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Phytochemical and Antimicrobial Analysis of the Stems of Cola Gigantea (Sterculiacea)

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----------ABSTRACT----------
Phytochemical and anti microbial analyses were carried out on the purified stems extract of Cola gigantea. The Harbone method was used in the extraction and the extract separated using a combination of column chromatography and preparative thin layer chromatography resulting in the isolation of four fractions with Rf values of 0.2667, 0.4133, 0.5667 and 0.7667 for stem fractions 1, 2, 3 and 4 respectively. The isolated fractions were subjected to structural elucidation using the combination of appropriate spectroscopic instruments; FTIR, UV, H^1-NMR, C^{13}-NMR and GC-MS which gave rise to the following suggested compounds: 4-((1E)-3-Hydroxyl-1-propenyl)-2-methoxyphenol-cyclopropanecarboxylicacid,2-pentyl-5,7-dodecadiyne-1,12-diol;Phenol,4-(3-hydroxy-1-propenyl)-2-methoxy-2-(2-nitrophenoxy) benzaldehyde-4-isopropyl-3-methyl phenoxy acetylhydrazone; 3,4,5-trimethyl-1-H-pyrano [2,3-C]pyrazol-6-onecyclopropaneoctanoicacid,2-[[2-[(2-ethylcyclopropyl)methyl] cyclopropyl]methyl]-methylester-5-methoxy-2-phenyl-7-chromanol and 1,2-benzene dicarboxylic acid,dioctyl-methyl-12-oxo-9-dodecenoate were contained in stem fractions 1-4 respectively. Results of the phytochemical analysis showed the presence of some secondary metabolites such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, steroids, tannins, terpenoids in various concentrations with flavonoids, steroids and resins in very high concentration. The values of the mineral elements; As (0.51mg/g), Cd (0.40mg/g), Cr (0.57mg/g), Fe (1.01mg/g) etc in the stems were above the WHO recommendations thus showing the need for further purification before therapeutic usage. The antimicrobial analyses (the anti fungal and anti bacterial analyses) using the Punched agar diffusion method was carried out on the four isolated fractions comparatively with a standard drug Cipromax fort (a broad spectrum antibiotic). A total of fourteen test organisms were used consisting of eleven bacteria strains and three fungi with stem fractions being active on all the test organisms given their average diameter zones of inhibition which ranged between 10mm and 28mm. Comparatively, the standard drug cipromax fort was of better antimicrobial effect than the stem extracts. However, these fractions could serve as antimicrobial to diseases caused by these test organisms as acclaimed by ethno medical practitioners and as confirmed from their MIC, MBC and MFC results.

KEYWORDS: *Cola gigantea, Phytochemical analysis, antimicrobial analysis, cipromax fort.*

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I. INTRODUCTION

There has been man's unending desire for good and healthy living from ancient days which has led to his curiosity to examine all aspects of his environment by trial and error (Daziel, 1961). This gave rise to traditional medicine practice which was the only way of saving life in the olden days before the advent of modern medicine as earliest humans used various plants to treat illness (Ajiwe *et al*., 2008). Unfortunately, the misuse of these life saving medications coupled with bacteria's amazing ability to adapt has led to an increase in the number of drug resistant organisms (Nester *et al*., 2004). Some people even speculate that we are in danger of seeing an end to the era of antimicrobial medications. In response, scientists are involved in much current research devoted to the phytochemical investigation of higher plants such as *Cola gigantea* which have ethno botanical information associated with them.

Cola gigantea a large forest tree found both in relatively dry and wet parts of the rain forest has been reported to have a high anti-microbial activity against *Staphylococcus albus, Bacillus subtilis, Aspergillus niger and Candida albicans* thus showing its potency as antibiotics. (Adeniyi *et al*.,2004; Agyare *et al*., 2012; Idu *et al*., 2000; Reid *et al*., 2005; Sonibare *et al*., 2009).

So far from the literature available, the isolation and structural elucidation of the active phytochemicals in the stems of Cola gigantea has not been done hence this present study which aims at identifying the antimicrobials, isolating and structurally elucidating the active components.

