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GC-MS Analysis and Antimicrobial Studies of the Methanol Extract of Aerial Parts of *Rauvolfia vomitoria* obtain from Agbarho, Delta State

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Abstract
The study was designed to investigate the antimicrobial and chemical compositions of the methanol extract of aerial parts of Rauvolfia vomitoria. The aerial parts of the plant were air dried, pounded using wooden mortar and pestle into coarse powder. The coarse powder was extracted successively with Soxhlet extractor. Qualitative phytochemical screening of the methanol extract revealed the presence of phytochemicals such as alkaloids, carbohydrates, terpenoids, cardiac glycosides, steroids, tannins, and saponins. The extract was investigated for in vitro antimicrobial activity against some organisms and their chemical constituents were also ascertained. The methanol extract was bactericidal against the following organisms: Salmonella typhi, Escherichia coli, Staphylococus aureus, Klebsiella pneumonia, Pseudomonas, Candida albicans and Candida krusei. The chemical composition of the methanol extract was established using Fourier Transform Infrared (FTIR) and Gas chromatography and mass spectroscopy (GC-MS).
Keyword: *Rauvolfia vomitoria*, phytochemicals, antimicrobial, methanol, GC-MS.

Introduction
Rauvolfia Vomitoria is a shrub or small tree. The generic name *Rauvolfia* commemorates a 16th century epithet, *vomitoria* refers to the purgative and emetic properties of the bark (1). The parts that are commonly used for herbal remedies are roots, root bark, leaves and stem-bark (2). The plant is of different species. The African species of the plant *Rauwolfia Vomitoria* had twice the amount of reserpine of the indian species, *Rauwolfia serpentine* (3).
Recent studies have demonstrated the efficacy of other *Rauvolfia species* used extensively for various ailments. It is useful in the lowering of blood pressure (4). It also possesses analgesic properties (5). In Nigeria and Ghana, herbalists used it for the management of typhoid fever. In some region, children are treated with this plant for cerebral cramps, jaundice and gastrointestinal disorders (6). Little or no information is found in literature on the active phytochemicals of *Rauvolfia vomitoria* despite its wide use for treatment of several ailments. The paucity of scientific data on identification and characterization of the active compounds in *Rauvolfia vomitoria* necessitated this study. The present study investigated the methanol extract of the aerial parts of *R. vomitoria* being consumed for medicinal purpose by the inhabitants of Agbarho community of Delta State.

Materials and Methods
Preparation of Extract
The aerial parts of the plant *Rauwolfia Vomitoria* were collected from Agbarho community, identified at the herbarium of University of Benin. Leaves and stem were dried in the laboratory at ambient temperature (25ºC); plant material was crushed using mortar and pestle to provide a greater surface area. The crushed plant was weighed (176g) and placed in container which were labeled and kept at room temperature. Crude plant extract was obtained by Soxhlet extraction method using methanol.
Phytochemical Screening
The methanol extract obtained from the Soxhlet extractor was subjected to phytochemical screening using standard techniques of plant secondary metabolites by Harborne (7), modified by Sofowora (8) and further modified by Trease and Evans (9). The crude plant extract was tested for alkaloids, saponins, phlobotannins, phytosterolds terpernoid, phenols, carbohydrate, tannins, steroids, flavonoids, cardiac glycosides.
Chromatography
Thin layer chromatography (TLC) was conducted on Silica gel (E-Merck and BDH) coated on a thin glass plate to enable the solvent combination and 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 60^0C. Column chromatography was carried out on the extract over silica gel using gradient elution method with different solvent

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systems in order of increasing polarity. In a combination of methanol and ethyl acetate in the ratio of (8:2), seven fractions were collected. Methanol: ethyl acetate (7:3) resulted in ten fractions being collected and methanol: ethyl acetate (3:2), five fractions. The column was finally washed with methanol (50 ml). 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, labeled M_1, was used for analysis.

Antimicrobial screening

The antimicrobial activity of the extract from the plant was determined using some pathogenic microorganisms. The test microbes such as *Esherichia coli*, *Staphylococcus aureus*, *Klebsiella Pneumonia*, *Pseudomonas*, *Salmonellae typhi*, *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*. (10).

