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Evalution of the Chemical Compositions of Extracts of the Plants *Palista Hirsota* and *Raovofovia Vomiti* use for the Management of Bacterial and Fungi Infections in Agbarho Community of Delta State

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ABSTRACT

Phytochemical screening, antimicrobial and GC-MS analysis of the ethyl acetate and methanol crude extracts of the aerial parts of Palista hirsota and Raovofovia vomiti were studied in this work. The aim of the work is to ascertain the strength of the different solvent extracts of both plants in terms of microbial potency and chemical composition since both plants are used in Agbarho community for the treatment is bacteria and fungi infections. The preliminary screening of the various extracts was carried out using standard methods and the results revealed the presence of steroids and saponins for the ethyl acetate extract of Raovofovia vomiti while terpenes, steroids, flavonoids carbohydrate and saponins were present in the methanol extract of Palista hirsota. The antimicrobial screening was carried out on both plant extracts using the organisms; Salmonellae Typhi, Esherichia coli, Staphylococcus aureus, Klebsiella Pneumonia, Pseudomonas, Candida Krusei and Candida albican. The extract from Raovofovia vomiti produced a zone of inhibition of 20 mm, 10 mm and 10 mm for the organisms Salmonellae Typhi, Staphylococcus aureus, and Candida albican respectively with a corresponding MBC/MFC of 50mg/ml each while the extract from Palista hirsota has significant zone of inhibition on all the organisms tested against with a corresponding MBC/MFC >100mg/ml for organisms. The major compound detected by the library search of the data base in the GC-MS analysis revealed that both compound G1 and D1 from the aerial parts of the plants P. hirsota and R. vomitoria is octadecanoic acid also called stearic acid with the molecular formula C18H36O2, molecular weight 284g/mol and with a percent peak area of 45.58% for compound D1 from the aerial parts of R. vomitoria and 29.27% for Palista hirsota..

Keywords: Antimicrobial, Chemical compositions, GC-MS, *Raovofovia vomiti*, Organisms, *Palista hirsota*.

INTRODUCTION

Bacteria and fungi diseases are caused by a pathogenic organisms present within the human environment. These diseases are common in most West African countries including Nigeria. It has been reported by WHO that approximately 60% of the inhabitants of most communities in the world believe in the use of medicinal herbs in the management of diseases. In many part of the world medicinal plant has been used for antibacterial, antifungal and antiviral activities for hundreds of years [1-3]. Rauvolfia Vomitoria and Palisota hirsuta are common herbs used by the indigenes of Agbarho community of Delta state for the management of bacteria and fungi infections. It is against this background the chemical constituents of the different extracts of the aerial plant parts of these two plants are checked to ascertain chemical potencies of each plant and where possible proffer recommendations. Rauvolfia Vomitoria and Palisota hirsuta are shrub or small tree found in different parts of West Africa including Nigeria. Recent studies have demonstrated the efficacy of different the Rauvolfia species used extensively for various ailments. It is useful in the lowering of blood pressure [4]. Palisota hirsuta are used in Ghana and other West African states for various painful and inflammatory conditions. The anti-inflammatory conditions of the ethanolic leaf extract of Palisota hirsuta was assessed and reported by woode.

Ogwuche and Adeyemi 2016, investigated the in vitro antimicrobial activity of the methanol extract of R. vomitoria used in Agbarho community of Delta state and it revealed that the extract was bactericidal against the following organisms, Staphylococus aureus, Klebsiella pneumonia, Pseudomonas, Candida albicans and Candida krusei at different concentrations and also other chemical constituents were also ascertained.

MATERIALS AND METHOD

Plant material

The aerial part of the plants *Palisota hirsuta* and *Rauwolfia Vomitoria* were collected from Agbarho community and identified at the herbarium of University of Benin. They were dried in the laboratory at ambient temperature of around 25ºC; plant materials were individually crushed using a mortar and pestle to provide a greater surface area. The crushed plant materials were weighed and it gave 176g for *Rauwolfia Vomitoria* and 157g for *Palisota hirsuta*, thereafter they were placed in container which were labelled and kept at room temperature. Crude plant extract was obtained by Soxhlet extraction method.

