

CHEMICAL AND MEDICINAL PROPERTIES OF RAUWOLFIA VOMITORIA (AFZEL) HARVESTED FROM THE SOUTH EASTERN NIGERIA

ABSTRACT

Aim ;This work was carried out to evaluate the chemical constituents of the leaf of *Rauwolfia vomitoria* and determine the activities on some selected human pathogens

Study design. The study was designed to characterize the compounds in the extract and to determine their medicinal applications

Place and duration of study; department of chemistry Federal University of Technology Owerri

Methodology. Standard assay technique was used to evaluate the phytochemical content of the sample. GC-MS analysis was carried out on the chloroform extract of the sample. The anti microbial activities were determines using the well agar diffusion method

Results

The analysis of the components of the leaf extract of *Rauwolfia vomitoria* revealed the presence of 34 compounds with their molecular masses , molecular weights and structures all elucidated. The initial phytochemical screening revealed the presence of alkaloids 1.92%, saponins 1.12% , flavonoids 1.15% and cardiac glycosides, the extract showed marked inhibition of the growth of certain human pathogens with inhibition diameters recorded against *Klebsiella pneumonia*, 22mm, *Pseudomonas aeruginosa* 18mm, *Aspergillus niger* 24mm and *Mucor specie* 9mm but was resistant to *Streptococcus specie*.The extract contain anti cancer,anti-tumour antioxidant,anti-inflammatory ,cardioprotectives compounds which includes phytol, Vitamin E, Campesterol,sistosterol, squalene, lupeol and vaccenic acid.

Keywords; *Rauwolfia vomitoria*, anti-cancer, anti-inflammation ,antitumour, phytochemicals, pathogens

INTRODUCTION

Rauwolfia vomitoria Afzel is a medicinal plant found growing in the wild in most parts of Nigeria. The plant has been reported to be full of phytochemicals with majority of the phytochemicals concentrating in the root of the plant. The compound Reserpine an alkaloid has been reportedly found in the root of the plant and is said to reduce blood pressure as well as treat mental illness.[1] The antitumor activities of the plant extract as well as its activities when combined with carboplatin on ovarian cancer has been reported. [2] . Similarly, other researchers had reported the effect of the plant extract on serum amino acid transferase and alkaline phosphatase and selected indices of liver and kidney functions activities. Their results showed an increase in serum amino transferases [aspartate amino transferase (AST) and alanine amino transferase (ALT)], alkaline phosphatase activities, serum conjugated bilirubin and urea concentration but a decrease in serum albumin and potassium concentrations .The plant is said to be useful as a sedative and in the treatment of psychotic tendency[3]. The Ethanolic root bark and leaf extracts of *Rauwolfia vomitoria* may lead to advanced skeletal development,[4] . The extracts of *the plant* has antiplasmodial effects, which however supports local claims on the efficacy of the plant's leaf in the treatment of malarial infections. Other research works [5] has shown that *Rauwolfia vomitoria* has potent sedative effect, which may result in severe unpleasant consequences when used without control measures. They concluded that the plant extract produces sedative behaviour, and cerebral cortical neurohistological changes. The anti-convulsant activity of the aqueous leaf extract of *Rauwolfia vomitoria* (Afzel) by testing the effects of the extract on strychnine-, picrotoxin and pentylenetetrazole induced seizures in mice has been studied, [3].The researchers reported that there is a possible efficacy potential of the aqueous leaf extract of *Rauwolfia vomitoria* in convulsions. The Effect Of Aqueous Extract Of *Rauwolfia Vomitoria* Root Bark On The Cyto-architecture Of The Cerebellum And Neurobehaviour Of adult male Wistar rats has been studied [6],it was reported that there were reductions in body weight and the neuro-behaviour test showed reduced locomotion and exploratory activities, while histological result showed distortions of the cerebellar cells and layers . They concluded that the extract affects the cerebellar cyto-architecture and neuro-behaviour.[7] Other