Determination of MIC of plant extract by Microdilution Method

The test-tubes were prepared by dispensing 50µl of nutrient broth for bacteria into each well. A 50µl from the stock solution of tested extracts (concentration of 200 mg/ml) was added into the first row of the plate. Then, two fold 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 approximately1.5 x 10^8 CFU/ml). Plant extract with media was used 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.

Gas Chromatography and mass spectrometry (GC-MS)

GC-MS analysis was carried out on the 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 Mass Spectrometric 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 were recorded on FTIR-8400S (Shimadzu Deutchland GmbH) Spectrophotometer in KBr and polyethylene pellets. Samples were weighed 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 obtain transparency pellet. Samples were scanned in the absorption area of 500-4000 cm-1. The analysis consisted of chemical structure, molecular binding form and certain functional group of tested sample as basic of spectrum type.

Results and Discussions

Phytochemical Screening

The Phytochemical studies carried out on the methanol extract of the aerial parts of *R.vomitoria* plant extract revealed the presence of active secondary metabolites such as saponins, alkaloid, terpernoid, steroid, carbohydrate, cardiac glycoside and tannin (Table 1). A recent study reported that tannins exhibit antiviral, antibacterial and antitumor activity and they are also used as diuretic (11). Cardiac glycosides are helpful to overcome various human diseases. Saponin has the property of precipitating and coagulating red blood cells (12). Alkaloids and their synthetic derivatives are being used as basic therapeutic agents for their analgesic, antispasmodic and bactericidal effects. Alkaloids have pharmacological activities which include antihypertensive effect, antimalarial activities and anticancer actions (13).

Table 1: Phytochemical screening result of methanol extract of *Rauvolfia vomitoria*

Constituents	**Inference**
Phenols	-
Steroid	+
Flavonoid	-
Alkaloids	+
Terpenoids	+
Carbohydrate	+
Cardiac glycosides	+
Phytosterol	-
Phlobatanin	-
Saponin	+
Tannin	+

Key: + = present, - = Absent

Antimicrobial Analysis

The antibacterial activity of extract on the micro-organisms compared favorably with that of commercial antibiotics as presented in Tables 2-4. The extract was bactericidal against the following organisms: *Salmonella typhi, Escherichia coli, Staphylococus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa,* and fungicidal against *Candida albicans* and *Candida krusei.* Gram-positive bacteria, *S. aureus* is known to cause serious diseases such as pneumonia, meningitis etc., in hospital patients (14). *E. coli* and *P.aeruginosa* cause urinary tract infections (UTI), pulmonary tract infections, burns, wounds, dysentery-like diarrhoea and other blood infections and similar also is true for *K.pneumonia, S.typhii* and *S. epidermidis* (15). *R. vomitoria* plant showed maximum inhibition of growth against all the antibiotic-resistant bacteria. The extract gave a higher zone of inhibition for *Salmonella typhi* and *Escherichia coli* than the antibiotics that was used. The Minimum inhibitory concentration study (MIC) shows that the extract of *Rauvolfia vomitoria* inhibited the growth of *Salmonella typhi, Escherichia coli, Staphylococus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* at a concentration of 50 mg/ml with the corresponding MBC at 50mg/ml as represented in Table 3.

Table 2: Zone of Inhibition (sensitivity test) for *Rauvolfia vomitoria* Extracts in diameter(mm)

Organisms	**ME(mm)**	AU	CPX	PN	CEP	OFX	NA	PEF	CN
Staphylococcus aureus	4	15	0	10	10	10	0	0	11
Escherichia coli	16	16	0	0	13	16	0	0	0
Salmonellae Typhi	28	10	0	0	12	18	0	0	10
Klebsiella pneumoniae	15	12	0	0	12	0	0	0	0
Pseudomonas aeruginosa	15	15	0	0	10	0	0	0	0