Phytochemical Screening

The extracts of both plants were obtained differently using Soxhlet extractor and they were subjected to phytochemical screening using standard techniques of plant secondary metabolites by Harborne [5], Sofowora [6] and Trease and Evans [7]. The crude plant extracts of the aerial plants of both plants were tested for Alkaloids, Saponins, Phlobotannins, Phytosterolds Terpernoid, Phenols, Carbohydrate, Tannins, Steroids, Flavonoids and Cardiac Glycosides.

Chromatography

Thin layer chromatography (TLC) was conducted on Silica gel (E-Merck and BDH) coated on a thin glass plate to determine the solvent combination to use for column chromatography and to view other components. Spots on TLC were detected by viewing under florescent lamp and further by spraying with 20% tetraoxosulphate (VI) acid, followed by heating at 60^{o}C. Column chromatography was carried out on the extract over silica gel using gradient elution method with different solvent systems in order of increasing polarity.

Methanol extract

A combination of methanol and ethyl acetate solvent was used to elude for the methanol extract. 5g of the methanol extract was loaded on the column. 100% ethyl acetate was initially used to run the column. Thereafter, by using gradient elution, the subsequent ratios used were 8:2, 7:3, 6:4, 5:5 up to 100% methanol and a total of ten fractions were collected at each solvent mixture. These fractions were allowed to evaporate to dryness. Another Thin layer chromatography (TLC) was conducted on the fractions collected, thereafter similar fractions were pooled together and the purer crystal was taken for analysis labeled, G_1.

Ethyl acetate extract

A combination of petroleum ether and ethyl acetate solvent was used to elude the ethyl acetate extract. 5g of the ethyl acetate extract was loaded on the column. 100% petroleum ether was initially used to run the column. Thereafter, by using gradient elution, the subsequent ratios used were 8:2, 7:3, 6:4, 5:5 up to 100% ethyl acetate and the column was finally washed with methanol and a total of ten fractions were collected at each solvent mixture. These fractions were allowed to evaporate to dryness. Another Thin layer chromatography (TLC) was conducted on the fractions collected, thereafter similar fractions were pooled together and the purer crystal was taken for analysis labeled, G_1.

A combination of petroleum ether to ethyl acetate solvent was used. At a ratio of 8:2, seven fractions were collected, at the ratio of 7:3, ten fractions was collected and at the ratio of 6:4 five fractions was collected. The column was finally washed with methanol (50ml). These fractions were allowed to evaporate to dryness. Thin layer chromatography (TLC) was conducted on the fractions collected, thereafter similar fractions were pooled together and the purer crystal was taken for analysis labeled, D_1.

Antimicrobial Screening

Zone of inhibition

The antimicrobial activities of the extracts from both plants were determined using some pathogenic microorganisms. The test microbes such as *Salmonellae Typhi*, *Esherichia coli*, *Staphylococcus aureus*, *Klebsiella Pneumonia, Pseudomonas*, *Candida Krusei* and *Candida albican* were obtained from Emma-Maria Biometric laboratory Abraka, Delta State. The zone of inhibition was conducted using the method of Kumara *et al*., [8].

Culture media

The culture media used were Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB). All the media were prepared according to manufacturer's specifications.

Preparation of inoculums of test organisms

The McFarland turbidity standard scale 1 was used to standardize the organisms. The scale was prepared by adding 9.9 ml of 1 % barium chloride ($BaCl_2$). Suspensions of the organisms were made in sterile distilled water and compared with the McFarland turbidity standard, until the opacity match with the scale number 1, which corresponds to 1.5 x 10^6 CFU/ml.

Determination of MIC of plant extract by micro dilution method

Test-tubes were prepared by dispensing 50µl of Nutrient broth for bacteria, into each well. A 50µl from the stock solution of tested extract (concentration of 200 mg/ml) was added into the first row of the plate. Then, twofold, serial dilutions were performed by using a micropipette. The obtained concentration range was from 100 to 25 mg/ml, and then added 10µl of inocula to each test-tube except a positive control (inocula were adjusted to contain approximately 1.5 x 10^8 CFU/mL). The extracts of both plants were individually used, with the media as a positive control and inoculum with media was used as a negative control. The test plates were incubated at 37^0C for 18 hours [9,10].

