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Phytoconstitution and Antimicrobial Activity of *Costus Lucanusianus* Floral Volatile Extract

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Abstract: *Information on volatile constituents of plant extracts have a significant backing to theseveral biological activities and applications of plants. Consequently, in this work, phyto content and the antimicrobial activity of the volatile floral extract of Costus lucanusianus are investigated in this work to complement the information database on plant volatile components. The volatile constituents of the floral part were extracted using the conventional hydrodistillation technique and its phyto-constitution was determined via Gas Chromatography-Mass Spectrometry (GCMS) instrumentation. The antimicrobial pharmacological activity was established using the pour method against eight (8) strains of microbes. The volatile extract has a total of twenty-three (23) phytochemicals comprising eight (8) sesquiterpenes, four (4) sesquiterpenoids and eleven (11) non-terpenoids. The major identified constituents are 4-(3-hydroxy-2-methoxyphenyl)-but-2-one (38.29%), 1-(4-Hydroxymethoxy-phenyl)dec-4-en-3-one (13.83%),[S-(R*,S*)] 5-(1,5-dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexad- iene (7.66%), 1-(1,5-dimethyl-4-hexenyl)-4-methyl- benzene (6.13%), decanal (4.35%),[S-(R*,S*)]-3-(1,5-dimethyl-4-hexenyl)-6-methy- lene- cyclohexene (3.47%) and β-bisabolene (2.76%). The antimicrobial activity witnessed a dose-dependent gradient extract–inhibitory relationship. The highest inhibitory activity was observed at 100 mg/ml for all the strains. There was no inhibition observed below 12.5 mg/ml for Escherichia coli and Fusarium spp.*
while Staphylococcus aureus, Bacillus subtillis, Pseudomonas aeruginosa, Salmonella typhi, Candida albicanandKlebsiela pneumonia showed no inhibition below 6.25 mg/ml. The present study has established that the floral part of Costus lucanusianus has volatile constituents with antimicrobial potency.

Keywords: *Phytochemical constituents, volatile components, plant extracts, Costus lucanusianus, antimicrobial activity*

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1.0 Introduction

The hovering challenge of microbial strains enabled resistance to drug's potency is a global apprehension. This has initiated several research and development programmes, in search of a wider range of substitute drugs to combat the menace of multidrug resistance. The untapped vast secondary metabolites resident in plant parts could be a spotlight in the search for alternate drug substances. However, some scratching phytochemicals have been explored, several ethnomedicinal claims made and lots are still latent. The known vast applications associated with plant and plant materials are a function of their phytochemical constitution (Eddy, 2010).

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Costus lucanusianus is a perennial plant found in the South-South and other geo-political zones of Nigeria. Locally, the plant is called *Ogboduo* and *mberitem* amongst the Ijaw and Efik ethnicity respectively. It is known for the treatment of headache, cough, stomach disturbance and eye infections (Peters and Chibueze, 2022a). Essential oils such as cis-carvone oxide, geranyl acetate, linanyl acetate, beta-myrcene and alpha-pinene are present in plant parts of *Costus lucanusianus* (Peters and Chibueze, 2022b). In view of the established roles in several industries and research profiling, the need for continuous search for readily available sources and the prediction of their application disciplines, the global research community is intensifying efforts in the population of phytochemical research databases especially concerning their applications in pharmaceutical, medicinal and other areas. In line with the required trend, the present work is designed to investigate and document information on the phytochemicals in an extract from *Costus lucanusianus* as well as their microbial reference activity.

2.0 Materials and Methods

2.1 Collection of sample

Costus lucanusianus floral parts wereharvested from the marshy area of Ogbia Local Government area of Bayelsa State, Nigeria. A whole plant was submitted to a Biologist with the Department of Biology, Federal University Otuoke for proper identification.

Plate 1. Floral part of *Costuslucanisianus*

2.2 *Chemicals and reagents*

The chemicals and reagents such as n-hexane, water, and anhydrous sodium sulphate were of the analar standard.