II. EXPERIMENTAL

Plant Collection, Identification and Preparation

The stems of the plant *Cola gigantea* used in this study were collected from Okpuno in Awka North Local Government Area of Anambra State, Nigeria. It was identified by Mr Ugwuozor a taxonomist of the Department of Botany, Nnamdi Azikiwe University, Awka and authenticated by Prof J.C Okafor as *Cola gigantea* of the *Stercliacea* family. Fresh stems samples were dried under shade for two weeks, pulverised and stored in a glass jar for subsequent analyses

Extraction and Fractionation into Different Classes

500g of the pulverized stem was macerated in 2500ml of methanol/water in a ratio of 4:1 for about 1hour 30minutes. The mixture was filtered and the filterate heated on a water bath to one-tenth of the volume at temperature of 40°C. The filterate was then acidified with 2ml of 2M H_2SO_4 and then extracted with chloroform. The mixture was separated using a separatory funnel. The chloroform extract was heated to dryness and re-dissolved with chloroform which gave the chloroform extract (Harbone, 1998). This extract was thereafter fractionated into four different fractions using a combination of column and preparative thin layer chromatography.

Phytochemical Screening

The crude stem extract was evaluated for the presence of acidic components, flavonoids, saponins, reducing sugar, carbohydrates, tannins, resins, steroids, terpenoids, alkaloids, proteins, cardiac glycosides and oil using standard procedures (Harbone, 1998).

Trace Metal Detemination

Using Atomic Absorption Spectrophotometer model varian AA 280, trace metal level of the stem was determined. Determined trace metals included As, Cd, Cr, Co, Fe, Pb, Mn, Hg, Ni and Zn.

Anti-Bacterial Assay

The sensitivity of the fractions and standard drug (cipromax fort) against the selected test organisms (*Bacillus typhi, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Staph albus, Staphylococcus aureus, Streptococcus muteus, Streptococcus pyogeus, Aspergillus flavis, Aspergillus niger* and *Candida albican*) was carried out using the Punched agar diffusion method (Bryant, 1972).

The MIC and MBC were determined using the serial dilution method while the MFC was determined using the Punched agar diffusion method (Bryant, 1972).

Structural Elucidation

Using a combination of these spectroscopic techniques such as FTIR, UV-visible, GCMS, H^1-NMR and C^{13}-NMR structures and molecular formulae were proposed for the four isolated fractions of the stem of *Cola gigantea*.

Results and Discussion

The results of the organoleptic examination of the stems are as given in Table 1

Table 1: Organoleptic Examination of the stems of *Cola gigantea*

Parameter	Colour	Odour	Taste
Stem	Cream	Odourless	Tasteless

The tasteless nature of this plant part gave an insight into the absence of the bitter pigment (tannin) as confirmed during the phytochemical screening.

Table 2: Phytichemical Compositon of the Stems of C.gigantea

Sample/ Tests	**Alkaloids**	**Cardiac Glycosides**	**Cyanogenic Glycosides**	**Flavonoids**	**Saponin**	**Steroids**	**Tannins**	**Terpenoids**
Fresh Stems	++	-	+++	++	-	++	-	-

Note: - Absent + Present in low concentration
++ Present in high concentration +++ Present in very high concentration

The results of the phytochemical analysis of *C.gigantea* showed the presence of alkaloids, flavonoids and steroids in high concentration while cardiac glycosides, saponin, tannins and terpenoids conspicuously absent. The very high presence if cyanogenic glycosides in this plant part is a cause for concern as it has the ability to release cyanohydric acid a very toxic substance (Harbone, 1998). The above phytochemicals are the main basis for the plant's medicinal properties and starting materials in the synthesis of new drugs today. Furthermore, the presence of alkaloids in high concentration in the stem signified possession of antimicrobial activity, cyto toxicity and sometimes neutralization of poisons within the herb. Flavonoids which are predominantly present help to reinforce capillary walls, improving exchange of nutrients and oxygen between the blood and tissues (Harbone. 1998).

Table 3: Result of Thin Layer Chromatography (TLC) of crude extract of the stems of Cola gigantea

Parameter	**R_f Value**	**Solvent Systems**
Stem fraction 1	0.2667	Chloroform: Methanol (80:5)
Stem fraction 2	0.4133	Chloroform: Methanol (80:5)
Stem fraction 3	0.5667	Chloroform: Methanol (80:5)
Stem fraction 2	0.7667	Chloroform: Methanol (80:5)

The thin layer chromatography of the stem extract showed four spots under iodine vapour with different Rf values as given in Table 3.