Gas Chromatography and Mass Spectrometry (GC-MS)

GC-MS analysis was carried out on each extract. It was analyzed using GC-MS QP2010 Plus Shimadzu under the following condition: column used were Rtx-5MS, 30m length and inner diameter of 0.25 mm and the initial column temperature was 80^0C and final temperature was 280^0C, while the injector temperature was 250^0C with split mode injector and split ratio of 1 and pressure of 108.0kPa. The flow rate was 6.2 ml/minute and the flow within the column was 1.58ml/minute. The detector temperature was 230^0C and using Helium as the gas carrier with FID (Flame ionization detector); and the samples volume injected was 8µl. Compounds were identified by comparing retention indices/comparing mass spectra of each compound with those of authentic samples and library

FTIR-84005 Fourier Transform Infrared Spectrophotometer

The Infra-red spectra's were recorded on FTIR-8400S (Shimadzu Deutchland GmbH) spectrophotometer in KBr and polyethylene pellets. The extract was weigh-in at 0.01 g and homogenized with 0.01 g KBr anhydrous by mortar agate. The mixture of sample and KBr were pressed by vacuum hydraulic at 1.2 psi (pounds per square inch) to obtained transparency pellet. Samples were usually scanned in the absorption area of 500-4000 cm^{-1}. The results of analysis consisted of chemical structure, molecular binding form and certain functional group of tested sample as basic of spectrum type.

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical screenings of the extracts of plants are shown in Table 1. It revealed the presence of Steroid, cardiac glycosides, phytosterol, terpenoid. Researchers have reported that some secondary metabolites can block tumour growth in rodent models, which further supports the idea that they have potential for cancer therapy and they also have pharmacological activities which include antihypertensive effect, anti-malarial activities and anticancer actions [11,12].

Table 1: Qualitative phytochemical screening of *Rauvolfia vomitoria* and palista histrus

Constituents	Test	Observation	Inference	
			Ethyl RA	ME PA
Alkaloid	Extract + 2ml 1% HCl. Filtrate +	Formation of yellow colouration, turn reddish brown	Absent	Absent

	Wagner reagent	on addition of Wagner reagent		
Terpernoid	Extract + 2ml Chloroform +3ml of conc. H2SO4	Formation of yellow colouration, turn green on addition of conc. H2SO4	Absent	Present
Phenols	Extract + 4 drops of FeCl3	Green colouration	Absent	Absent
Steroid	Extract + 2ml chloroform + 3 drops conc. H2SO4	Yellow colour, on addition of conc. H2SO4 turn green	Present	Absent
Flavonoid	Extract + 2ml of 2% NaOH	Green colouration, turn light green	Absent	Present
Phytosterol	.Extract + 2ml Chloroform. Filtrate + 3 drops of conc. H2SO4	Yellow colouration, turn green on addition of conc. H2SO4	Absent	Absent
Carbohydrate	Extract + 2ml Bennedict solution + heat	Blue colouration. on heating turn green	Absent	Present
Phlobatanin	Extract + 1% HCl	Light green colouration	Absent	Absent
Saponin	Extract + 20ml distilled H2O, shake for 15 minute	Colourless	present	Present
Tannin	Extract + 10ml distilled H2O. Filtrate + 2ml FeCl3	Colourless and turn yellow on addition of FeCl3	Absent	Absent

Antimicrobial Screening

Table 2: Comparison of the zone of Inhibition (sensitivity test) for *Rauvolfia vomitoria* extract and palista histrus extracts

Organisms	**EAc RA(mm)**	**ME PA(mm)**
Staphylococcus aureus	12	4
Escherichia coli	0	16
Salmonellae Typhi	10	28
Klebsiella pneumoniae	0	15
Pseudomonas aeruginosa	0	15
Candida albicans	10	18
Candida krusei	0	14
Key: 0 = No zone of inhibition, EAc RA = Ethylacetate extract of *Raufofovia vomite*, M.E = Methanol extract of *Palista histrus.*		

Table 3: Zone of Inhibition (sensitivity test) for the antibiotic disc (drugs) Extract in diameter (mm)