researchers reported that the plant has a lot of medical potential in curing and preventing ailments like malaria, typhoid, and jaundice . It also has aesthetic effects on human beings and their environment. [8] The In Vitro Assessment of Anthelmintic Activities of *Rauwolfia vomitoria* (Apocynaceae) Stem Bark and Roots against Parasitic Stages of *Schistosoma mansoni* and Cytotoxic Study has been documented. it was reported that Both plant parts were found to be active against cercariae and adult worms. Within two hours of exposure, all cercariae were killed by the plant extracts which were inhibitory to the proliferation of cell lines, providing evidence of in vitro Schistosomicidal potency of *Rauwolfia vomitoria*, with the stem bark being moderately, but relatively, more active and selective against Schistosome parasites [9]. The plant has shown so many medicinal and pharmaceutical applications yet the constituents the plant has not been fully characterized. We are on a journey of total elucidation of the components and uses of the plant starting from the leaf

MATERIALS AND METHOD

Sample collection

Fresh leaves of *Rauwolfia vomitoria* were collected in July 2020 at the department of Forestry and wildlife field, Federal University of Technology Owerri in Imo state in the eastern part of Nigeria. The plant was identified by Mr Iwueze Francis a taxonomist of the same department. The plant leaves was room dried , milled into fine particles with the aid of an electric blender and then stored in an air tight container.

Test for Flavonoids

5g of the sample was soaked with 50cm³ of water for 2hours and then filtered and to the filtrate was added drops of ammonia and 3cm³ of concentrated H₂SO₄ was added. A yellow precipitate which disappears on storage indicates the presence of flavonoids.[10]

Test for Alkaloids

5g of the sample was extracted using 20% acetic acid in ethanol .5cm³ of the extract was treated with Wagner's reagent (iodine crystals and KI). A yellowish brown precipitate indicates the presence of alkaloids. [10]

Test for Tannins

5g of the root sample was weighed into a beaker and 50cm³ of water was added and allowed to soak properly for two hours and extracted. The extract was treated with drops of ferric chloride. A blue-black precipitate indicates the presence of tannins.[11]

Test for Steroids

5cm³ of the water extract was treated with concentrated H₂SO₄ in acetic anhydride. The formation of a blue-green color indicates the presence of steroids. [12]

Test for Glycosides

20cm³ of the water extract was treated with Fehling solutions of A and B in equal amount and boiled. A brownish red precipitate indicates the presence of glycoside.[12]

Alkaloid determination

10 g of the sample was weighed into a 250 cm³ beaker and 200 cm³ of 20 % acetic acid in ethanol was added and covered to stand for 6hrs. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. The alkaloid was precipitated out using concentrated ammonium hydroxide which was added drop by drop until precipitation was complete. The solution was allowed to settle and the precipitation was collected by filtration using whatman filter paper, the precipitate was dried and weighed.[13,14]

Saponin Determination

20 g of the sample was weighed into a 250 cm³ beaker and 200 cm³ of 20 % ethanol was added and stirred using glass rod. The mixture was heated over water bath for 4hrs with continuous stirring while the temperature was maintained at 55 °C. The mixture was extracted and the residue was extracted with 200 cm³ of 20 % ethanol. The combined extract was reduced to 40 cm³ over water bath at 90 °C. The concentrated extract was transferred into a 250 cm³ separation funnel and 20cm³ of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This process was repeated thrice. 60 cm³ of n-butanol was added. The mixture was washed twice with 10 cm³ of 5 % sodium chloride. The remaining solution was heated over water bath and the residue dried to constant weight. The saponin content was calculated in percentages [13,14]

Flavonoid Determination

10 g of the plant sample were extracted repeatedly with 100 cm³ of 80% of aqueous methanol at room temperature. The solution obtained was filtered with whatman filter paper no 45. The filtrates were later transferred into a crucible and evaporated to dryness over a water bath and weighed [14,15]

Preparation of Samples for GC-MS Analysis

Two hundred grams of the sample was repeatedly extracted with ethanol using soxhlet extractor, another 200 g of sample was soaked in ethanol for 48 hour and extracted. The extracts from the soxhlet extracts and that obtained from cold extracts for the sample were combined and re-extracted using chloroform to obtain chloroform soluble extract. This was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant oil was subjected to GC and GC-MS analysis.