Table 4: Results of the Mineral Elements in the Stems of Cola gigantea

Element	**As**	**Cd**	**Cr**	**Co**	**Fe**	**Pb**	**Mn**	**Hg**	**Ni**	**Zn**
Stems(mg/g)	0.03	0.04	0.57	0.70	1.01	2.88	0.31	0.28	4.95	0.00
WHO Standard	0.01	0.003	0.005			0.01	0.50	0.001	0.02	-

The values of the elements found in the stems of *C.gigantea* were above the WHO recommendations hence there is the need for reduction of the trace metal levels to permissible levels before human consumption this would mitigate the adverse effects of these on human body as a result of their gradual accumulation. Other useful elements like Fe was equally present in substantial amount with Zn conspicuously absent (Table 4).

Table 5: Results of Antimicrobial activity of fractions of the Stems of C.gigantea

Extracts	Vol.Used (cm^3)	Average Diameter (mm) Zones of Inhibition on Test Organisms									
		E.Coli (NCTC 10481)	S.Au	P.A L.C.I	K.P L.C.I	P.V L.C.I	S.M L.C.I	S.P L.C.I	B.T L.C.I	S.T L.C.I	E.A L.C.I	S.A L.C.I
Cipromax	0.05	18	22	14	18	30	14	16	14	24	35	24
Stem Fraction1	0.05	14	18	12	12	16	14	12	NA	14	28	20
Stem Fraction2	0.05	18	20	16	14	18	16	14	10	12	22	18
Stem Fraction3	0.05	12	24	18	16	24	14	14	12	15	18	16
Stem Fraction4	0.05	20	28	16	16	28	16	12	12	14	24	18

S.Au= *Staphylococcus Aureus,* P.A=*Pseudomonas aeroginosa,* K.P = *Klebsiella pneumonia,* P.V=*Proteus vulgaris,* S.M= *Strept muteus,* B.T=*Bacillus typhi,* S.T=*Salmonella typhi,* E.A=*Enterobacter aerogenes,* S.A= *Staph albus,* S.P= *Strept pyogenes*
NCTC = National Collection of Type Cultures. L.C.I = Local Clinical Isolate. NA= No Action

Table 6: Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the stem extracts of *C.gigantea*

Extracts	**Average Diameter (mm) Zones of Inhibition on Test Organisms**										
	E.Coli (NCTC 10481)	S.Au L.C.I	P.A L.C.I	K.P L.C.I	P.V L.C.I	S.M L.C.I	S.P L.C.I	B.T L.C.I	S.T L.C.I	E.A L.C.I	S.A L.C.I
Cipromax	MIC 0.0625	0.0313	0.125	0.0625	0.0156	0.125	0.125	0.125	0.0313	0.0156	0.0313
	MBC 0.125	0.0625	0.250	0.125	0.0313	0.250	0.250	0.250	0.0625	0.0313	0.0625
Stem Fraction1	MIC 0.125	0.0625	0.250	0.250	0.125	0.250	0.250	NA	0.250	0.0156	0.0313
	MBC 0.250	0.125	0.50	0.50	0.250	0.500	0.500	NA	0.500	0.0313	0.625
Stem Fraction2	MIC 0.0625	0.0625	0.0625	0.125	0.0625	0.6250	0.125	0.250	0.250	0.0625	0.6250
	MBC 0.125	0.125	0.125	0.250	0.125	0.125	0.250	0.500	0.500	0.125	0.125
Stem Fraction3	MIC 0.250	0.03125	0.0625	0.0625	0.03125	0.250	0.250	0.125	0.0625	0.063	0.0313
	MBC 0.1250	0.0313	0.250	0.250	0.0313	0.250	0.500	0.500	0.500	0.0625	0.125
Stem Fraction4	MIC 0.0625	0.0156	0.125	0.125	0.0156	0.125	0.250	0.250	0.250	0.0313	0.063
	MBC 0.125	0.0313	0.250	0.250	0.0313	0.250	0.500	0.500	0.500	0.0625	0.125

S.Au= *Staphylococcus Aureus,* P.A=*Pseudomonas aeroginosa,* K.P = *Klebsiella pneumonia,* P.V=*Proteus vulgaris,* S.M= *Strept muteus,* B.T=*Bacillus typhi,* S.T=*Salmonella typhi,* E.A=*Enterobacter aerogenes,* S.A= *Staph albus,* S.P= *Strept pyogenes*
NCTC = National Collection of Type Cultures L.C.I = Local Clinical Isolate NA= No Action