Organisms	**AU**	**CPX**	**PN**	**CEP**	**OFX**	**NA**	**PEF**	**CN**
Staphylococcus aureus	15	0	10	10	10	0	0	11
Escherichia coli	16	0	0	13	16	0	0	0
Salmonellae Typhi	10	0	0	12	18	0	0	10
Klebsiella pneumonia	12	0	0	12	0	0	0	0
Pseudomonas aeruginosa	15	0	0	10	0	0	0	0
Key: AU-AUGUMETIN, CPX-CIPROFLOX, PN- AMPLICIN, CEP-CEPOREX, NA-NALIDIXIC ACID, PEF-REFLACINE, OFX-TARIVID, CN-GENTAMYCIN								

Table 4: Zone of Inhibition (sensitivity test) for the antifungal disc (drugs) Extract in diameter (mm)

ORGANISMS	**Nystatin 1000UI**	**Fluconazole 230mg**
Candida albicans	0	12
Candida krusei	10	6

Table 5: Comparison of the Minimum Inhibitory Concentration of *Rauvolfia vomitoria* extract and *Palista histrus* extracts on some selected bacteria isolates

Organisms	**100mg/ml**	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**	**MBC**
Methanol extract PA						
Staphylococcus aureus	++-	+--	---	---	---	50mg/ml
Escherichia coli	+++	+--	---	---	---	50mg/ml
Salmonellae Typhi	+++	++-	---	---	---	50mg/ml
Klebsiella pneumonia	+++	++-	---	---	---	50mg/ml
Pseudomonas aeruginosa	+++	++-	---	---	---	50mg/ml
Ethyl Acetate extract RA						
Staphylococcus aureus	---	---	---	---	---	>100mg/ml
Escherichia coli	---	---	---	---	---	>100mg/ml
Salmonellae Typhi	---	---	---	---	---	>100mg/ml
Klebsiella pneumonia	---	---	---	---	---	>100mg/ml
Pseudomonas aeruginosa	---	---	---	---	---	>100mg/ml

Key: ++=No inhibition on both runs, +- =Inhibition on one run and no inhibition on the second run, - - = Inhibition on both runs, PA= Palista histrus RA = Raufofovia vomite

Spectroscopic measurement

The both compounds G_1 from *Palista* crude extract and D1 from *Rauvofovia* crude extracts were analyzed using FTIR-8400S Fourier Transformer Infrared Spectrophometer and an Agilent Gas Chromatography (6890N model) coupled to 5973 Mass Selective detector (MSD), using chloroform as solvent at the National Research Institute of Chemical Technology Zaria. The results obtained were compared with an inbuilt main library C:/Database/NIST02.L) and this library enabled the confirmation of the compound(s) present in the plant.

