome of *Costus pictus* have also been reported to contain fatty acids.[13] Also, biological activities such as antimicrobial activity of cold aqueous methanol extract of the leaf[14], antihyperglycemic, renoprotective and hepatoprotective of the leaf aqueous extract[15], antifertility of stem methanol extract[16] and antimalarial of root methanol extract[17] have been reported for these plants.

In traditional medicine, the application of *Costus* species in the treatment of renal disorder, jaundice, abdominal pain, diabetes, and hypertension have been reported.[3,18-20] The rhizomes of *Costus* are a good source of lipids, protein and carbohydrate and the aerial parts are eaten as vegetables.[21] *Costus lucanusianus* J. Braun & K. Schum is an evergreen, perennial, rhizomatous, herbaceous and aromatic plant species with a thin stem that grows nearly vertically. Inflorescence infusion of *C. lucanusianus* is used as a remedy for tachycardia and stomach problem. The sap from the stem is used as a remedy for venereal disease, urethral discharge and in Gabon, as an eye drop to control filariasis. Pulped stem in water is taken as a diuretic in Southern Nigeria, the Ijaw uses the sap from the stem for malaria and to clear urine.[21-23]

The chemical compositions of the essential oils (EOs) of some *Costus* species have been reported. The major constituents of *Costus speciosus* (Koen ex. Retz) Sm. rhizome oil were α-humulene (20.55%) and zerumbone (55.11%).[5] It was also reported that the rhizome oil of *C. speciosus* contained pinocarveol (59.9%), cadinene (22.6%) and cineole (10.7%) as the major constituent.[24] Hexadecanoic acid, 9, 12 octadecadienoic acid, 2-pentanol and dodecanoic acid were the main constituents identified in the leaf, stem and rhizome oils of *Costus pictus* D. Don plant. Hexadecanoic acid constituted 24.51%, 28.30% and 25.26% of the leaf, stem and rhizome oils respectively. In addition to hexadecanoic acid, the leaf oil also contained 22.48% pentanol. The stem and rhizome oils also contained 9,12-octadecadienoic acid (18.33%) and dodecanoic acid 16.56% respectively, the root EO of *C. pictus* have also been reported to contain long-chain hydrocarbon (11.04%) as part of the major component.[13,25] Sesquiterpenoids were the most abundant group of *Costus afer* Ker Gawl Eos.[26] Antimicrobial activity of

C. pictus, *C. speciosus* and *C. afer* essential oil has been reported.[5, 25-28]

To the best of our knowledge, there are no reports on the EOs profile of *Costus lucanusianus* J. Braun & K. Schum. Therefore, in this present study, we analyzed the chemical constituents of essential oils obtained from *Costus lucanusianus* J. Braun & K. Schum aerial and rhizome parts using GC-MS and also investigated the *in vitro* antimicrobial activity.

Figure 1a. The plant *Costus lucanusianus.*

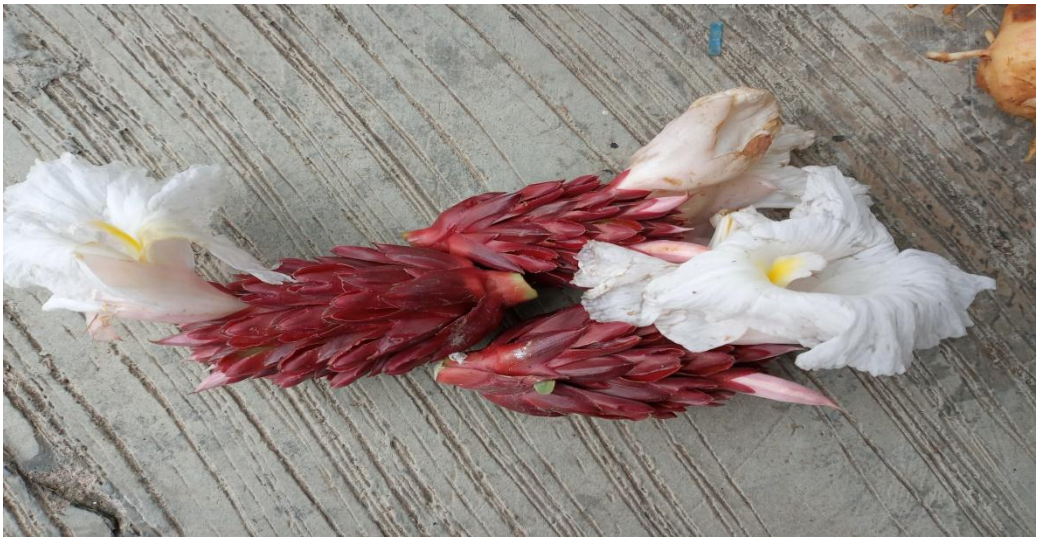

Figure 1b. The inflorescence organ.

Figure 1c. The rhizome organ.

Figure 1d. The leaf organ.