GC-MS Experimental Procedures

GC- analysis was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m) and the conditions are as follows: Temperature programming from 80 – 200°C held at 80 °C for 1 minute, the rate is 5 °C/min and at 200 °C for 20 minutes. FID Temperature of 300 °C, injection temperature of 250 °C, carrier gas is Nitrogen at a flow rate of 1 cm³/min and split ratio of 1: 75. GC-MS Gas chromatography Mass spectrum analysis were conducted using GC-MS QP 2010 Plus Shimazu Japan with injector Temperature at 230 °C and carrier gas pressure of 100kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. The eluents were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5 kv and sampling rate of 0.2seconds. The Mass Spectrometer was also equipped with a computer fed Mass Spectra data bank, HERMCE Z 233 M-Z centrifuge Germany was used. Reagents and solvents such as Ethanol, Chloroform, Diethyl ether, hexane all of analytics grade was obtained from Merck Germany. [12]

ANTIMICROBIAL ANALYSIS

Bacterial isolates and identification

Clinical bacterial isolates were obtained from Federal Medical Centre Owerri microbiology laboratory. The bacteria isolates includes; *Klebsiella pneumonia*, *Staphylococcus aureus* and

Streptococcus pneumoniae . Each of these bacteria were sub-cultured on a sterile plate of nutrient agar, Mac-Conkey agar, blood agar and chocolate agar according to the type of bacteria. The identification of the bacteria was confirmed using the following gram staining and biochemical test such as methyl red test, motility test and indole test

Gram staining test

The bacterial colonies were collected using a sterile inoculating loop and emulsified onto a drop of normal saline on clean grease free slides. The smear was allowed to air dry and then heat fixed by passing over flame the slide containing the heat fixed smear was placed on a staining rack and flooded with primary stain crystal violet and allowed to stand for 60 seconds before being washed with water. After, the smear was flooded with Lugol's iodine and allowed to stand for 60 seconds before being washed with water. Acetone was also used to flood the smear in order to decolorize it, after being flooded with acetone, the smear was quickly washed with water. The slide was also flooded with secondary stain safranin and allowed to react for 60 seconds before being washed with water the back of the slide was cleaned and allowed to air dry. After drying, the smear, a drop of oil was placed on the surface of the smear and examined using oil immersion objective lens

Motility TEST

There are some bacteria genera that can move actively from one place to another, others that lack locomotive structures such as flagella cannot move. The ability to test this is important in their identification as it helps to differentiate motile from non-motile bacteria. Stab culture technique was used to demonstrate motility of using straight wire, the inoculum were picked up and a straight line stab of the inoculum made into a semi solid medium so that the stab stopped at about the center of the medium. This was done in duplicate for the test bacteria isolates. It was incubated at 39°C for 24 hrs and the tube examined non motile bacteria grew only along the line of stabbing while the motile bacteria grew along the line of the stabbing and diffused into the medium away from the line of stabbing causing turbidity and rendering the medium opaque. The non-motile bacteria growth was confined to the path of inoculation

Catalase Test

Aerobic bacteria produce varying levels of catalase enzymes that break down hydrogen peroxide to water and oxygen. This test was performed by bringing the test bacteria in contact with hydrogen peroxide. The production of effervescence indicated that the organism is a catalase producer. Two drops of hydrogen peroxide was made on a clean grease free glass slide using a clean glass rod, the bacteria was transferred to the first drop, the second was used as control

Coagulase Test

The test was carried out using a slide test method. A colony of the bacteria was emulsified on a glass of physiological saline on a glass slide to make a thick suspension. A drop of plasma was added to the suspension and mixed gently. Clumping of the of organism which did not re-emulsify indicates the presence of coagulase positive test

Indole Test

2ml of peptone water was inoculated with 5ml of bacterial culture and incubated at 35°C for 48hrs. Kovacs; reagent 0.5ml was added well shaken and examined after 60 seconds. A red colour in the reagent layer indicates indole.