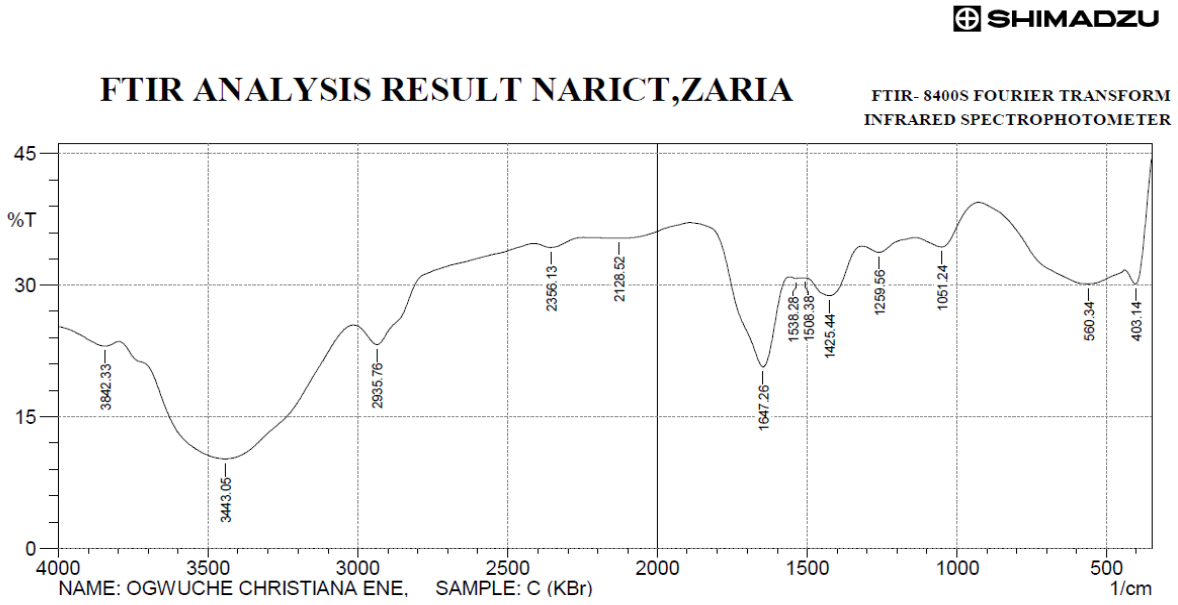

Figure 1: FTIR spectrum of Compound G_1

Table 6: Functional groups in Compound G_1 analyzed by using FTIR

Bands (cm^{-1})	**Functional group**
3443.05	O-H Stretch
2935.76	C-H Stretch of alkanes
1647.26	C=C Stretch of alkenes
1425.44	-CH_3 bend f alkanes
1051.24	C-O Stretch present in compounds with C-O bonds ethers, esters etc.

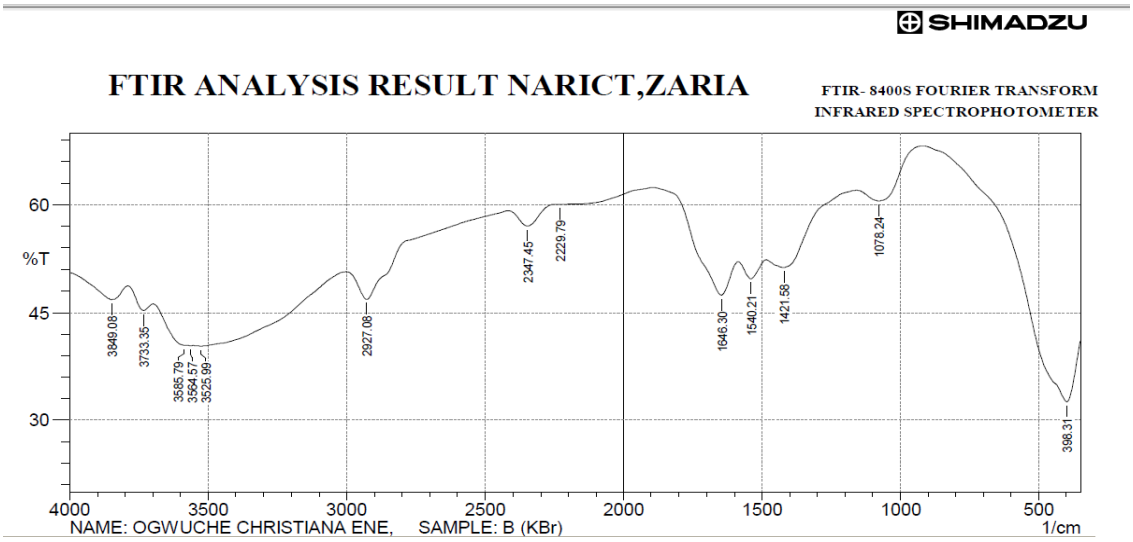

Figure 2: FITR spectrum of compound D_1

Table 7: Functional groups present in compound D_1 analyzed by using FTIR

Bands (cm^{-1})	**Functional group**
2927.08	O-H Stretch
1646.30-1540. 21	N-H bend of primary and secondary amine
1421.50	C-H Stretch of a -CH_2- bend of
1078. 24	C-O Stretch of esters, ethers, carboxylic acids and anhydride