2.3 Extraction of volatile oil

Volatile oil, CLE of the floral part of *Costus lucanusianus* was achieved using the simple hydrodistillation technique as reported by Hamilton-Amachree and Odokwo, 2022; Odokwo and Onifade, 2021. The percentage yield, A% was calculated using the relationship:

$$A\% = \frac{A_O}{A_F} \times 100 \qquad (1)$$

where, A_o – is the weight of the volatile oil and A_F – is the weight of the floral material

2.4 Identification and characterization

CLE was identified using a gas chromatography coupled mass spectrometry, GC-MS equipment with a NIST 14.0 library. The various candidates present in CLE are reported in Table 1.

2.5 Antimicrobial analysis

The antimicrobial analysis was carried out using the pour method as reported by Odokwo and Salawu, 2021. Microbial strains of *Staphylococcus aureus, Escherichia coli, Bacillus subtillis, Pseudomonas aeruginosa, Salmonella typhi, Candida albican, Klebsiela pneumonia* and *Fusarium spp.* were cultured and probed for the effectiveness of CLE.

The gradient concentration of CLE was done by transferring 100 mg of it into an n-hexane and a serial dilution was ensured. The negative control was DMSO and the positive control was a solution of ciprofloxacin

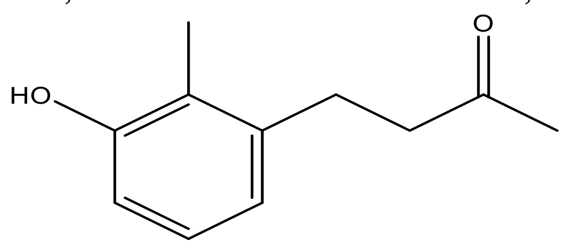
The pour method used witnessed the inoculation of microbes of interest on a sterile broth nutrient for one (1) day at 37 ^oC.

A functional 1:100 bacterium solution was obtained by mixing 9.9 ml of clinically cleaned distilled water and 0.1 ml of the cultured bacterium. Bored wells (4 mm in diameter) on the sample (the microbe) were exposed to CLE and to both the positive and negative controls.

This was done with precision, stayed on the bench for 2hrs and the plates incubated for one day at 37 ^oC, the inhibition zones were observed and reported in Table 2.

3.0 Results and Discussion

The percentage yield of 0.30 (w/w) was obtained for CLE. The physical characteristics of CLE were those typical of essential oil. The physical characteristic observed includes the ease of volatility and that of an essential smell. In Table 1, information deduced from the GCMS of the plant extract is presented. The constituent with the highest concentration of 38.29% is 4-(3-hydroxy-2-methyl phenyl)-but-2-one, whose structure is as shown below,



4-(3-hydroxy-2-methyl phenyl)-but-2-one

Table 1.The Phytochemical constitution of CLE

SN.	P.C	MF	MWt	R.T	Conc.
1	Decanal	$C_{10}H_{20}O$	156.2652	6.086	4.35
2	1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene	$C_{15}H_{22}$	202.3352	9.775	6.13
3	[S-(R*,S*)]-5-(1,5-dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene (zingibere)	$C_{15}H_{24}$	204.3511	9.931	7.66
4	1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	$C_{15}H_{24}$	204.3511	10.003	1.00
5	beta-Bisabolene	$C_{15}H_{24}$	204.3511	10.086	2.78
6	[S-(R*,S*)]-3-(1,5-dimethyl-4-hexenyl)-6-methylene-cyclohexene	$C_{15}H_{24}$	204.3511	10.283	3.47