2. Experimental

2.1 Plant materials

Samples of inflorescence, leaf, rhizome and stem of *C. lucanusianus* were obtained fresh from Akobo Ibadan, Oyo state, South-west, Nigeria (at a geographical coordinate of 7^{o} 25! 45.4" N 3^{o} 56! 10.9" E; altitude 830 ft.) on 14th October 2015. The taxonomic identification of the plant materials was confirmed by a senior plant taxonomist, Mr L.T Soyewo of Forestry Research Institute of Nigeria (FRIN) herbarium, Ibadan. Voucher specimens (FH110048) were deposited at the FRIN herbarium.

2.2 Isolation of the essential oils

Extraction of Essential Oils from the Inflorescence, Leaf, Stem and Rhizome of *C. lucanusianus*.

The fresh leaves (325.45 g), inflorescence (416.77 g), stem (596.25 g) and rhizome (265.0 g) were chopped and separately subjected to extraction using hydrodistillation method with Clevenger type apparatus for three hours following British Pharmacopoeia specifications with modifications (British Pharmacpoeiae, 1988). All plant parts used for the experiment were fresh.[5,13] The samples were added into a 3 L round-bottomed flask containing 1.6 L distilled water and heated to boiling. When foaming was observed during the extraction, the temperature of the heating mantle was slightly reduced for further extraction. There was the evaporation of the essential oils together with water vapour, and these were collected in a condenser. Several organic solvents have been reported to aid in the trapping of the essential oils. Diethyl ether (DEE) was used for the collection of the Eos.[5,13, 24,28] The upper phase that contained the EOs in diethyl ether was separated from the lower one, and anhydrous sodium sulphate was used for drying the oils isolated, and the ether evaporated. The DEE was removed by evaporation. This was done by placing the

glass bottles used to collect the EOs opened in a 25 mL beaker containing water at 35 ^{o}C. The boiling point of DEE is 34.6 ^{o}C, and this is why it was chosen. Extracted oils were conserved in a sealed amber glass vial at 4^{o}C until analyses. The percentage yields (w/w) were determined.

2.3 GC-MS analysis of the EOs

GC-MS analyses of the EOs were performed using Agilent Technologies 7890B coupled to a 5975 VLMSD mass spectrometer. An injector 7890B series device was utilized for the analyses of the obtained oils. EOs (3 µL) was introduced into a gas chromatograph GC system. The system was equipped with flame ionisation detector and Agilent (9091)-413:325^{o}C HP-5 MS capillary column (30 mm x 320 µm id, film thickness 0.50 µm) interfaced with 5975 VLMSD mass spectrometer system. The oven temperature was set at 70-240 ^{o}C and at the rate of 50 ^{o}C/min. The ion source was fixed at 240 ^{o}C and electron ionization set at 70 eV. Helium, the carrier gas used was set at a flow rate of 1.4 mL/min with 50:1 split ratio, and 70.615 mL/min split flow. The scanning range was from 30-500 amu. The sample of oil (3.0 µL) diluted in hexane was injected manually into the GC-MS. The components of the essential oils were separated and identified by gas chromatography-mass spectrometry.

2.4 Identification of components

Most of the constituents of the EOs were identified by comparison of their retention indices (RI) with those reported in the literature. Others were identified by comparing their mass spectra with those of standards obtained on a non-polar HP-5MS column, Wiley Library Mass Spectra database of the GC/MS system.[30-31]

Antibacterial and antifungal assays

The positive control for bacteria was gentamicin 10 µg/mL while that for the fungi was tioconazole 70%. The different concentrations of EOs (12.5-100 µg/mL) were prepared in dimethyl sulphoxide (DMSO) (10 % of the final volume) which did not influence the growth of bacteria and fungi by serial doubling dilution.[32]

2.5 The antimicrobial activity

Clinical strains were supplied by the Pharmaceutical Microbiology Laboratory, University of Ibadan. Six bacteria strains used were; *Salmonellae typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Klebsiella pneumonia.* While *Penicillium notatum, Candida albicans, Aspergillus niger* and *Rhizopus stolonifer* were the four fungi strains used. The antimicrobial activity of the EOs was determined using Agar well diffusion method.

2.5.1 Preparation of bacterial inoculum

The strains were preserved as stock strains in 50% glycerol and sustained at 33 ^{o}C until resuscitation. The resuscitation of the organisms was carried out by obtaining a loop full of each glycerol stock-culture and inoculating into 5 mL each of sterile Mueller-Hinton broth (MHB) in a tube and vortexed carefully well, following this was incubation at 37 ^{o}C for 24 hours. Standardization of the bacterial suspension (inoculum) was performed with sterile distilled water to 10^{8} CFU/mL (turbidity= McFarland barium sulphate 0.5). An initial 1:100 dilution of the organisms was prepared by adding 0.1 mL of the overnight culture to sterile distilled water (9.9 mL). After that, 0.2 mL of the solution of the organism was taken and added into sterile nutrient agar (20 mL) which was at 45 ^{o}C[33] with modification.

2.5.2 Antibacterial assay (Agar well diffusion method)

The mixture of sterile nutrient agar, and the organisms were carefully transferred into sterile petri dishes and solidification for about 45 minutes took place. 30 µL of EOs, gentamicin and 10% DMSO (negative control) were introduced into the wells (8 mm diameter hole pierced in the Agar at 25 mm apart from one another using sterilized cork borer). The EOs was permitted to diffuse properly for an hour at room temperature. Subsequently, the plates were incubated for 24 hours at 37 ^{o}C. After incubation, the bacterial growth zone was observed. The bacterial inhibition zone was measured with a metre ruler in mm. Tests were carried out in triplicate.