GC-MS analysis of compound $G_{1\ and}$ D_1

GC-MS analysis was carried out on Agilent Technologies 6890N Network GC System and Agilent Technologies 5973 Network Mass Selective Detector coupled with 7683B Series Injector. The model number of the column used was Agilent 122-5533 capillary column with specification: DB-5ms, 0.25mm*30m*1µm. The carrier gas used was Helium at a flow rate of 1.2ml/min. The injection volume was 1µ. The inlet temperature was maintained at 230^0C. The oven temperature was programmed initially at 5^0C for 5 minutes, Then programmed to increase to 300^0C at a rate of 10^0C ending with 25 minutes. Total run time was 45 minutes. The MS transfer line was maintained at a temperature of 300^0C. The source temperature was maintained at 230^0C and the MS Quad at 150^0C. The ionization mode used was electron ionization mode at 70eV. Total Ion Count (TIC) was used to evaluate for compound identification and quantitation. The Spectrum of the separated compound was compared with the database of the spectrum of known compound saved in the NIST02 Reference Spectra Library. Data analysis and peak area measurement was carried out using Agilent Chemstation Software.

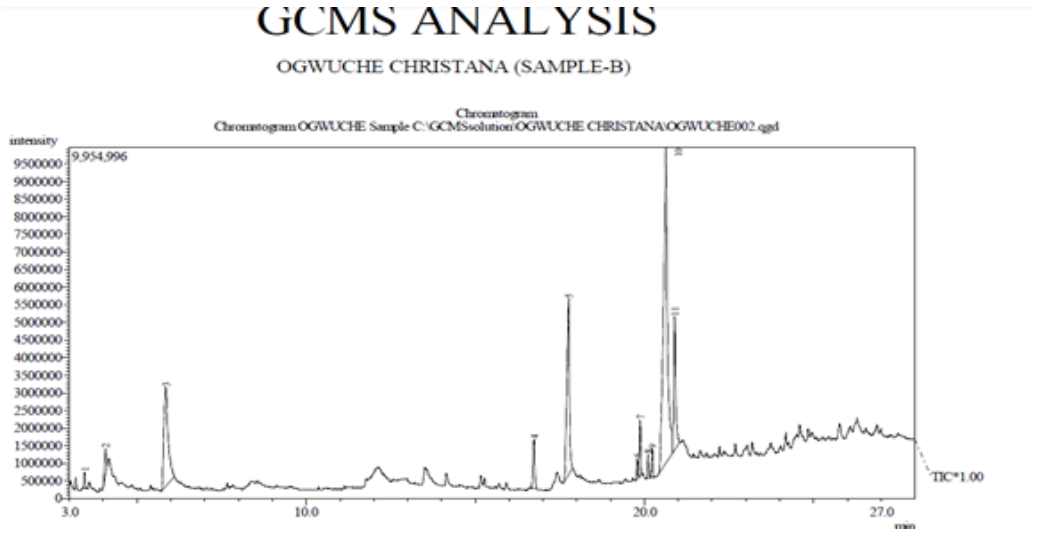

Figure 3: GCMS analysis

Identification of components

The database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns was used to identify the compound. The spectrum of the unknown components was compared with the spectrum of the known compounds stored in the NIST library.

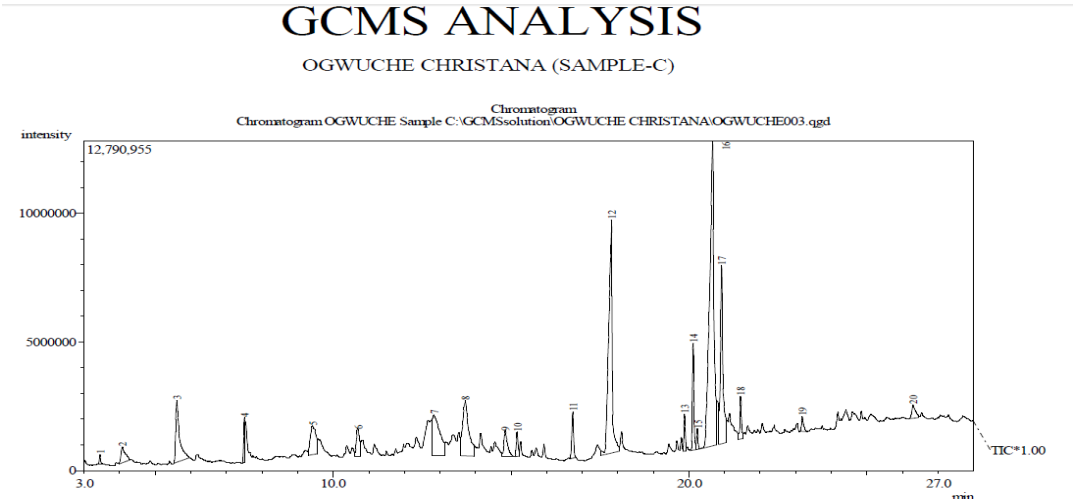

Figure 4: GC-MS of compound G_1 (*Palista hirsota*)