7	1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-cyclohexane (ELEMENE)	$C_{15}H_{24}$	204.3511	10.610	1.04
8	Nerolidol	$C_{15}H_{26}O$	222.37	10.730	1.01
9	(1R,4R,5S)-1,8-Dimethyl-4-(prop-1- en-2-yl)spiro[4.5]dec-7-ene	$C_{15}H_{24}$	204.3511	11.072	0.55
10.	Octahydro- 1,4,9,9-tetramethyl-1H-3a,7-methanoazulene	$C_{15}H_{26}$	206.3669	11.549	1.38
11.	4-(3-hydroxy-2-methoxyphenyl)- butan-2-one	$C_{11}H_{14}O_3$	194.23	11.892	38.29
12.	7-epi-cis-sesquisabinene hydrate	$C_{15}H_{26}O$	222.3663	12.214	1.40
13.	cis-1-ethylideneoctahydro-7a-methyl-1H-Indene	$C_{12}H_{20}$	164/287	13.614	0.57
14.	(R,Z)-2-Methyl-6-(4-methylcyclohexa-1,4-dien-1-yl)hept-2-en-1-ol	$C_{15}H_{24}O$	220.35	14.133	0.92
15.	n-Hexadecanoic acid	$C_{16}H_{32}0_2$	256.4241	15.031	1.06
16.	(Z)-9,17-Octadecadienal,	$C_{18}H_{32}O$	264.4461	16.712	1.25
17.	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	$C_{15}H_{26}O$	222.37	16.800	0.45
18.	(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one	$C_{17}H_{24}O_3$	276.4	17.459	1.39
19.	1-(4-hydroxy-3-methoxyphenyl)-3-decanone	$C_{17}H_{26}O_3$	278.3865	17.537	1.48
20.	1-(4-Hydroxymethoxyphenyl)dec-4-en-3-one	$C_{17}H_{24}O_3$	276.376	18.123	13.83
21.	Gingerol	$C_{17}H_{26}O_4$	294.38	18.995	1.67
22.	1-(4-Hydroxy-3-methoxyphenyl)dodec -4-en-3-one	$C_{19}H_{28}O$	304.4238	20.266	1.30
23.	Diisooctyl phthalate	$C_{24}H_{38}O_4$		20.853	1.88

****SN. Serial number, P.C- Phytochemical constitution, M.F-Molecular formula, M.Wt-Molecular weight, R.T –Retention time, Conc.-Concentration**

The chromatogram of the GCMS analysis of the plant extract showed thepresence of twenty-three (23) phytochemicals with several essential oils components. The major constituents are4-(3-hydroxy-2-methoxyphenyl)-butan-2-one (38.29%), 1-(4-

Hydroxymethoxyphenyl)dec-4-en-3-one (13.83%),[S-(R*,S*)] 5-(1,5-dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene (7.66%), 1-(1,5-dimethyl-4-hexenyl)-4-methyl- benzene (6.13%), decanal (4.35%),[S-(R*,S*)]-3-(1,5-dimethyl-4-hexenyl)-6-methylene- cyclohexene (3.47%) and β-bisabolene (2.76%). Also, eight (8) sesquiterpenes were identified in the extract, ibcluding. They include: -(1,5-dimethyl-4-hexenyl)-4-methyl-benzene, [S-(R*,S*)]-5-(1,5- dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene, β-bisabolene, [S-(R*,S*)]-3-(1,5-dimethyl-4-hexenyl)-6-methylene- cyclohexene, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-cyclohexane, (1R,4R,5S)-1,8-dimethyl-4-(prop-1- en-2-yl)spiro[4.5]dec-7-ene and octahydro- 1,4,9,9-tetramethyl-1H-3a,7-methanoazulene.

Four (4) sequiterpenolswerealso found which arenerolidol, 7-epi-cis-sesquisabinene hydrate, (R,Z)-2-Methyl-6-(4-methylcyclohexa-1,4-dien-1-yl)hept-2-en-1-ol and (R,Z)-2-Methyl-6-(4-methylcyclohexa-1,4-dien-1-yl)hept-2-en-1-ol.

