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ANTIMICROBIAL PROPERTIES AND CHEMICAL COMPOSITIONS OF THE PETROLEUM ETHER EXTRACT OF THE AERIAL PARTS OF *Rauvolfia vomitoria* OBTAINED FROM AGBARHO COMMUNITY OF DELTA STATE.

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ABSTRACT

The study was designed to investigate the antimicrobial and chemical compositions of the petroleum ether extract of theaerial parts of Rauvolfia vomitoria. The aerial parts of the plant were air dried under shade, pounded using wooden mortar and pestle into coarse powder. The coarse powder was extracted in aSoxhlet extractor using petroleum ether. Qualitative phytochemical screening of the plant extract reveals the presence of phytochemicals such as Alkaloids, Terpenoids, Cardiac Glycosides and Steroids. The extract was investigated for in vitro antimicrobial activity against some organisms and their chemical constituents were also ascertained. The extract was bactericidal against the following organisms: salmonella Typhi, Candida krusei and candida albican at a zone of inhibition of 10 mm, 16 mm and 10 mm respectively andthe minimum inhibitory concentration studies showed that the petroleum ether extract of the aerial parts of Rauvolfia vomitoria inhibited grow that a concentration above 100 mg/ml with the corresponding MBC above 100mg/ml.The percentage yield of the petroleum ether extract was 2.5%. The chemical compositions of the petroleum ether extract were established using Fourier Transform Infrared (FTIR)spectroscopy and Gas chromatography and mass spectroscopy (GC-MS).

Key word: *Rauvolfia vomitoria*, phytochemicals, antibacterial, GC-MS, Delta State.

INRODUCTION

*Rauvolfia vomitoria*is a shrub or small tree up to 8m.The parts that are commonly used for herbal remedies are roots, root bark, leaves and stem-bark (Gill, 1992). The plant is of different species. The Indian species is called *Rauwolfia serpentina.* The African species of the plant *Rauwolfia vomitoria* had twice the amount of reserpine of the Indian species, *Rauwolfia serpentine* (Kutalek and Prinz, 2007).

Medicinal Plants have recently become of great interest owing to their versatile applications. These plants are considered as rich resources of ingredients which can be used in drug development and synthesis. In many part of the world medicinal plants have been used for antibacterial, antifungal and antiviral activities for hundreds of years (Ali *et al.,* 1998; Barhour*et al.,* 2004; Yasunaka*et al.,* 2005). In spite of these, researches are still ongoing to bring to light

plants with antimicrobial and antiviral properties.

Traditionally *R. vomitoria*, it is used against snakebite, fever, and nervous disorders. In Nigeria and Ghana, herbalists used it as an emetic and purgative for typhoid. A bioactive carboline alkaloid, alstonine, present in the root of *Rauvolfia vomitoria* was reported to have anti-cancer activity. (Denis *et al.*, 2006; Pettit *et al.*,1994).

MATERIALS AND METHOD

Preparation of Extract

The aerial parts of the plant were collected from Agbarho community and identified at theherbarium of University of Benin. The aerial plant partswere air dried in the laboratory at ambient temperature of around 25ºC.The plant materials were crushed using a mortar and pestle to provide a greater surface area. The crushed plant was weighed, labeled and kept ina container at room temperature in a dark place. The crushed plant was weighed (176g) and placed in a container which was labeled and kept at room temperature. Crude plant extract was obtained by Soxhlet extraction method. The weight of the extract was obtained to be 4.4g.

Phytochemical Screening

The petroleum ether extract obtained from the Soxhlet extractor wassubjected to phytochemical screening using standard techniques of plant secondary metabolites by Harborne (1997), Sofowora (2008) and Trease and Evans (2009). The crude plant extract was tested for Alkaloids, Saponins, Phlobotannins, Phytosterolds Terpernoid, Phenols, Carbohydrate, Tannins, Steroids, Flavonoids, Cardiac Glycosides. These phytochemicals were qualitatively tested.