Table 8: Sample C (*Palista hirsota*)

Peak Number	Retention Time 3	Area%	Height%	Formula	Molecular Weight	Compound Name
1	3.525	0.24	0.67	$C_6H_{14}O_2$	118	1-Propanol, 2-(1-methylethoxy)-
2	4.300	1.45	1.18	C_8H_{16}	112	2-Octene
3	5.967	5.29	4.64	$C_5H_{10}O_4$	134	1,2,3-Propanetriol
4	7.533	1.46	3.36	$C_5H_{10}O_4$	134	1,2,3-Propanetriol
5	9.558	3.55	2.17	$C_4H_8O_3$	104	1,2-Ethanediol, monoacetate
6	10.775	1.93	2.12	$C_8H_{12}O_6$	204	1,1,2-Triacetoxyethane
7	13.142	6.87	3.06	$C_6H_{13}ClO_2$	152	2-Propanol, 1-chloro-3-propoxy
8	13.958	7.46	4.13	$C_4H_8O_3$	104	1,2- Ethanediol monoacetate
9	15.067	2.00	1.93	$C_{11}H_{20}O_2$	184	3,6-Dimethyl-5-hepten-1-ol acetate
10	15.225	0.85	1.82	$C_{14}H_{28}O$	212	E-2-Tetradecen-1-ol
11	16.808	1.81	3.46	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester
12	18.042	18.95	17.50	$C_{15}H_{30}O_2$	242	Pentadecanoic acid
13	19.933	1.20	2.81	$C_{19}H_{36}O_2$	296	6-Octadecenoic acid, methyl ester
14	20.192	3.65	8.00	$C_{20}H_{40}O$	296	Phytol $$ 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,
15	20.308	0.67	1.55	$C_{22}H_{44}O_2$	340	Heneicosanoic acid, methyl ester

16	20.783	9.95	13.38	$C_{16}H_{30}O_2$	254	Hexadecenoic acid,
17	21.058	29.27	22.84	$C_{18}H_{36}O_2$	284	Octadecanoic acid OR Stearic acid
18	21.517	1.63	3.22	$C_{17}H_{32}O_2$	268	Heptadecenoic acid
19	23.325	23.184	0.68	$C_{19}H_{38}O_2$	298	Nonadecanoic acid
20	26.450	1.07	1.01	$C_{21}H_{40}O_3$	340	Oleic acid, 3-hydroxypropyl ester

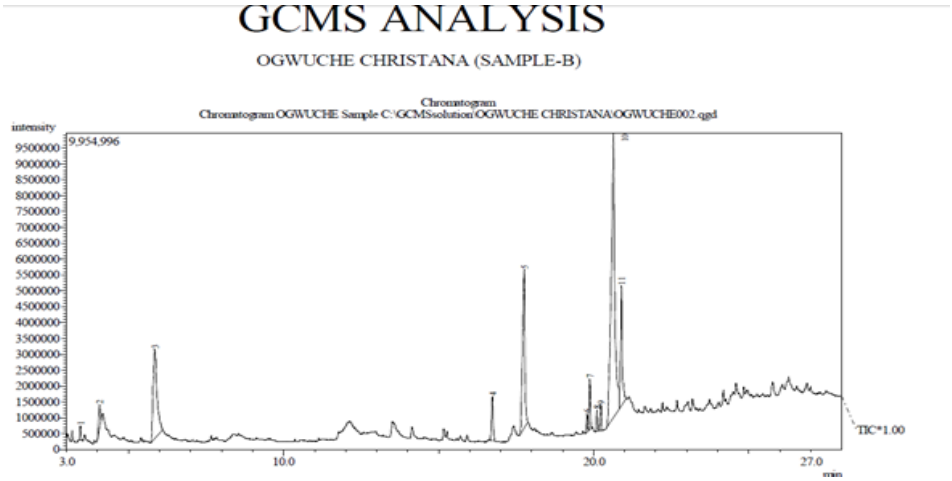

Figure 5: GC-MS of compound G_1 (*Rauvolfia vomitoria*)