2.5.3 Antifungal assay: Preparation of inoculum

The fungi strains were taken from the stock and subcultured on to Sabourand dextrose agar (SDA). The mixture was incubated at 35 ^{o}C for three days. The yeast spores and cells obtained were suspended in sterile distilled water (5 mL) to get 10^{5}cells/mL. Tween 20 (1%) was added to the distilled water containing only *Aspergillus* spp. and *Penicillium* spp which are hydrophobic in nature. This was to facilitate the preparation of the inoculum. Dilutions of the organisms to 1:100 were carried out, and 0.2 mL of 1:100 dilution of the adjusted inoculum was taken and spread over the Agar using a sterile spreader.[34]

2.5.4 Surface plate method

The prepared sterile Sabouraud Dextrose Agar (SDA) (62 g/L) was allowed to solidify for 45 minutes in sterile plates in triplicate. The antifungal activity of the EOs was determined by using 30 µL of the oils (12.5-100 µg/mL) in 10% DMSO. The experiment was performed in triplicate on Sabourand Dextrose Agar (SDA) impregnated with clinical fungal strains. Wells were made inside the set plates using 8 mm diameter sterile cork borer. Different concentrations of EOs, DMSO (10% total volume) and the control (tioconazole 70%) were poured into the wells, and there was a perfect diffusion of the oils into the Agar for 120 minutes. After that, the incubation of the plates uprightly for 48 hours at 28^{o}C took place. The inhibition zone diameter (IZD) in mm was measured.[35]

3.0 RESULTS AND DISCUSSION

3.1 Essential oil composition

The physical properties of the EOs of *C. lucanusianus* are listed in Table 1. As indicated in Table 1, hydrodistillation of the inflorescence, leaf, stem and rhizome samples of *C. lucanusianus* afforded colourless oils of yields 0.026-0.18%.

Table 1: Physical properties of EOs of *Costus lucanusianus.*

Sample	Yield (%)	Colour	Odour
Inflorescence	0.11	Colourless	Aromatic
Leaf	0.05	Colourless	Spicy
Stem	0.026	Colourless	Herbaceous
Rhizome	0.18	Colourless	Herbaceous

The quantitative and qualitative chemical compositions of the EOs are listed in Table 2. The oils were rich in long-chain saturated and unsaturated alkanes, oxygenated terpenoids, fatty acids and its derivatives, chlorine and nitrogen compounds.

A total of 8 constituents (86.15%) were identified in the inflorescence oil (Figure 2). Heptacosane (30.23%), Phytol (17.41%) and (*z*)-9-tricosene, (10.27%) were its major components. Fourteen constituents (89.05%) were identified in the leaf oil (Figure 3). The most abund ant constituent was 11-octadecenoic acid, methyl ester (41%), followed by squalene (16.40%), hexadecanoic acid, methyl ester (10.19%), methyl salicylate (2.91%) and *n*-hexadecanoic acid (2.24%). In the stem oil (Figure 4), 11 constituents (90.72%) were identified with 4-(1,3,3-trimethyl-bicyclo[4.1.0] hept-2-yl)-but-3-en-2-one, an oxygenated sesquiterpene (28.10%) and 3',8,8'-trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone (22.06%), caryophyllene (8.66%) and *cis*-vaccenic acid (4.00%) as the main constituents**.** The rhizome oil (Figure 5) which contained 10 constituents (48.03%) was dominated by octadecane, 3-ethyl-5-(2-ethylbutyl)- (18.23%). Other constituents are 2-tetradecanal (7.10%), heneicosane (3.46%) and *trans*-13-octadecenoic acid (0.64%).

Analyses of the oils show that they were acidic in nature, like some other species in the genus *Costus* due to the presence of acidic chemical constituents such as *cis*-vaccenic acid, *trans*-13-octadecenoic acid, *n*-hexadecanoic acid and docosahexaenoic acid among others. Fatty acids have been reported as the major constituents of *Costus* species essential oils.[13, 24-25] All parts of *C. lucanusianus* have been reported to have an acidulous taste.[23] The *C. lucanusianus* EOs were characterized by the presence of fatty acids and its derivatives (6.49-60.35%). The percentage composition of fatty acids and its derivatives in the leaf and stem oils were (60.35% and 28.23%) respectively. The inflorescence and rhizome oils contained higher amount of saturated and unsaturated alkanes (51.88% and 34.44%) respectively than the leaf and stem oils. The long-chain saturated and unsaturated alkanes were absent in the stem oil. Oxygenated diterpene (17.41%) was detected only in the inflorescence oil while triterpene (16.40%) was also detected only in the leaf oil.