Chromatography

Thin layer chromatography (TLC) was conducted on Silica gel (E-Merck and BDH) coated on a thin glass plate using 0.1 g of the extract. Spots on TLC were detected by spraying with 20% tetraoxosulphate (VI) acid, followed by heating at 60^0C. They were also viewed using florescence lamp.Column chromatography was carried out on the extract over silica gel using gradient elution method with different solvent systems in order of increasing polarity. A combination of petroleum ether and ethyl acetate at the ratio of 8:2, seven fractions were collected, at the ratio of 7:3, ten fractions were collected and at the ratio of 5:5, five fractions were 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 fraction was taken for analysis labeled as P_2.

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.

Preparation of Culture Media

Nutrient Agar Preparation

2.8g of powdered agar was weighed and suspended in 100ml of distilled water. The mixture was heated gently to dissolve the agar to clear. 100ml of the agar solution was poured into bottles and sterilized by autoclaving at 121°C, 15P for 15 minutes.

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Paper Disk Diffusion Assay

A suspension of testing microorganisms was spread on Nutrient Agar (NA) medium. The filter paper discs (5mm in diameter)was placed on the agar plates which was inoculated with the tested microorganisms and then impregnating with 20µl of plant extract (concentration200 mg/ml). The plates were subsequently incubated at 37^0C for 24 Hrs. After incubation the growth inhibition zone were quantified by measuring thediameter of the zone of inhibition in mm (Kumara*et al*., 2009)

Minimum Inhibitory Concentration Determination

The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC) and at this concentration the antibiotic is bacteriostatic.

Determination of MIC of Plant Extract by Microdilution Method

The method adopted was that of Radojević*et al*., 2012.The test-tubes were prepared by dispensing 50µl of Nutrient broth for bacteria and fungi into each well. A 50µl from the stock solution of tested extracts (concentrationof 200 mg/ml) was added into the first row of the plate. Then, twofold, seria ldilutions were performed by using a micropipette. The obtained concentrationrange 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 andinoculum with media was used as a negative control. The test plates wereincubated at 37^0C for 18 h. MIC was defined as the lowest sample concentration showed no color change (clear) and exhibited a complete inhibition of growth.

Minimum Bactericidal/Bacteriostatic Concentration Determination

This was performed as an adjunct to the MIC and is used to determine the concentration of the antibiotic that is lethal to the target bacteria/fungi in vitro.

MBC/MFC Procedure

Broth from the MIC broth tube (100ml) was aliquoted onto Nutrient agar / Sabouraud dextrose agar and spread. The plates were incubated at 35°C until the next day when they were examined for colony growth or lack of it. No growth indicated that the antibiotic was bactericidal/fungicidal and growth indicated that it was bacteriostatic/fungistatic.

Gas Chromatography and mass spectrometry (GC-MS)

GC-MS analysis was carried out on the most pure fraction from the conventional column chromatography. 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 sample 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). 0.01 g of the Sample was weighed and homogenized with 0.01 g KBr anhydrous by mortar agate. The mixture was pressed by vacuum hydraulicat 1.2 psi (pounds per square inch) to obtain transparent pellet. Sample was scanned in the absorption area of 500-4000 cm-1. The spectrum is shown as Figure 3 with a wave length rangebetween 411.82 cm-1 to 3980.24 cm-1 and interpreted on table 6.

RESULT AND DISCUSSION

Percentage Yield of Extract

The aerial parts of the plant *R.vomitoria* yielded some quantities of the extract in petroleum ether. About 176g of the aerial parts was extracted and it yielded 4.4 g of the extract. this translated to a percentage yield of 2.5% as shown below.

$$\frac{4.4}{176} \times 100 = 2.5\%$$

Phytochemical Composition

The Phytochemical studies carried out the aerial parts of *R.vomitoria* plant extract revealed the presence of active secondary metabolites such as alkaloids,terpenoids and steroids. Researchers have reported that alkaloid have pharmacological activities which include antihypertensive effect, antimalarial activities and anticancer actions (Margaret and Micheal, 1998).