Key: 0 = No zone of inhibition, ME= methanol extract, AU-AUGUMETIN, CPX-CIPROFLOX, PN- AMPLICIN, CEP-CEPOREX, NA-NALIDIXIC ACID, PEF-REFLACINE, OFX-TARIVID, CN-GENTAMYCIN

Table 3: Zone of Inhibition of the antifungal disc (drugs) and the extract in diameter (mm)

ORGANISMS	**ME(mm)**	**Nystatin 1000UI**	**Fluconazole 230mg**
Candida albicans	18	0	12
Candida krusei	14	10	6

Table 4: Minimum Inhibitory Concentration of the extract

Organisms	**Methanol extract**					
	100mg/ml	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**	**MBC**
Staphylococcus aureus	++	+-	---	---	---	50mg/ml
Escherichia coli	++	+-	---	---	---	50mg/ml
Salmonellae Typhi	++	+-	---	---	---	50mg/ml
Klebsiella pneumonia	++	+-	---	---	---	50mg/ml

Key: ++= Inhibition on both runs, +-=Inhibition on one run and no inhibition on the second run, --- = No inhibition on both runs

Gas Chromatography and mass spectrometry (GC-MS)

The gas chromatogram is shown in Figure 1 while Table 5 shows the retention time (RT) of compounds identified in the methanol extract of *Rauvolfia vomitoria.* The major compound identified were Erucic acid ($C_{22}H_{42}O_2$) (Figure 2) with RT (20.631) has peak area 26.09%, followed by 3-Methylmannoside ($C_7H_{14}O_6$) with RT(14.773) has peak area 17.83%, Octadecanoic acid ($C_{18}H_{36}O_2$) (Figure 2) with RT (14.150) has peak area 14.17%, Pentadecanoic acid ($C_{15}H_{30}O_2$) with RT (17.575) has peak area 12.58%. The results confirm the presence of constituents which are known to exhibit physiological activities. Erucic acids (RT 20.631) possesses antibacterial activity, that could be used for the preparation of drugs required for human and animal health (16). 3- Tridecanoic acid (Figure 2), methyl ester (RT 14.773) can act as antioxidant, hypocholesterolemic, nematicide, pesticide and lubricant.