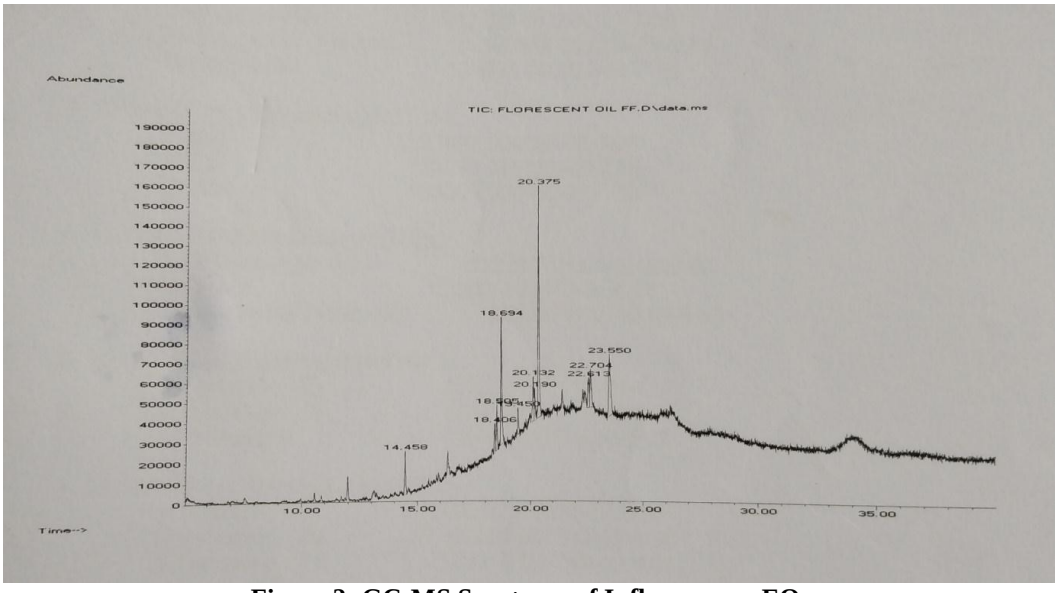

Figure 2: GC-MS Spectrum of Inflorescence EO.

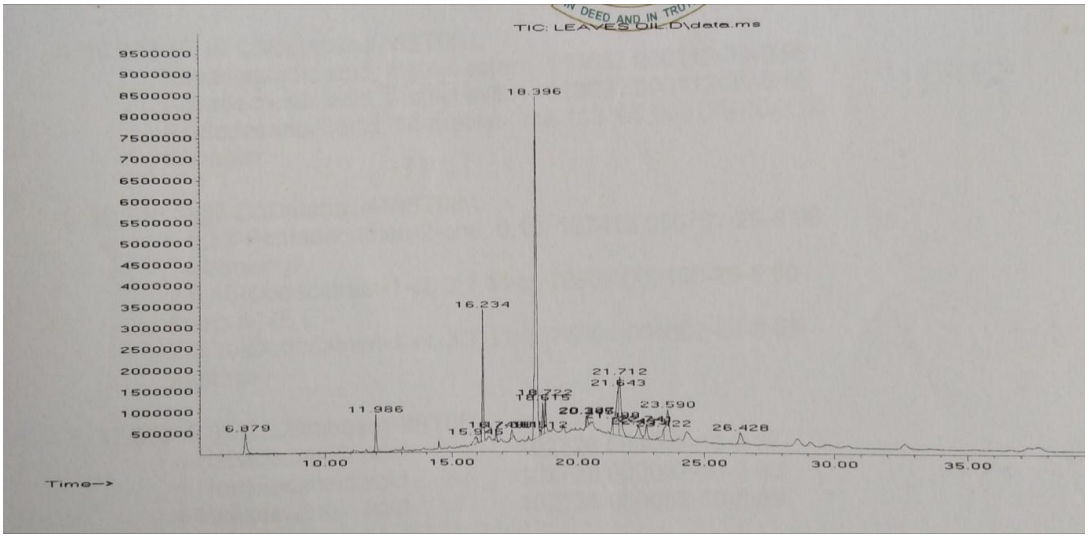

Figure 3: GC-MS Spectrum of Leaf EO.