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Table 1: Phytochemicals Preliminaryfrom the plant *R.vomitoria*

Constituents	Test	Observation	Inference
Alkaloid	Extract + 2ml 1% HCl. Filtrate + Wagner reagent	Formation of yellow colouration, turn reddish brown on addition of Wagner reagent	Present
Terpernoid	Extract + 2ml Chloroform +3ml of conc. H_2SO_4	Yellow colouration, reddish brown colour of the interface on addition of conc. H_2SO_4	Present
Phenols	Extract + 4 drops of $FeCl_3$	Green black colouration	Absent
Steroid	Extract + 2ml chloroform + 3 drops conc. H_2SO_4	Yellow colouration, on addition of conc. H_2SO_4 produced red in the lower layer	Present
Flavonoid	Extract + 2ml of 2% NaOH	Green colouration	Absent
Phytosterol	.Extract + 2ml chloroform. Filtrate + 3 drops of conc. H_2SO_4	Light green. Formation of a dark green layer at the bottom on addition of conc. H_2SO_4	Absent
Carbohydrate	Extract + 2ml Bennedict solution + heat	Green colouration, on heating turn reddish brown	Absent
Phlobatanin	Extract + 1% HCl	Formation of brown colour, on heating brown colouration persist	Absent
Saponin	Extract + 20ml distilled H_2O, shake for 15 minute	Formation of 1cm layer of foam	Absent Absent
Cardiac glycoside	Extract + 2ml glacial acetic acid + 2 drops of 2% $FeCl_3$ + 2ml conc. H_2SO_4	Formation of yellow colouration on $FeCl_3$, turn brown on addition of conc. H_2SO_4	Absent
Tannin	Extract + 10ml distilled H2O. Filtrate + 2ml $FeCl_3$	Light green colouration. On addition of $FeCl_3$ turn into condensed greenish colour.	Absent

Antimicrobial Poperties

The antibacterial activities of extract on the micro-organisms used compare favorably with those of commercial antibiotics as represented in tables 2.The extract were bactericidal and fungicidal against the following organisms: S*almonella typhi,Candida krusei*and *Candida albican.*The Minimum inhibitory concentration studies showedthat the petroleum ether extract of the aerial parts of *Rauvolfiavomitoria*inhibited growthat a concentrationabove 100 mg/ml with thecorresponding MBC above 100mg/ml as represented in Table 4. From the result shown, the inhibitory activities exhibited by theextracts tends to agree with the reports of Levin *et al*.(1979) and Elmahmood*et al*. (2008), all of whom linkedantimicrobial properties of plants to the presence of bioactivesecondary metabolites Researcher has revealed that plant with high zone of inhibition has potential to be used for treatment of various diseases as shown by the extract for *Salmonella Typhi, Candida krusei and candida albican*as such can be used for the treatment of typhoid and other fungi infections.

Table 2: Zone of Inhibition of extracts in diameter(mm)

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

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

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

ORGANISMS	**PE(mm)**	**Nystatin 1000UI**	**Fluconazole 230mg**
Candida albicans	10	0	12
Candida krusei	16	10	6

Key: 0 = No zone of inhibition, P.E= Petroleum ether extract

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Table 4: Minimum Inhibitory Concentration of extract.

Organisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	MBC
Staphylococcus aureus	---	---	---	---	---	**>100mg/ml**
Escherichia coli	---	---	---	---	---	**>100mg/ml**
Salmonellae Typhi	---	---	---	---	---	**>100mg/ml**
Klebsiella pneumonia	---	---	---	---	---	**>100mg/ml**
Pseudomonas aeruginosa	---	---	---	---	---	**>100mg/ml**

Key:---= Inhibition on both runs

Gas Chromatography and mass spectrometry (GC-MS)

The GC-MS analysis revealed the presence of thirty five (35) bioactive phytochemical compoundsThe active principles with their retention time (RT), molecular formula, molecular weight (MW) and the peak area (%) of 0.23-8.02 are presented in (Table 5andFig 2).