Citrate utilization test

This test assists in the differentiation of entobacteriaceae. The test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as only its source of nitrogen. This was carried out by inoculating 4ml of sterile Simon citrate medium with 24hrs culture of the test organism using a sterile wire loop. The inoculated agar was incubated at 35⁰ C for 48hrs and observed for colour change from green to royal blue

Methyl Red Test

Colonies of the test bacteria were inoculated in 0.5ml sterile glucose phosphate broth, after overnight incubation at 37°C a drop of methyl red solution was added .a red colouration was positive and indicated an acid phi resulting from fermentation of glucose. A yellow colouration indicates negative result

Oxidase Test

Oxidase test was used to differentiate bacteria that produce oxidase enzyme from the non-oxidase producing type. Many facultative organism lack cytochrome C which can be detected using oxidase test. Oxidase catalyze the electron transport between electron donor in the bacteria and a redox dye, di tetraethyl-p-phenylenediaminedehydrochloride is produced which is reduced to a purple colour. A piece of filter paper was placed on a clean petri-dish .3 drops of oxidase reagent was added on the filter paper using a piece of glass rod , the test bacteria was smeared on the filter paper. Appearance of a bluish –purple colour within 10 seconds indicates positive reaction

Mycological Examination of Fungal Isolates

Two fungal isolates were sub-cultured on sterile plates of sabouraud dextrose agar. The two fungi includes *Aspergillus niger* and *Mucor specie*. The identity of the fungus was determined using the needle mouth technique. With the help of a sterile inoculating needle, small portion of each fungal structure was collected and dropped at the center of a sterile grease free glass slide. With the help of Pasteurs' pipette, a drop of ethanol was made on the fungal structure .the ethanol was allowed to evaporate. A drop of lactophenol cotton blue stain was made on the fungal structure using Pasteurs' pipette. Two inoculating needles were used to tease the fungal structure. The preparation was carefully covered with cover slip. It was examined using low and high power magnification, the fungal isolates were identified using types of conidia, chlamydosperm and hyphae

Evaluation of Antimicrobial Activity

Preparation of Bacterial and Fungal Suspensions

2mls of normal saline was aseptically poured into sterile 5ml test tube. The test tube. The test tubes were labeled with the names of each isolate; bacteria and fungi. Two gram positive bacteria , *Staphylococcus aureus* and *Streptococcus pneumonia*, one gram negative bacteria (*Klebsiella specie* , two fungi specie *Aspergillus niger* and *Mucors specie*. These five test tubes were set up on test tube rack. With the help of a sterile wire loop, each fungal and bacteria were transferred to each tube bearing each isolates name

according to the previous labeling of the test tubes. The test tubes were swirled after each inoculation until the isolate suspension becomes turbid. The color of each tube was compared with that of a 5% Marc Farland standard. Bacterial suspension had four test tubes while the fungal suspensions had two test tubes

Dilution of the plant extract

Four sterile test tubes were set up on a test tube rack and labeled accordingly. 1 ml of the plant extract was added to the first test tube that is the stock. 1 ml of distilled water was added into each test tube from test tube 2-4. 1 ml of plant extract. 1 ml of plant extract was added into test tube 2 (1/20). the 1ml distilled water and 1 ml extract were carefully shaken to obtain homogenous mixture. 1 ml of this mixture was transferred to the third test tube and well shaken. With a new pipette 1ml of the content of test tube 3 was transferred to test tube 4 and carefully shaken. 1ml of its content was discarded using another sterile pipette. With this method four different concentrations of plant extracts were obtained in each test tube