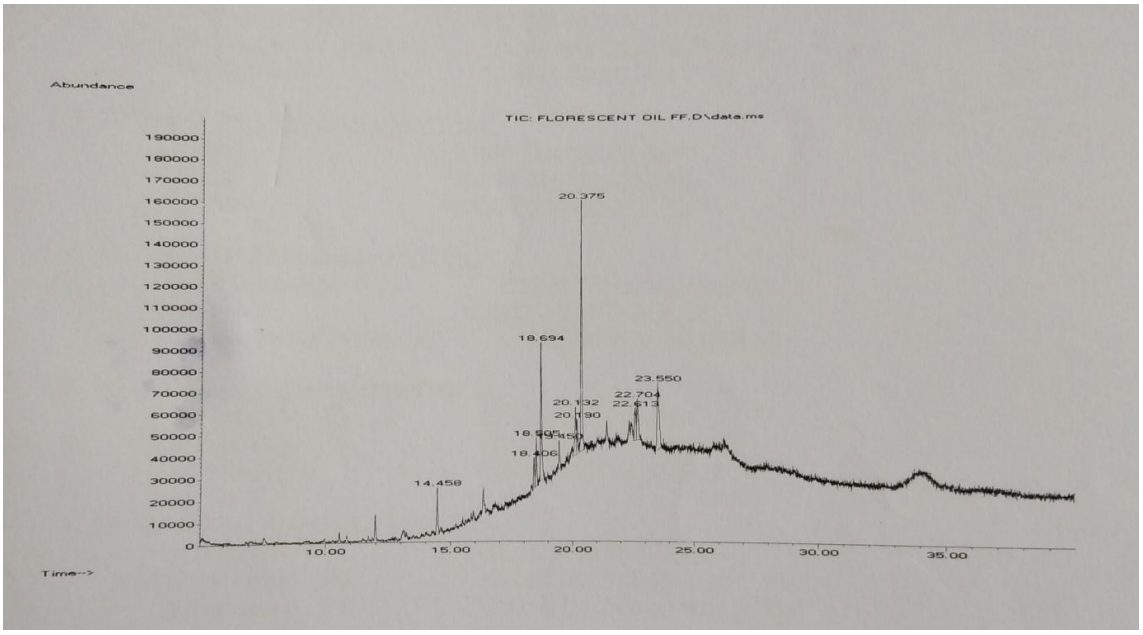

Figure 4: GC-MS Spectrum of stem EO.

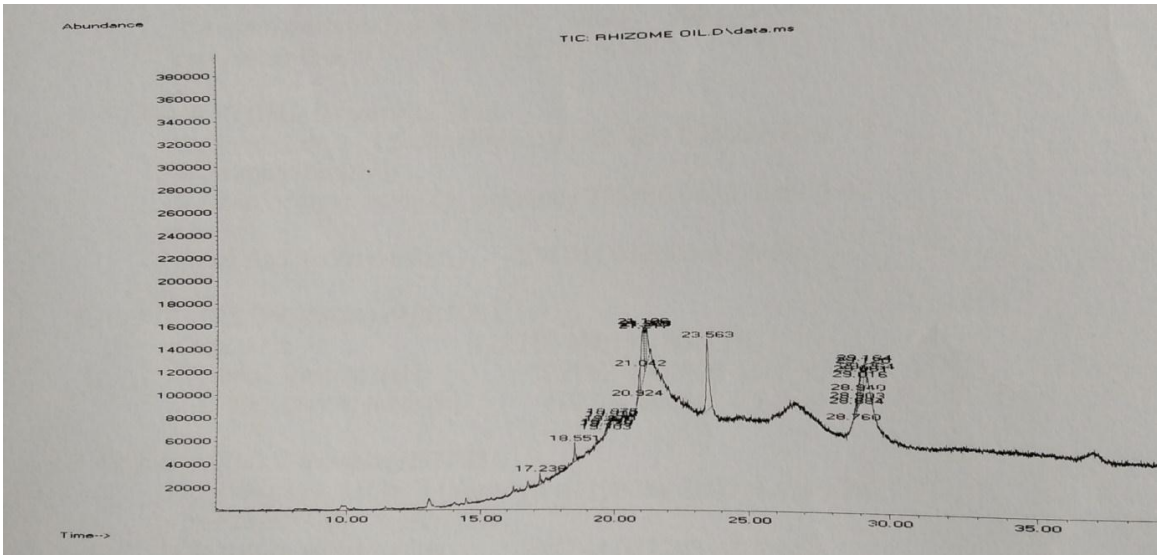

Figure 5: GC-MS Spectrum of rhizome EO.

The uses of *C. lucanusianus* organs in ethnomedicine in the treatment of various ailments could be attributed to the presence of potent chemical constituents amongst which are phytol, squalene and caryophyllene. The anticancer and anti-inflammatory activity of phytol[36], and antioxidant, emollient, anticancer and detoxifier property of squalene have been reported.[37] *Beta*-caryophyllene also is known for its antimicrobial, anti-inflammatory an antibacterial property.

A low dosage would be required of the leaf oil due to the presence of hexadecanoic acid (palmitic acid) and its ester which have been reported to increase the levels of low-density lipoprotein (LDL) and the ratio of LDL to high-density lipoprotein (HDL) cholesterol, thereby elevating the risk of development of coronary heart disease.[13]

Some of the constituents identified in this study had important resemblances with the EOs constituents of other reported *Costus* species:- *C. pictus* and *C. igneus*. These constituents include *n*-hexadecanoic acid, which was the principal constituent of *C. igneus* and *C. pictus*, oleic acid, squalene, phytol and tritetracontane.[24,26] However, the chemical constituents of *C. speciosus* root EO and *C. afer* leaf EO were different from those of *C. lucanusianus* Eos.[5,26] The major constituents of *C. lucanusianus* EOs that have not been reported in the composition of EOs of other *Costus* genus are 4-(1,3,3-trimethyl-bicyclo[4.1.0] hept-2-yl)-but-3-en-2-one,3',8,8'-trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone, octadecane, 3-ethyl-5-(2-ethylbutyl)-, 11-octadecenoic acid, methyl ester.[6,13,25,26] The differences in the EO compositions are influenced by several factors amongst which are environmental, biological, methodological and instrumental.[38] 1, 2-Benzenedicarboxylic acid (2-ethylhexyl) (phthalates) ester that been reported to be detected from the rhizome extract of *C. speciosus*, butanol fraction of *C. afer* leaf and ether fraction of *C. pictus* leaf.[6-8] These phthalates could have been originated from the plastic containers used for the chemical analysis or from polluted environment or may have been the artefacts.[38]