The major compound identified wereCyclopropanepentanoic acid, 2-undecyl, methyl ester ($C_{20}H_{38}O_2$) with RT (20.096) has peak area 8.02%, followed by 2-Methyl-1-1-octanol ($C_9H_{20}O$) with RT(16.232) has peak area 7.94%, Hexadecenoic acid ($C_{16}H_{34}O_2$) with RT (20.802) has peak area 7.88%,9,12-Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) with RT (19.637) has peak area 7.76%, 8-Octadecanone ($C_{18}H_{36}O$) with RT (24.339) has peak area 7.15%, Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) with RT (16.963) has peak area 5.88%. Theresults confirm the presence of constituents which are known to exhibit medicinal value as well as physiological activities.These compounds have been reported as potential antioxidant and anticancer agents (Grace *et al*., 2002, Sermakhani and thangapandian, 2012, and Sharafzadeh*et al*., 2011).

Table 5: Major components from *Rauvolfiavomitoria*petroleum etherextract for 60 minutes at 80^0

Peak	RT	Name of the compound	Molecular formula	Molecular weight	Peak area(%)	Nature of the compound
1	5.852	Methyl 6-heptanoate	$C_9H_{18}O_2$	158	1.33	Ester
2	8.308	Hexadecane	$C_{16}H_{34}$	226	0.23	Alkane
3	8.617	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	1.14	Ester
4	9.644	2,6-Dimethylheptadecane	$C_{19}H_{40}$	268	0.67	Alkane
5	10.447	2.5,11-trimethyldodecane	$C_{15}H_{32}$	158	0.33	Alkane
6	10.707	2-ethyl-4-methylpentanol	$C_{18}H_{18}O$	130	0.68	Alcohol
7	10.918	Dodecane	$C_{12}H_{26}$	170	0.56	Alkane
8	11.246	Cyclopentaundecanoic acid	$C_{17}H_{32}O_2$	268	4.73	Ester
9	11.994	Tradecanoic acid	$C_{14}H_{28}O_2$	228	5.35	Fatty acid
10	12.131	Tetradecane	$C_{14}H_{30}$	198	0.42	Alkane
11	13.120	Decanoic acid, decyl ester	$C_{20}H_{40}O_2$	312	1.23	Ester
12	13.623	Hexadecanoic acid, 15-methyl ester	$C_{18}H_{36}O_2$	284	4.13	Ester
13	14.334	Heptanoic acid	$C_{17}H_{34}O_2$	270	2.69	Ester
14	14.632	Tetradecane	$C_{14}H_{30}$	198	0.41	Alkane
15	16.232	2-Methyl-1-octanol	$C_9H_{20}O$	144	7.94	Alcohol
16	16.963	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	5.88	Fatty acid
17	17.977	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.51	Fatty acid
18	18.363	Dodecane	$C_{12}H_{26}$	170	0.36	Alkane
19	14.334	Dodecanone	$C_{12}H_{24}O$	184	0.72	Ketone
20	19.637	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	7.76	Ester
21	20.096	Cyclopropanepentanoic acid, 2-undecyl, methyl ester	$C_{20}H_{38}O_2$	310	8.02	Ester
22	20.392	Docosanoic acid, methyl ester	$C_{23}H_{47}O_2$	354	3.75	Ester
23	20.802	Hexadecenoic acid	$C_{16}H_{34}O_2$	254	7.88	Fatty acid
24	21.041	Nonadecanoic acid	$C_{19}H_{38}O_2$	298	1.64	Ester
25	21.190	9,11-Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	294	1.20	Fatty acid
26	22.265	6-Pentadecanone	$C_{15}H_{30}O$	226	1.01	Ketone
27	22.493	1-Flourodecane	$C_{10}H_{21}F$	160	4.49	Alkane
28	22.799	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	0.96	Ester
29	23.048	3-Methyl-1-hexanol	$C_7H_{16}O$	116	0.54	Alcohol
30	23.530	Pentadecane	$C_{15}H_{32}$	212	0.46	Alkane
31	24.339	8-Octadecanone	$C_{18}H_{36}O$	268	7.15	Ketone
32	24.517	Nonanyl Chloride	$C_9H_{17}ClO$	176	4.66	Ester
33	24.752	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	3.07	Fatty acid
34	26.187	Myristic acid vinyl ester	$C_{16}H_{30}O_2$	254	3.27	Fatty acid
35	27.216	Oxalic acid,isobutylnonyl ester	$C_{15}H_{28}O_4$	272	2.85	Fatty acid