The results of the antibacterial activity on eleven bacteria species both gram positive bacteria (*Staphylococcus albus, Bacillus typhi, Streptococcus pyogenes* etc) and gram negative bacteria (*Escherichia coli and Pseudomonas aeroginosa*) showed that the various fractions from this plant cell could serve as broad spectrum anti-microbial (Cunha, 2009). The high presence of flavonoids in the plant part as shown from the preliminary tests could account for this high antimicrobial effect as one of the undisputed functions of flavonoids and related polyphenols is their role in protection against microbial invasion. Several recent papers report the regular presence of antibacterial activity among flavonoids (Alinnor, 2007; Penecilla *et al*., 2011). Specifically, the value of the fractions on *Staphylococcus aureus* and *Staphylococcus albus* confirmed the work done by Haraguchi on the effect of flavonoids on *Staphylococcus aureus* a causative organism for skin and wound infections, abscess and osteomyelitis which according to Greenwood *et al*.,(1992) could account for its use in the treatment of the aforementioned diseases. Comparatively, all stem fractions had similar antimicrobial activity with the standard drug Cipromax fort except stem fraction 1 which showed no activity on *Bacillus typhi*.

Table 7: Results of Antifungal activities of the Stem fractions of *C.gigantea*

Extracts	**Vol.Used (cm^3)**	**Average Diameter (mm) Zones of Inhibition on Test Organisms**		
		Candida Albican L.C.I	*Aspergillus flavis* L.C.I	*Aspergillus Niger* L.C.I
Cipromax	0.05	NA	NA	NA
Stem fraction 1	0.05	14	10	12
Stem Fraction 2	0.05	12	12	10
Stem fraction 3	0.05	10	12	10
Stem Fraction 4	0.05	8	10	13

L.C.I = Local Clinical Isolate NA= No Action

Table 8: Results of MIC and MFC of the stem fraction of *C.gigantea*

Extracts		**Presence or Absence of growth on Test Organisms**		
		Candida Albican L.C.I	*Aspergillus flavus* L.C.I	*Aspergillus Niger* L.C.I
Cipromax fort	MIC	-	-	-
	MFC	-	-	-
Stem Fraction 1	MIC	0.25	0.25	0.25
	MFC	0.50	0.50	0.50
Stem Fraction2	MIC	0.25	0.25	0.25
	MFC	0.50	0.50	0.50
Stem Fraction 3	MIC	0.25	0.25	0.25
	MFC	0.50	0.50	0.50
Stem Fraction 4	MIC	-	0.25	0.25
	MFC	-	0.50	0.50

All the stem fractions showed similar activities on the test organisms with the stem fraction 4 totally inactive on *Candida albican* confirming the report by Ibeh *et al*., 2003 that an inhibitory diameter of 10mm or less indicated that the organism was resistant. An inhibitory zone diameter of 11-15mm showed intermediate effect while a 16mm and above indicated that the organism was susceptible to the compound (Ibeh et al., 2003). Hence, the stem of Cola gigantea had an intermediate antimicrobial effect as most values fell between 11-14mm as shown in Table 7.

Spectroscopic Analysis And Structural Elucidatiom

Table 9: FTIR results of stem fraction 1

Wave band (cm^{-1})	Description
3346.61	OH Stretch of alcohols, Phenols and esters
2898.14	C-H Stretch for alkanes and aromatics
1435.09	C=O stretch for esters
1063.78	C-O deformation bonds of esters
881.50	C-H deformation bonds of alkyl groups
438.82	C-H deformation of methyl groups

Table 10: UV-Visible results of stem fraction 1

λ max (nm)	Chromophore description
741.00	C-OH Stretch of Phenols
651.50	C=C of Aromatic ($\pi \longrightarrow \pi^*$)
604.00	
278.50	C=O ($n \longrightarrow \pi^*$)

Table 11: Summary of the H^1 and C^{13} NMR results of stem fraction 1

H^1 δ (ppm) & Multiplicity	**Coupling Constant (MHz)**	**Types of Proton**	**C^{13} δ (ppm)**	**Types of Carbon**
9.9 (d)	0.0176	RCO_2H	177.73	C=O
7.8 (t)		PhOH	153.48	ArC
7.2 (s)		ARH	153.24	ArC
6.6(multiplet)	1.1365	ARH	118.83	ArC
5.2		ROH	114.41	ArC
4.25 (multiplet)	4.9389	RO-CH_2	114.27	ArC
3.89		CH_2	103.09	ArC