Table 2: Chemical composition of essential oil of *C. lucanusianus**

S/N	Compound	% composition				RRI	GC-MS-RT	Class of compound
		INF	LF	STM	RHIZ			
1	Methyl Salicylate	-	2.91	-	-	1192.9	6.878	Other
2	Butylated hydroxytoluene	-	2.84	-	-	1533.3	11.988	Other
3	Tetradecanal	5.09	-	-	-	1612.3	14.459	Long-chain aldehyde
4	1- Heptadecene	-	2.08	-	-	1694	22.739	Unsaturated long-chain alkane
5	1-Octadecene	-	-	-	4.51	1795	21.040	Long-chain unsaturated alkane
6	5,9,13-pentadecatrien-2-one,6,10,14-trimethyl-	-	0.85	-	-	1921	16.748	Unsaturated fatty acid derivative
7	n-hexadecanoic acid	-	2.24	-	-	1968	15.947	Fatty acid
8	Eicosane	6.70	-	-	-	2000	22.705	Acyclic alkane
9	6-Octadecenoic acid	3.15	-	-	-	2073	19.449	Fatty acid
10	11-octadecenoic acid, methyl ester	-	41.00	-	-	2089	18.396	Fatty acid ester
11	Heneicosane	-	-	-	3.46	2109	21.217	Long-chain saturated aliphatic hydrocarbon
12	Phytol	17.41	-	-	-	2116	18.694	Acyclic diterpene alcohol.
13	Octadecanoic acid, methyl ester	-	2.35	-	-	2130	18.614	Fatty acid derivative
14	Oleic acid	-	-	4.56	1.24	2133.2	$21.022^{STM}/19.701^{RHIZ}$	Monounsaturated fatty acid
15	9-Octadecenoic acid (E)	-	-	2.99	0.44	2141	$22.470^{STM}/28.759^{RHIZ}$	Fatty acid
16	trans-13-octadecenoic acid	-	3.33	-	0.64	2163.6	$18.722^{LF}/19.741^{RHIZ}$	Fatty acid
17	Tricosane	-	2.40	-	-	2300	26.430	Long-chain alkane
18	9-tricosene, (z)-	10.27	1.20	-	-	2335	$20.130^{INF}/21.400^{LF}$	Long-chain unsaturated alkane
19	Doconexent	-	-	4.21	-	2520.9	15.180	
20	Heptacosane	30.23	-	-	-	2700	20.376	Long-chain alkane
21	Squalene	-	16.40	-	-	2847.1	21.709	Triterpene
22	Tritetracontane	4.68	-	-	-	3401	18.505	Long-chain alkane
23	Oleic acid, eicosyl ester	-	-	5.91	0.93	3922.1	$19.981^{STM}/19.769^{RHIZ}$	Fatty acid derivative

24	Chromone, 3-methyl-7-nitro-	-	-	3.67	-	-	11.982	Other
25	4-(1,3,3-Trimethyl-bicyclo[4.1.0]hept-2-yl)-but-3-en-2-one	-	-	28.10	-	-	13.858	Oxygenated bicyclic Sesquiterpene
26								
27	Hexadecanoic acid, methyl ester	-	10.19	-	-	1928	18.396	Fatty acid derivative
27	Hexadecane, 8-hexyl-8-pentyl-	-	-	-	8.24	-	21.263	Long-chain alkane
28	3',8,8'-trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	-	-	22.06	-	-	23.557	Other
29	Tetrapentacontane, 1,54-dibromo-	-	0.87	-	-	-	20.387	Long-chain alkane derivative
30	n-Propyl 11-octadecenoate	-	-	-	3.24	-	18.551	Fatty acid ester
31	Caryophyllene (z)	-	-	8.66	-	1406.5	14.877	Bicyclic sesquiterpene
32	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	-	-	-	18.23	-	21.189	Alkane
33	2-tetradecanol	-	-	-	7.10	-	21.320	Long-chain fatty alcohol
34	Octadec-9-enoic acid	-	-	2.28	-	-	26.435	Fatty acid
35	Carbonic acid, octadecyl 2,2,2-tri chloroethyl ester	-	-	4.28	-	-	20.399	Fatty acid derivative
36	cis-vaccenic acid	-	0.39	4.00	-	-	$20.347^{LF}/18.556^{STM}$	Fatty acid
37	Carbonic acid, hexadecyl 2,2,2-tri chloroethyl ester	8.62	-	-	-	-	18.408	Fatty acid derivative
Total(%)		86.15	89.05	90.72	48.03			
Yield (% w/w)		0.11	0.05	0.026	0.18			
Sesquiterpene hydrocarbon		-	-	8.66	-			
Triterpene		-	16.40	-	-			
Oxygenated sesquiterpene		5.09	-	28.10	-			
Oxygenated diterpene		17.41	-	-	-			
Long-chain saturated and unsaturated		51.88	5.68	-	34.44			
Fatty acid and its derivatives		11.77	60.35	28.23	6.49			
Others		13.80	5.75	25.73	32.68			