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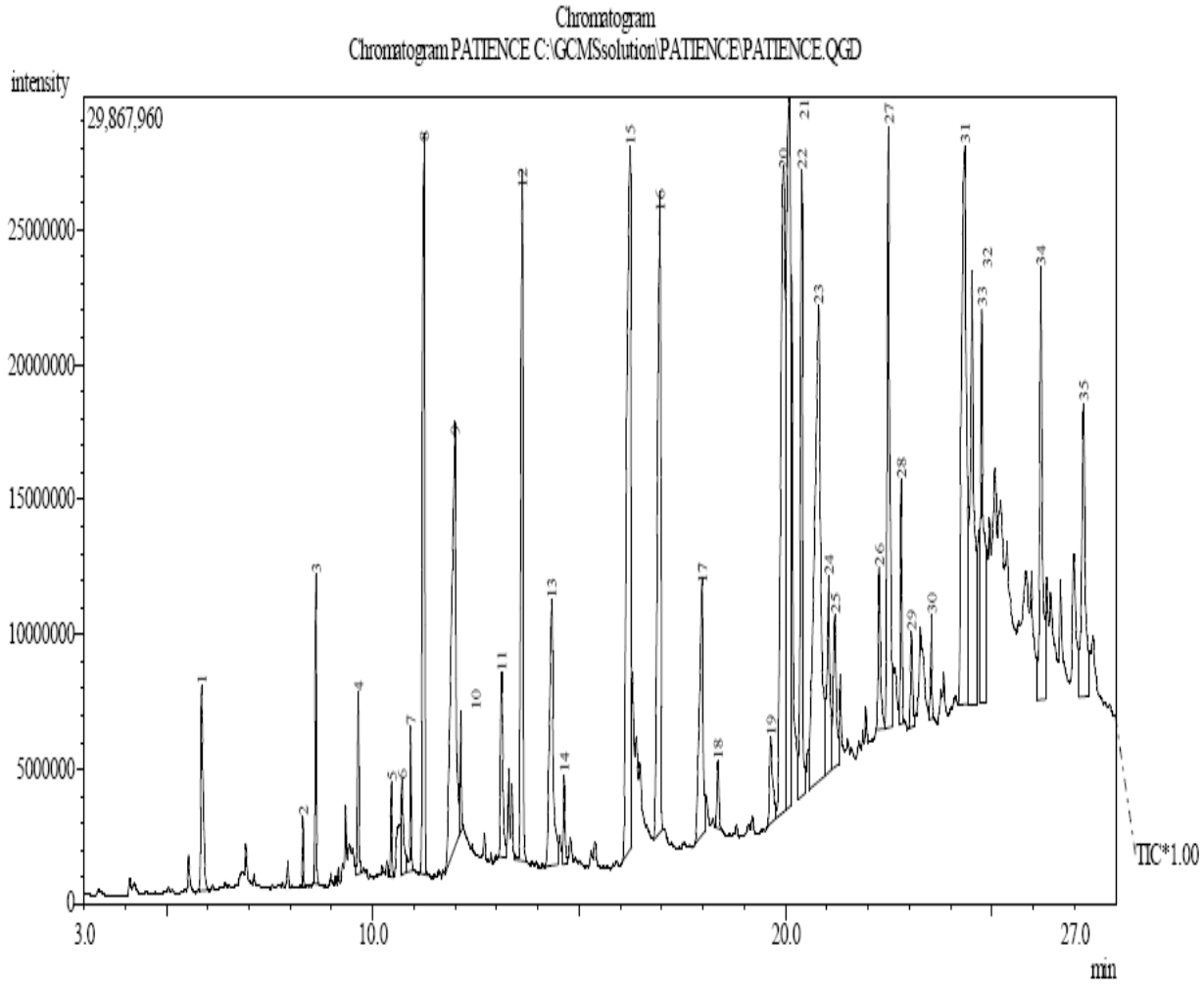

Fig. 1. Gas chromatogram of the P_2 fraction of the petroleum ether extract

FTIR – 84005 Fourier Transform Infrared Spectrophotometer

The result of the FTIR confirmed the presence of alcohol, alkyl halide, alkene and alkane.The dominant IR peaks in the spectra is the broad IR peak centered around 3430 cm-1, whichis most likely due to O-H group.FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bimolecular composition.