2.06	CH_2	86.97	C-O
		77.35	C-O
		77.03	C-O
		76.72	C-O
		72.58	C-O
		60.55	C-O
		56.27	C-O
		56.00	C-O
		34.12	CH_2
		33.85	CH_2
		31.91	CH_2
		31.42	CH_2
		30.21	CH_2
		30.02	CH_2
		29.67	CH_2
		29.44	CH_2
		29.33	CH_2
		29.24	CH_2
		29.10	CH_2
		24.90	CH_2
		24.77	CH_2
		22.66	CH_2
		14.17	CH_2
		14.07	CH_2

The combination of the FTIR, UV-VS, H1-NMR, C13 NMR results with major fragments in GCMS gave rise to the proposed structure for the compound of fraction 1 (fig 1.0)

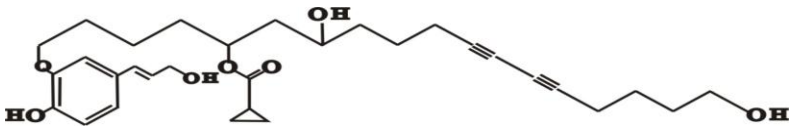


Fig 1.0. 4-((1E)-3-Hydroxyl-1-propenyl)-2-methoxy phenol-cyclopropane carboxylic acid, 2-pentyl-5,7-dodecadiyne-1,12-diol

A part of the above compound (4-((1E)-3-Hydroxyl-1-propenyl)-2-methoxy phenol) has been reported to have antimicrobial effect, anti-oxidant and anti-inflammatory (Ravikumor *et al*, 2012).
This could account for the antimicrobial effect of this plant part as seen in the result (Tables 5 and 6)

Table 12: FTIR Results of stem fraction 2

Wave band (cm^{-1})	Chromophore description
3338.89	NH Stretch of amines and amides
2964.69	C-H Stretch of alkanes and aromatics
1399.0	C=O stretch of amides and imides
1064.78	C-O deformation bonds for alcohols and esters
880.53	C-H deformation bonds for aromatics and alkyl groups
441.71	C-H deformation bond of methyl groups

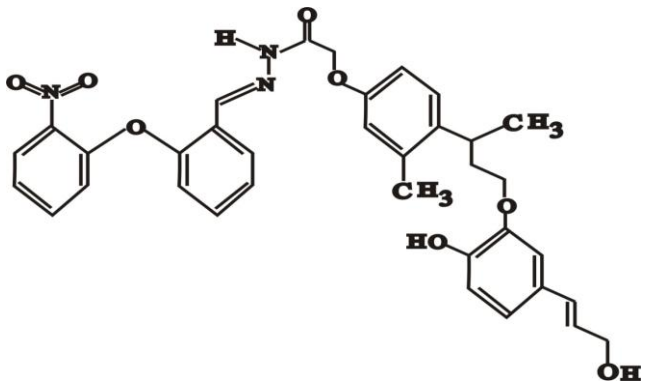
Table 13: UV-visible results of stem fraction 2

λ max (nm)	Description
740.00	C-NO_2 absorption bonds
655.00	-C=C- for aromatics (n ⟶ π*)
605.00	-C=C- for aromatic (π ⟶ π*)
537.00	C=N (n ⟶ π*)
502.00	HN-C=O (n ⟶ π*)

Table 14: Summary of the H^1 and C^{13} NMR results of stem fraction 2

H^1 δ (ppm) & Multiplicity	**Coupling Constant (MHz)**	**Types of Proton**	**C^{13} δ (ppm)**	**Types of Carbon**
9.65 (d)	1.5329	ArCH	178.4376	C=O
7.5		ArCH	153.4795	C=N
6.75 (multiplet)	172.611	ArCH	118.8586	
5.3080		H-C=N	114.4370	C
4.25		HN-C=O	114.2895	C-C=C
3.9020		ArCH	108.6722	,,
3.8945		ArCH	103.1469	,,
3.8886		ArCH	102.8906	,,
3.8837	(multiplet) 288.6066	ArCH	77.3760	C-O
3.8675		ArCH	77.0583	C-O
2.3379		ArCH	76.7464	C-O
1.6(d)		ArCH	72.5560	C-O
1.3518		ArCH	70.2710	C-O
1.3208		R-C-OH	65.1115	C-O
1.3031		$R-CH_2OH$	63.3675	C-O
1.2767		$O=C-CH_2$	60.5403	C-O
0.9141		$R-CH_2O$	56.2633	CH
0.9073		$R-CH_2$	55.9952	CH
0.8976		$=C-CH_3$	37.2578	CH
0.8800		$-C-CH_3$	34.1495	CH
			33.9627	CH
			31.9002	CH
			31.4185	CH
			30.2048	CH
			29.6641	CH
			29.5837	CH
			29.4312	CH
			29.3253	CH
			29.2366	CH
			29.0890	CH
			28.9472	CH_2
			27.1991	CH_2
			24.8883	CH_2
			24.7542	CH_2
			22.6562	CH_3
			14.0662	CH_3