3.2 Antibacterial activity of essential oils

The antibacterial screening results of the EOs revealed that the oils inhibited bacteria with varying sensitivity, as shown in Table 3. The inflorescence oil at 12.5 µg/mL showed inhibition zone diameter (IZD) against *S. aureus* (13.3 mm), *E. coli* (12.0 mm), *B. subtilis* (12.0 mm), *P. aeruginosa* (14.0 mm), *K. pneumonia* (10.0 mm) and *S. typhi* (10.0 mm). At 25 µg/mL, the leaf oil inhibited *S. aureus* (10.7 mm), *E. coli* (10.0 mm), *K. pneumonia* (10.7 mm) and *S. typhi* (14.3 mm) and at 50 µg/mL, *P. aeruginosa* and *B. subtilis* (10.0 mm). Stem oil showed IZD against *S. aureus*, *B. subtilis* (10.0 mm) at 25 µg/mL, *E. coli*, *S. typhi* and *K. pneumonia* (10.0 mm) at 50 µg/mL. Stem oil displayed no activity against *P. aeruginosa*. Rhizome oil also showed IZD against *S. aureus* (21.0 mm), *P. aeruginosa* (20.0 mm) *E. coli* (22.3 mm), *K. pneumonia* (21.7 mm), *B. subtilis* (19.0 mm) and *S. typhi* (23.0 mm) at 12.5 µg /mL.`

The four EOs which were assayed showed different antimicrobial activity against medically significant microorganisms. This might have been as a result of the diversity of the chemical composition of the oils, the nature of the constituents, the proportion of volatile chemical constituents and their interactions.[38-39] In this present study, rhizome oils exhibited highest antibacterial activity among oils from other plant parts. The antibacterial activity of *C. speciosus* and *C. pictus* plant aerial, rhizome and root has been reported.[5,24] The rhizome oil of *C. speciosus* at a concentration of 1 mg/mL showed antibacterial activity against tested bacterial with IZD between 10 mm-16 mm while the rhizome oil of *C. pictus* displayed antibacterial activity with IZD between 6 mm-15 mm.

Table 3: Antibacterial activity of essential oils.

Part of the plant	***S. aureus***				***E. coli***				***P. aeruginosa***				***K. pneumonia***				***B. subtilis***				***S. typhi***			
Concentration (µg/mL)	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
Inflorescence	19.3 ± 0.533	17.3 ± 0.533	15.3 ± 0.533	13.3 ± 0.533	18.7 ± 1.067	16.7 ±1.067	14.6 ± 1.067	12.0 ± 0.00	20.7 ± 1.067	18 ± 0.00	16 ± 0.00	14 ± 0.00	16.7 ± 1.067	14 ± 0.00	12 ± 0.00	10 ± 0.00	18.7 ± 1.067	16.7 ± 1.067	14 ± 0.00	12 ± 0.00	17.3 ± 1.067	15.3 ± 1.067	12.6 ± 1.067	10 ± 0.00
Leaf	14.0 ± 0.00	12.3 ± 0.411	10.7 ± 1.067	-	14.3 ± 0.533	12.7 ± 1.067	10.0 ± 0.00	-	11.3 ± 1.067	10.0 ± 0.00	-	-	14.3 ± 0.533	12.3 ± 0.533	10.7 ± 1.067	-	11.7 ± 0.533	10 ± 0.00	-	-	17.3 ± 1.067	15.6 ± 0.533	14.3 ± 0.533	12.3 ± 0.533
Stem	14.7 ± 1.067	12.7 ± 1.067	10.0 ± 0.00	-	12.7 ± 1.067	10.0 ± 0.00	-	-	-	-	-	-	12.7 ± 1.067	10.0 ± 0.00	-	-	14.0 ± 0.00	12.0 ± 0.00	10 ± 0.00	-	13.3 ± 1.067	10.0 ± 0.00	-	-
Rhizome	30.3 ± 0.533	26.7 ± 1.067	23.7 ± 0.533	21 ± 0.00	32 ± 0.00	28.3 ± 0.533	25.6 ± 0.533	22.3 ± 0.533	30 ± 0.00	26.7 ± 0.533	23.3 ± 0.133	20.0 ± 0.445	29.3 ± 1.067	26.0 ± 0.533	24.7 ± 1.067	21.7 ± 1.067	28 ± 0.00	25 ± 0.00	21.7 ± 0.533	19.0 ± 0.600	31.3 ± 1.067	28.3 ± 0.533	26.0 ± 0.533	23.0 ± 0.533
-ve standard DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+ve standard Gentamicin 10 µg/mL	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	40	40	40	40

-=Beyond detectable limit. Values are mean ± SE (n=3) at P<0.05

3.3 Antifungal activity of essential oils

Different concentrations of *C. lucanusianus* oils repressed all the fungal species with varying degrees of sensitivity except for the stem oil, which was inactive against every tested fungal species as shown in Table 4. The inflorescence oil at 12.5 µg/mL displayed IZD against *C. albicans* (14.0 mm), *A. niger* (12.7 mm), and at 25 µg/mL against *P. notatum* (10.0 mm) and *R. stolonifer* (10.0 mm). The leaf oil at 25 µg/mL inhibited *C. albicans* and *A. niger* (10.0 mm), *R. stolonifer* (10.0 mm) at 100 µg/mL and *P. notatum* (10.0 mm) at 50 µg/mL. Rhizome oil showed activity against *C. albicans*, *P. notatum* and *R. stolonifer* (14.0 mm) and *A. niger* (15.3 mm) at concentration 12.5 µg/mL. The inflorescence and rhizome oils similarly inhibited *C. albican*s.