Table 9: Sample B (*Rauvolfia vomitoria*)

Peak Number	Retention Time 3	Area%	Height%	Formula	Molecular Weight	Compound Name
1	3.508	0.72	1.69	$C_8H_{18}O$	130	2-Heptanol, 3-methyl
2	4.125	1.93	2.95	C_7H_{14}	98	1-Heptene
3	6.108	15.36	10.46	$C_7H_{14}O_2$	130	1-Butanol, 3-methyl-, acetate
4	16.817	3.11	5.16	$C_{17}H_{34}O_2$	270	Pentadecanoic acid, 14-methyl-, methyl ester
5	17.883	17.64	18.57	$C_{16}H_{30}O_2$	254	Hexadecenoic acid,
6	19.825	0.99	2.18	$C_{12}H_{20}$,	164	1,6,11-Dodecatriene
7	19.925	2.78	6.01	$C_{19}H_{36}O_2$	296	9-Octadecenoic acid, methyl ester
8	20.167	1.20	2.53	$C_{20}H_{40}O$	296	Phytol or 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl
9	20.292	45.58	33.36	$C_{18}H_{36}O_2$	284	Octadecanoic acid OR Stearic acid
10	20.833	1.35	3.01	$C_{22}H_{42}O_2$	338	Erucic acid or Docosenoic acid
11	21.042	1 9.34	14.09	$C_{16}H_{30}O_2$	254	Hexadecenoic acid

DISCUSSION

The major compound detected by the library search of the data base in the GC-MS analysis revealed that both compound G1 and D1 from the aerial parts of the plants *Palista hirsota* and *Rauvolfia vomitoria* respectively is octadecanoic acid also called stearic acid with the molecular formula $C_{18}H_{36}O_{2,}$ molecular weight 284g/mol and with a percent peak area of 45.58% for compound D1 from the aerial parts of *R. vomitoria* and 29.27% for *Palista hirsota*. Stearic Acid is a saturated long-chain fatty acid with an 18-carbon backbone. Stearic acid is found in various animal and plant fats.

Stearic acid It has been reported to have antibacterial, antifungi, antiviral and anti-inflammatory activities, as a result, cream formulations containing n-docosanol (docosanol) or stearic acid were tested for effects on chemically-induced burns in mice [13].

Also from the hexane-soluble fraction of an ethanol extract from leaves and stems of Stemodia foliosa (Scrophulariaceae), the new stearic acid 4-[(n-pentoxy) phenethyl] ester (1) was isolated. This compound exhibited antibacterial properties at 10 microg/mL concentration by using disc diffusion method against Gram-positive bacteria Bacillus cereus and Bacillus subtilis and fast-acid bacterium Mycobacterium fortuitum [14].

Manivachagam, et al., [15], reported the gas chromatographic analysis of fatty acid methyl esters from Sesuvium (S.) portulacastrum antimicrobial activity against human pathogenic microorganisms. The analysis revealed the presence of palmitic acid with the highest relative percentage (31.18%), followed by oleic acid (21.15%), linolenic acid (14.18%) linoleic acid (10.63%), myristic acid (6.91%) and behenic acid (2.42%). The saturated fatty acids were higher than the unsaturated fatty acids. The result showed the highest antibacterial and anticandidal activities and moderate antifungal activity against the tested microorganisms *Bacillus subtilis*, *Aspergillus fumigatus and Aspergillus niger* at different zones of inhibition [16].

CONCLUSION

This study illustrates that the extracts of the aerial parts of *Palista hirsota* and *Raovofovia vomiti* used for the management of bacterial and fungi diseases in Abgarho community of Delta state are good sources of metabolites with antimicrobial activities worthy of further investigations. The extracts were bactericidal against the causative organism *Salmonella typhi* in which they are mostly used for the treatment of typhoid fever and also against other organisms tested against such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae Candida albicans* and *Candida krusei* at different MIC and MBC/MFCs.

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