A combination of the FTIR, UV-Visible, ^{1}H-NMR and ^{13}C-NMR results and the fragments generated from GCMS spectrum gave rise to the proposed structure shown in fig 2



$C_{35}H_{34}O_8$

Fig 2.0: Phenol,4-(3-hydroxy-1-propenyl)-2-methoxy-2-(2-nitrophenoxy) benzaldehyde-4-isopropyl-3-methyl phenoxy acetylhydrazone

Table 15: FTIR results of stem fraction 3

Wave band (cm^{-1})	Description
3339.86	NH Stretch of amines, amides and imides
2964.69	C-H Stretch for alkanes and aromatics
1399.40	C=O stretch of esters
1065.71	C-O deformation bonds of esters
881.50	C-H deformation bonds of aromatics and alkyl groups
498.78	C-H deformation of methyl groups

Table 16: UV-Visible Results of Stem fraction 3

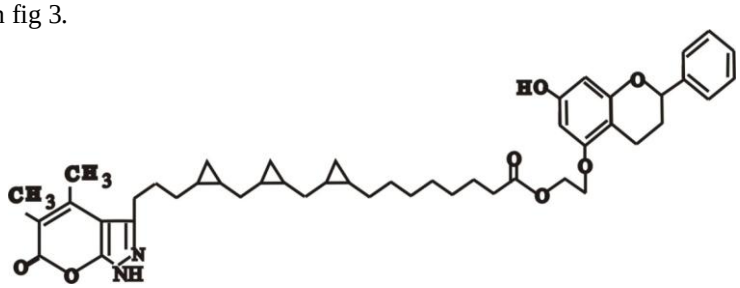
λ max (nm)	Chromophore description
741.50	C=N (n $\longrightarrow$ π*)
651.00	C=C of Aromatic (π $\longrightarrow$ π*)
605.50	
521.00	C=N (n $\longrightarrow$ π*)
305.00	C=O (n $\longrightarrow$ π*)

Table 17: Summary of H^1 and C^{13} NMR results of Stem fraction 3

H^1 δ (ppm) & Multiplicity	**Coupling Constant (MHz)**	**Types of Proton**	**C^{13} δ (ppm)**	**Types of Carbon**
9.7000 (d)	0.0119	RCO_2H	179.0917	C=O
7.2921 (n)	1.061	RCONH	130.1955	C=O
5.3769		PhOH	130.0046	ArC
5.3708		ArH	129.7186	ArC
5.3618		ArH	128.0714	ArC
5.3466		ArH	127.9112	ArC
3.9583		RCHO	102.8978	ArC
3.9316 (multiplet)	0.8532	RCH_2O	77.3650	C-O
3.8987		RCH_2	77.0472	C-O
2.7500		RCH_2	76.7295	C-O
2.2500 (multiplet)	5.9412	RCH_2	70.2923	C-O
1.6453		RCH_2	65.1104	C-O
1.6285		RCH_2	63.3624	C-O
1.3299		RCH_2	60.5357	C-O
1.2780		RCH_2	56.2604	C=NH
1.2074		RCH_2	55.9973	O-C-NH
1.1821		RCH_2	37.2615	CH_2
1.0230		RCH_2	34.0054	CH_2
0.9506		RCH_2	31.8988	CH_2
0.9090		RCH_2	31.5088	CH_2
0.8989		RCH_2	30.1913	CH_2
0.8814		RCH_2	30.1058	CH_2
0.8481		RCH_2	29.7430	CH_2
0.8284		CH_3	29.6645	CH_2
			29.5757	CH_2
			29.4969	CH_2
			29.4226	CH_2
			29.3243	CH_2
			29.2972	CH_2
			29.2294	CH_2
			29.1250	CH_2
			29.0687	CH_2
			28.7094	CH_2
			28.4851	CH_2
			27.1957	CH_2
			25.6276	CH_2

	24.8811	CH_2
	24.7192	CH_2
	22.6540	CH_2
	22.5417	CH_2
	14.0605	CH_3

Based on the above results and the major fragments of the GCMS Spectra, a structure was suggested for the compound as given in fig 3.