Table 2. Antimicrobial activity of CLE and the inhibition zones

	Inhibition zone						
Microbes	100 .0 mg/L	50 .0 mg/L	25.0 mg/L	12.5 mg/L	6.25 mg/L	3.13 mg/L	10 .0 mg/L*
S.a	22	18	16	14	12	0	28
B.s	20	14	12	10	10	0	24
E.c	14	12	10	0	0	0	20
P.a	16	14	12	12	10	0	25
S.t	18	12	10	8	6	0	18
K.s	16	10	10	8	6	0	22
C.a	16	14	10	10	8	0	0
F.s	12	10	8	0	0	0	NA

- **standard**

Standard: 10mg/ml ciprofloxacin**,** Sa-*Staphylococcus aureus*, Ec-*Escherichia coli*,Bs-*Bacillus subtillis,* Pa- *Pseudomonas aeruginosa*, St-*Salmonella typhi,* Ca- *Candida albican*,Kp-*Klebsiela pneumonia* and Fs-*Fusarium spp*

Decanal is found in the essential oil of coriander, citrus and buckwheat (James *et al*., 2018; Nurzynska-Wierdak, 2013). 1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene is also reported to be a phytoconstituent of *Cassia angustifolia* and is know for its significant synergistic roles is useful as an antimicrobial agent (Al-Marzoqi *et al*., 2016). [S-(R*,S*)]-5-(1,5-dimethyl-4-hexenyl)-2-methyl-1,3-cyclohexadiene also known as zingiberene, is a sesquiterpene which has been documented as a useful compound because of its binding affinity towards ACE2 receptor and as a promising SARS-CoV-2 lead drug substance (Murya *et al*., 2020).

β-bisabolene is also a known constituent in *Rattusrattus*essential oil especially in bisabolol, cubeb, lemon and oregano. They can also be synthesized by lower organisms such as fungi, insects and stink bugs. It is a useful sweetener and fragrance (Sparkowicz and Strobel, 2015; Lu and Teal, 2001). Also, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-cyclohexane, also known as β-elemene is sesquiterpene used as a chemotherapeutic agent in medicine (Chen *et al*., 2022).

Nerolidol is a sesquiterpenol also known as farnesol, peneriol or penetrol. It is found in many flower parts of plants and other parts. It has been reported to be present in the essential oils of neroli, ginger, jasmine, lavender, tea tree, *Cannabis sativa* and lemongrass. It has numerous applications such as flavouring agents, perfumery, and non-cosmetic products such as detergents. Pharmacologically, it has been implicated to have exhibited antioxidant, anticancer and anti-microbial activity (Chan *et al*., 2016).

Literature report published by Asraf *et al*. (2017) indicated that 7-epi-cis-sesquisabinene hydrate has some antimicrobial activity towards several organisms. It is also present in *Zingiber officinale* extract (Shareef *et al*., 2016). Also N-hexanoic acid (i.e palmitic acid) is a fatty acid with known pharmacologically anti-inflammatory properties(Aparna *et al*., 2012) while 1-(4-Hydroxymethoxyphenyl) dec-4-en-3-one has been documented in several published works as an anticancer agent (Chen *et al*., 2007).

The antimicrobial activity decreases as the concentration of the floral extract, CLE decreases for all the strains of microbes investigated. The highest inhibitory activity was observed at 100 mg/ml for all the strains with *Staphylococcus aureus* having the highest activity and *Fusarium spp* having the least activity. There was no inhibition observed below 12.5 mg/ml for *Escherichia coli* and *Fusarium spp.* while *Staphylococcus aureus, Bacillus subtillis, Pseudomonas aeruginosa, Salmonella typhi, Candida albican* and *Klebsiela pneumonia* showed no inhibition below 6.25 mg/ml.

4.0 Conclusions

The volatile phytoconstituents and the antimicrobial activity of the volatile floral extract of *Costus lucanusianus* have been established. The floral volatile constituents consist of twenty-three (23) phytochemicals comprising eight (8) sesquiterpenes, four (4) sesquiterpenoids and eleven (11) non-terpenoids. The floral extract has shown a wide spectrum of antimicrobial activity as its concentration increases. The floral part of *Costus lucanusianus* is a good source of the medicinal herb.

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