Of all the tested oils, the rhizome oil exhibited the most IZD against the fungi tested. Furthermore, the highest antifungal activity was against *A. niger* (16 mm). However, only the leaf and root EOs of *C. pictus* was reported to possess antifungal activity.[25]

In this present study, the stem oil which was inactive against the fungal strains contained 28.10% oxygenated sesquiterpenes which have been reported to possess high antimicrobial property.[40-41] *C. afer* (a related species) which contained oxygenated sesquiterpene, sesquilavandulyl acetate (17.0%) as the highest abundant constituent in its leaf EO have been reported to display antimicrobial inactivity.[25] The antibacterial activity displayed by stem oil was ascribed to the existence of fatty acids and its derivative, which are recognized to exhibit antimicrobial activity.[42] The antifungal activity displayed by the inflorescence, leaf and rhizome oils could have been due to the synergistically reaction of the long-chain saturated and unsaturated alkanes which were present in the oils. The antibacterial and antimicrobial properties of hexadecane, which is a long-chain alkane, eicosane, octadecanoic acid, and hexadecanoic acid have been reported.[43-44] *C. pictus* root oil which displayed antifungal activity was reported to contain fatty acid and long-chain hydrocarbon as the main constituents.[13]

Table 4: Antifungal activity of essential oils.

Part of the plant	***C. albicans***				***A. niger***				***P. notatum***				***R. stolonifer***			
Concentration (µg/mL)	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
Inflorescence	19.3 ± 1.067	18.0 ± 0.00	16 ± 0.00	14 ± 0.00	18.7 ± 1.067	16.7 ± 1.067	14.67 ± 1.067	12.7 ± 1.067	14.7 ± 1.067	12.7 ± 1.067	10 ± 0.00	-	14.7 ± 1.067	12 ± 0.00	10 ± 0.00	-
Leaf	14.0 ± 0.00	12.0 ± 0.00	10.0 ± 0.00	-	14.3 ± 1.067	12.3 ± 1.067	10.0 ± 0.00	-	12 ± 0.00	10 ± 0.00	-	-	10.0 ± 0.00	-	-	-
Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizome	20 ± 0.00	18 ± 0.00	16 ± 0.00	14 ± 0.00	20.3 ± 0.533	18.3 ± 0.533	17.3 ± 1.411	15.3 ± 0.533	19.3 ± 0.533	17.3 ± 0.533	15.3 ± 0.533	14.0 ± 0.00	20 ± 0.00	18 ± 0.00	16 ± 0.00	14 ± 0.00
-ve standard DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+ve standard Tioconazole 70%	28	28	28	28	28	28	28	28	26	26	26	26	28	28	28	28

-= Beyond detectable limit, DMSO=Dimethylsulphoxide. Values are mean ± SE (n=3) at $P<0.05$.

The antimicrobial activity results revealed that the rhizome and inflorescence oils amongst others possessed broad-spectrum activity against *E. coli* and *C. albicans*. The existence of fatty acid and its derivatives and a considerable amount of the long-chain saturated and unsaturated alkanes with different polarity in both the inflorescence and rhizome oils characteristically act synergetically to enhance the antimicrobial activities of the rhizome and inflorescence oils.[44-45] Essential oils which are secondary metabolites with strong odour obtained from aromatic plants[46] were found to be present in the aerial and rhizome parts of *C. lucanusianus*.

3. CONCLUSION

This study revealed that the aerial and rhizome parts of *C. lucanusianus* contained colourless EOs of yield ranging from 0.02%-0.18% w/w of fresh weights. GC-MS analyzed the chemical constituents of *C. lucanusianus* EOs. The essential oils consisted of a wide range of chemical constituents with fatty acids and its derivatives as the main components. The leaf contained the highest concentration of fatty acids and its derivatives. These oils displayed broad-spectrum antimicrobial activities, especially against *C. albicans* and *E. coli*. However, stem oil showed no antifungal activity. This study has revealed that EOs from *C. lucanusianus* plant is able to inhibit the growth of medically important microorganisms. The *in vitro*

antimicrobial activity displayed by the oils from the inflorescence, leaf and rhizome organs of *C. lucanusianus* against 6 clinical bacterial and 4 fungal strains justifies the use of these plant organs in ethnomedicine the treatment of infectious diseases such as urinary tract infection, venereal disease and cough.

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Conflict of interest

The authors declare that there is no conflict of interest.

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