Table 6: Functional compounds of *Rauvolfiavomitoria* extract analyzed by using FTIR

Bands	**Functional group**
3430.51cm^{-1}	O-H stretch, H- bonded of alcohols
2946.36 cm^{-1} and 2642.57cm^{-1}	C-H stretch of alkanes indicative of methylene groups ($(CH_2)_n$)
1643.41cm^{-1} and 1462.09cm^{-1}	Medium, -C=C- stretch of alkenes
1118cm^{-1} and 1031.95cm^{-1}	C-H bend of alkanes
721.40cm-1	Medium, C-H rock of alkane and C–Cl stretch of alkyl halides

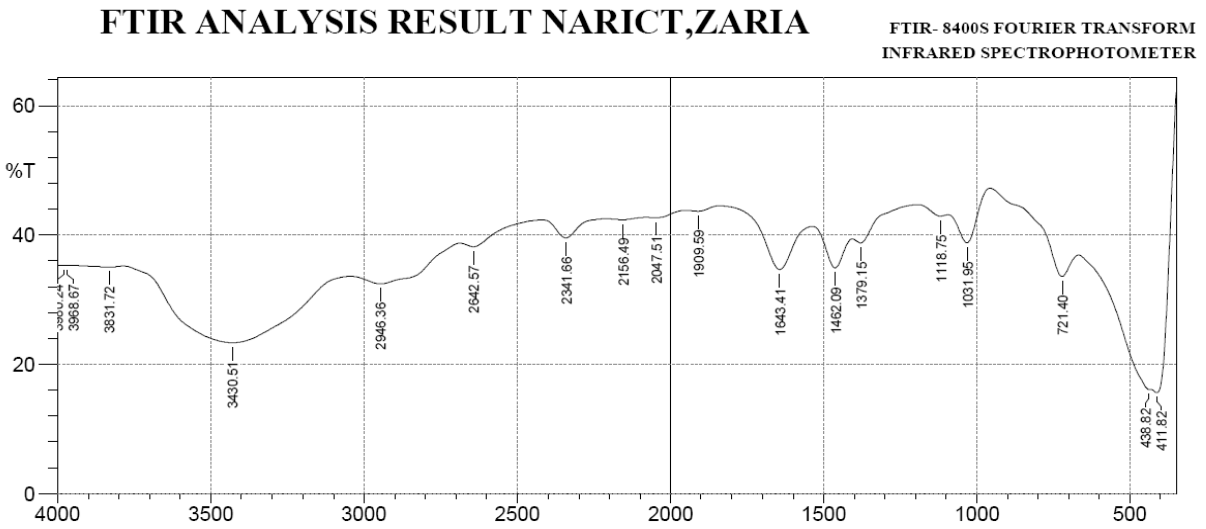

Fig 2. The infra red spectrumof petroleum ether extractof*Rauvolfiavomitoria*

The result of this study revealed the presence of bioactive components such as Alkaloids, Terpenoids and Steroids. The presence of these bioactive compounds in plant have been reported to make them relevant as potential therapeutic agents.

The antibacterial study of extract illustrates that the leaf extracts of the *RauvolfiaVomitoria* could be a good source of metabolites with antimicrobial activities worthy of further investigations.The petroleum extract were bactericidal against the following organisms: *salmonella Typhi* and *candida albican.*

The GC-MS analysis confirms the presence of different chemical compounds that are present in the plant, and the IR analysis was used to reveal the various functional group.
The present study has verified the usefulness of the aerial parts of *R.vomitoria* plant extracts for medicinal purposes. This preliminary study justifies the usefulness of this plants in the treatment typhoid in Agbarho community.

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