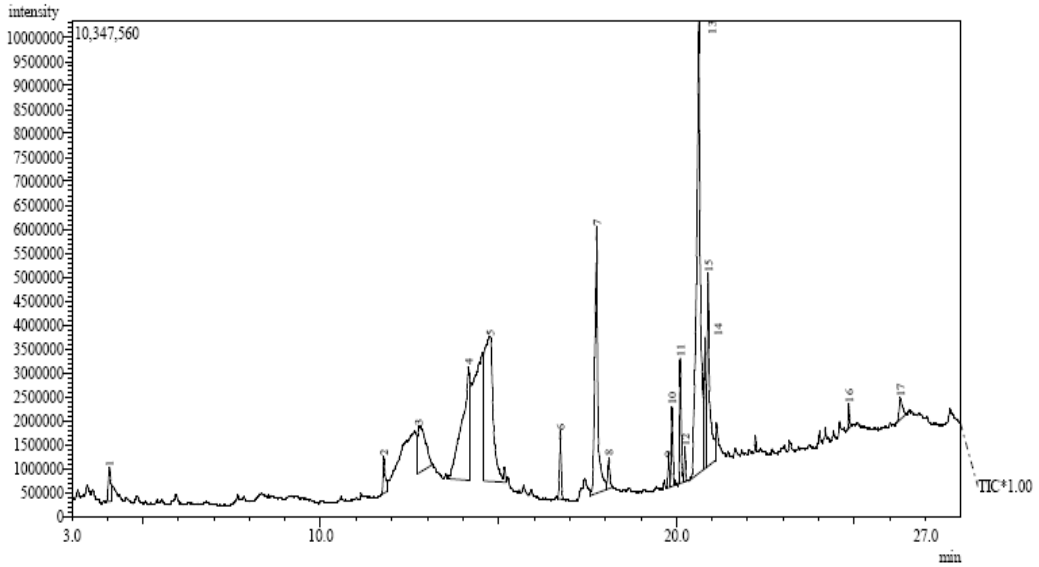

Figure 1: Gas chromatogram of the M1 fraction of the methanol extract

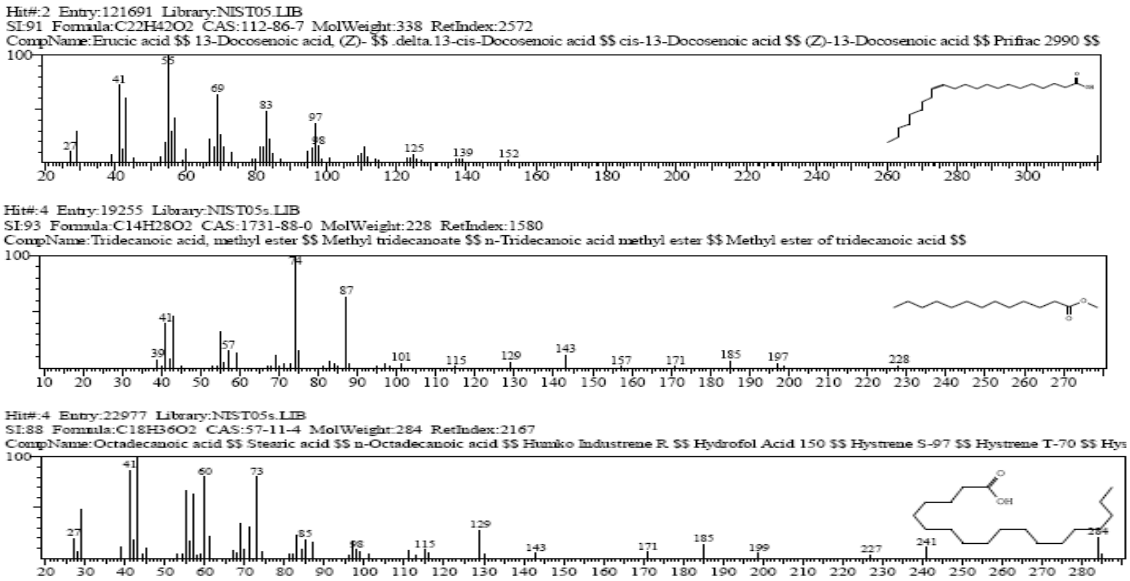

Figure 2: Spectrum of (a) erucic acid, (b) tridecanoic acid and (c) octadecanoic acid