$C_{47}H_{59}N_2O_7$

Fig 3: 3,4,5-trimethyl-1-H-pyrano [2,3-C] pyrazol-6-one cyclopropane octanoic acid,2-[[2-[(2-ethylcyclopropyl)methyl] cyclopropyl]methyl]-methylester-5-methoxy-2-phenyl-7-chromanol

Table 18: FTIR Results of stem fraction 4

Wave band (cm^{-1})	Description
3366.86	OH Stretch (H-bonded) for carboxylic acids and alcohols
2905.86	C-H Stretch for aromatics and alkanes
1701.27	C=O stretch for esters and acids
1401.33	C=C stretch of alkanes and aromatics
1061.85	C-H deformation bonds of aromatics and alkyl groups
879.57	C-H deformation of alkyl and methyl groups
454.25	C-H deformation of methyl groups

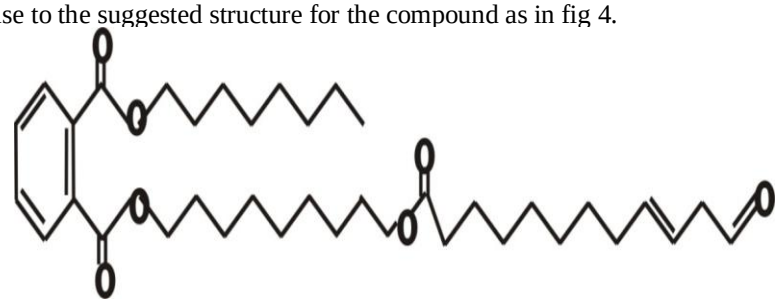
Table 19: UV-Visible results of Stem fraction 4

λ max (nm)	Chromophore description
795.50 740.50	-HC=O absorption bonds (n $\longrightarrow$ π*)
661.50 604.50	C=C of aromatics (π $\longrightarrow$ π*)
532.00 504.50	C=C of alkenes (π $\longrightarrow$ π*)
427.50 408.00	C=O (n $\longrightarrow$ π*)

Table 20: Summary of the H^1 and C^{13} NMR Results of stem fraction 4

H^1 δ (ppm) & Multiplicity	**Coupling Constant (MHz)**	**Types of Proton**	**C^{13} δ (ppm)**	**Types of Carbon**
9.75 (t)	0.1418	RCHO	130.8487	C=O
7.2917 (multiplet)	2.1453	ArH	130.0154	C=O
5.200 (multiplet)	6.6961	$R_2C=CH$	129.7237	RC=CR
3.6893		RCH_2	77.3360	C-O
2.3638		RCH_2	77.0184	C-O
2.1000		RCH_2	76.7011	C-O
1.3592		RCH_2	38.7752	CH_2
1.3385		RCH_2	37.2754	CH_2
1.3306 (multiplet)		RCH_2	34.1142	CH_2
1.3111	45.2820	RCH_2	33.9400	CH_2
1.2844		RCH_2	32.1528	CH_2
0.9227		RCH_2	31.9098	CH_2
0.9166		RCH_2	31.5958	CH_2
0.9068		RCH_2	31.5155	CH_2
0.8891		CH_3	30.3939	CH_2
			29.6733	CH_2
			29.5803	CH_2
			29.5038	CH_2
		1	29.4284	CH_2
			29.3322	CH_2
			29.2316	CH_2
			29.1272	CH_2
			29.0693	CH_2
			28.9362	CH_2
			28.9362	CH_2
			27.9780	CH_2
			27.3428	CH_2
			27.2052	CH_2
			25.6333	CH_2
			24.7053	CH_2
			23.7840	CH_2
			23.1092	CH_2
			22.6613	CH_2
			22.5479	CH_2
			21.0429	CH_2
			14.0667	CH_3

A combination of the FTIR, UV-Visible, H^1-NMR and C^{13}-NMR results with the major fragments of the GCMS spectra gave rise to the suggested structure for the compound as in fig 4.