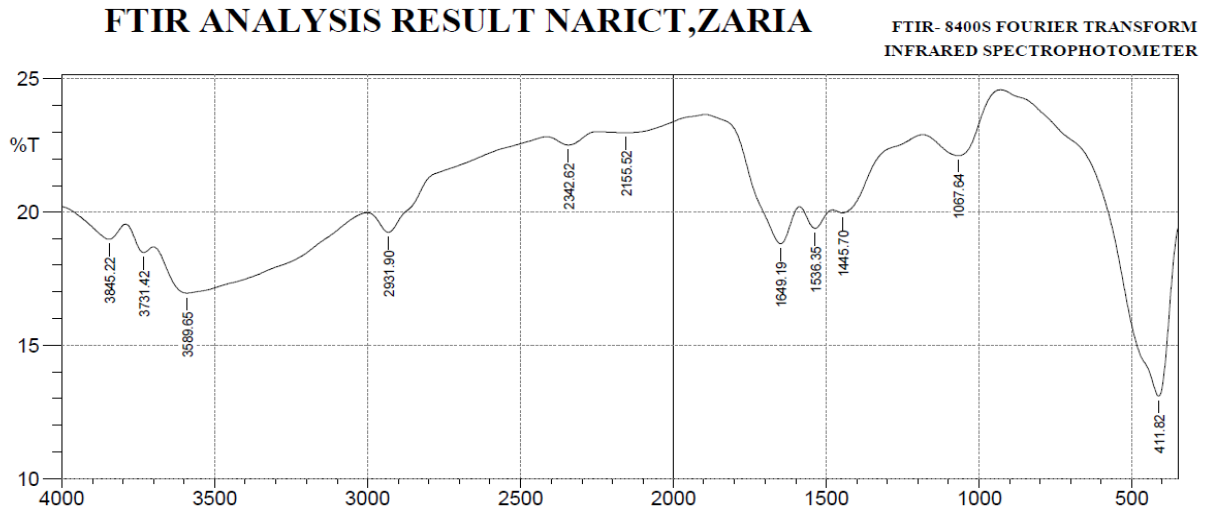

Figure 3: The infra-red spectrum of methanol extract of the aerial parts of Rauvolfia vomitoria

FTIR – 84005 Fourier Transform Infrared Spectrophotometer

FTIR shows the bands of the different functional groups that are present in the plant extract. The result further suggests that the extract could be a wide spectrum antibiotics based on earlier study (13). It was earlier reported that stretches of O-H and C-O could be indicative of antidiabetic principles (17). However, further investigation would be needed, in this case, especially where there is no report of the use of *Rauvolfia vomitoria* for that purpose.

Table 5: Major components from *Rauvolfia vomitoria* methanol extract for 60 minutes at 80^0C

Peak	RT	Molecular formula	Name of the compound	Molecular weight	Peak area(%)
1	4.063	$C_{12}H_{24}$	7-methyl-3-Undecene	168	1.30
2	11.755	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	256	1.27
3	12.766	$C_8H_{12}O_3$	2,7-Dioxatricyclo[4.3.1.0(3,8)] decan-4-ol	156	5.14
4	14.150	$C_{18}H_{36}O_2$	Octadecanoic acid	284	14.17
5	14.773	$C_{14}H_{28}O_2$	Tridecanoic acid, methyl ester	228	17.83
6	16.737	$C_{18}H_{36}O_2$	Methyl 15-methylhexadecanoate	284	1.89
7	17.575	$C_{15}H_{30}O_2$	Pentadecanoic acid	242	12.58
8	18.102	$C_{24}H_{48}O_2$	Docosanoic acid, ethyl ester	368	0.95
9	19.785	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester	294	0.70
10	19.878	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	296	1.79
11	20.114	$C_{20}H_{40}O$	Phytol	296	2.98
12	20.241	$C_{18}H_{36}O_2$	Hexadecanoic acid, 15-methyl-, methyl ester	284	0.73
13	20.631	$C_{22}H_{42}O_2$	Erucic acid	338	26.09
14	20.804	$C_{18}H_{34}O_2$	E-11-Hexadecenoic acid, ethyl ester	282	3.34
15	20.893	$C_{18}H_{36}O_2$	Octadecanoic acid	284	7.74
16	24.851	$C_{10}H_{17}Cl_3O_2$	Acetic acid, trichloro-, octyl ester	274	0.49
17	26.296	$C_{14}H_{26}O$	13-Tetradecenal	210	1.02

Table 6: Functional groups in *Rauvolfia vomitoria* extract

Bands (cm^{-1})	**Functional group**
2927.08	O-H Stretch, very broad
1646.30- 1540. 21	N-H bend of primary and secondary amine
1421.50	C-H Stretch of a -CH_2- bend of
1070. 24	C-O Stretch of esters, ethers, carboxylic acids and anhydride

Conclusion

Experimental evidence from this study revealed that *Rauvolfia vomitoria* contains some bioactive components such as alkaloids, terpenoids, cardiac glycosides, carbohydrates, saponin, tannin and steroids. The study illustrates that the methanol extract of the aerial parts of *Rauvolfia vomitoria* is a good source of metabolites with antibacterial and antifungal activities worthy of further investigations. The GC-MS analysis confirmed the presence of different compound that are present in the plant, and the IR analysis was used to reveal the various functional group. The trend observed in this study supports the traditional use of *Rauvolfia vomitoria* in the treatment of typhoid in Agbarho community. However, isolation of the bioactive principle against *Salmonellae typhi* and *Candida spp* as well as its toxicological evaluation are major areas for further studies.

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