$C_{37}H_{59}O_7$

Fig 4: 1,2-benzene dicarboxylic acid,dioctyl-methyl-12-oxo-9-dodecenoate

III. CONCLUSION

The stem of the plant *Cola gigantea* has shown to be potent medicinal plant for antimicrobial/pharmaceutical applications and that the effectiveness of the plant in the treatment of veneral diseases, abscess, oesteomytlitis, wound infection etc was due to the presence of some secondary metabolities. The active isolates from this plant parts: 4-((1E)-3-Hydroxyl-1-propenyl)-2-methoxy phenol-cyclopropane carboxylic acid,2-pentyl-5,7-dodecadiyne-1,12-diol; Phenol,4-(3-hydroxy-1-propenyl)-2-methoxy-2-(2-nitrophenoxy) benzaldehyde-4-isopropyl-3-methyl phenoxy acetylhydrazone; 3,4,5-trimethyl-1-H-pyrano [2,3-C] pyrazol-6-one cyclopropane octanoic acid,2-[[2-[(2-ethylcyclopropyl)methyl] cyclopropyl]methyl]-methylester-5-methoxy-2-phenyl-7-chromanol and 1,2-benzene dicarboxylic acid,dioctyl-methyl-12-oxo-9-dodecenoate could serve as precursors for drug production.

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REFERENCES

[1]. Adeniyi, B.A.,Groves, M.J., and Gangadharam, P.R.J., (2004). Invitro antimycobacterial activities of three species of cola plant extracts, (sterculiaceae). Phytotherap Res., 18(5), 414-418.
[2]. Adodo, A., (1998). Nature Power (Revised Edition), Don Bosco Publishers, Akure , Nigeria, p.41.
[3]. Agyare, C.,Koffuor, G.A., Boamah, V.E, Adu, F., Mensah, K.B, Adu-Amoah L., (2012). Evidence Based Complementary and Alternative Medicine,Hindawi Publishing Corporation, India, p.902394
[4]. Ajiwe,V.I.E., Dimonyejiaku, N., Ajiwe ,A.C., Chinweuba, A.J., and Chendo, N.M., (2008). Preliminary study on the pharmaceutical constituents of e*milia sonchifolia* leaf, Anachem Journal, 2(2), 302-309.
[5]. Alinnor, I.J., (2007). Preliminary Phytochemical and antibacterial activity screening of seeds of *Garcinia Cola*, Journal Chemical Society Of Nigeria, 32(2), 41-47.
[6]. Benjamin, L.T., (1991). Coca-Cola, Caffeine and Mental Deficiency- Harry Hollingworth and the Chattanooga trial of 1999, Journal Histol Behavioural Science, 27(1), 42-45.
[7]. Blades, C., (2000). Functional foods or Neutraceutics, Nutrition and Food Sci., 30(2), 73-75.
[8]. Cunha, B.A., (2009). Antibiotic Essentials, 8th edn., Jones and Barlett Learning Publishers, United States, p.180.
[9]. Dalziel,J.M., (1961). The Useful Plants of West Tropical Africa, the Crown Agents, London, p.308.
[10]. Harbone, J.B., (1998). Phytochemical Methods- A guide to Modern Techniques of plant Analysis, 3rd edn., Chapman and Hall, London, pp.36-89.
[11]. Hoffman, K.L, Han, I.Y, and Dawson P.L., (2001). Antimicrobial effects of Corn Zein Films Impregnated with Nisin, Lauric acid and EDTA, Journal food protection, 64(6), 885-889.
[12]. Ibeh, I.N and Uraih, N. (2003). Practical Microbiology, Vol.1, Ambik Press Ltd, pp82-93.
[13]. Idu, M., (2010). Documentationon Medicinal Plants sold in markets in Abeokuta, Nigeria, Tropical Journal of Pharmaceutical Research, 9 (2), 110-118.
[14]. Nestler, M.T and Hurley,D., (2004). Microbiology; A Perspective, 4th edn., Mc Graw-Hill, New York, pp 230-253.
[15]. Ouattar, B, Simard R.E. ,Piett, G., Begin, A., and Holley, R.A., (2000). Anti-listerial activity of a polymeric film coated with hybrid coatings doped with Enterocin 416K1 for use as bioactive food packaging, International Journal of food microbial, 62 (1-2), 139-148.
[16]. Penecilla, G.L., Magno, C.P.,(2011). Antibacterial activity of extracts of twelve common medicinal plants from phillipines. J.med.plants research, 5(16),3975-3981.
[17]. Reid, K.A., Jager,A.K., Light,M.E., Mulholland, D.A., and Staden, J.V., (2005). Phytochemical and Pharmacological screening of Sterculiacea Species and Isolation of antibacterial compounds, J. Ethnopharmacol, 97(2), 285-291.
[18]. Sonibare, M.A., Soladoye, M.O., Esan, O.O., and Sonibare, O.O., (2009). Phytochemical and Antimicrobial Studies of four Species of *cola schott and End*l. *(sterculiaceae)*. Afr. J. Trad Complementary Alternative Medicine, 6 (4), 518-525.