ANTIBACTERIAL ACTIVITY USING WELL-IN- AGAR METHOD

4 plates of sterile Muller Hinton agar plates were prepared according to the manufacturer's instruction. The plates were allowed to cool and solidify. With the help of a pipette 0.1ml. *Klebsiella specie* suspension was introduced on the surface of the plate of prepared Muller Hinton agar. In like manner, equal volume of other bacterial suspensions were made on the remaining agar plates. Using a sterile 6mm cork borer, four wells were made on each agar plate. The wells were labeled according to the dilution of plant extract made (stock, 1/10, 1/20, 1/40). With different pipette, each extract of the plant extract was filled into each well as labeled until each well is filled to the brim. This was left for 1 hour to ensure that the extract has been absorbed by the agar. The plates were incubated at 37°C for 24 hours. Zones of inhibition of the different the plant extract on each bacteria were observed and recorded. The process was repeated with each of the bacteria isolates.

ANTIFUNGAL ACTIVITY USING WELL IN AGAR METHOD

Two plates of Sabouraud dextrose agar were freshly prepared. Two plates of the agar were smeared with *Aspergillus niger* while the

other two plate were also smeared with *Mucor specie*, four well agar were also bored on each plate. Each well containing different dilution of each extract just as was done to the bacteria isolates earlier. The plates were incubated at 37° C temperature for 48 hours. Plates were observed and zones of inhibition of different plant extracts on the two fungi plates were recorded.

RESULT AND DISCUSSION

The results obtaining from the screening and quantitativ determination of the constituents of the leaf of *Rauwolfia vomitoria* are contained in table 1 and 2 bellow

Table 1 results of phytochemical screening of the leaf of *Rauwolfia vomitoria*

Phytochemical	Inference
Saponins	++
Flavonoids	++
Alkaloids	++
Cardiac glycoside	++
Tannins	--
Steriods	--

Key; + present - absent

Table 2: Phytochemical quantification of the leaf *Rauwolfia vomitoria*

Phytochemic al constituent	Solvent used in extraction	Percentage yield(weight/weight)
Saponins	20% ethanol	1.12%
Flavonoids	80% aqueous methanol	1.15%
Alkaloids	20% acetic acid in ethanol	1.92%

The results reveal the presence of alkaloids 1.92%, saponins 1.12% , flavonoids 1.15% and cardiac glycosides, while tannins and steroids were not detected. Alkaloids have reported to be present in most green leafy plants and they have many therapeutic properties. Most alkaloids have found their way in medicinal application as antimalarial, antimicrobial ,antibacterial,antifungal an

d antiparasitic activities .[9] It has been reported that the plant extract has a lot of medical potential in curing and preventing ailments like malaria, typhoid, and jaundice. Their result could only be justified because of the presence of alkaloids Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever [16].

Flavonoids are distributed group of polycyclic compounds characterized by a common Benzo pyrone ring structure that has been reported to act as antioxidants in many biological systems. The family encompasses flavonoids, flavones, chalcones, catchins, anthocyanidins and isoflavonoids [17] . In addition to their free radical scavenging activities, Flavonoids have multiple biological activities including – vasodilatory, anti-carcinogenic, anti-allergic, antiviral, estrogenic effects as well as being inhibitors of phospholipase H2, cyclooxygenase, glutathione reductase and xanthine oxidase [18]. flavonoids support lactogenesis. These properties therefore support the use of plant extract in cancer therapy[19] . Flavonoids in intestinal tracks lower the risk of heart diseases. As anti-oxidant, flavonoids provide anti-inflammatory actions.

Saponins was found to be available in the leaf of *Rauwolfia vomitoria*, Some of the general characteristic of saponins includes; formation of forms in aqueous solutions, hemolytic activity and cholesterol binding properties[20]. Saponin has the natural tendency to ward off microbes and this makes them good candidates for treating fungal and yeast infections. These compounds serve as natural antibiotic, helping the body to fight infections and microbial invasion.. [14]

ANTIMICROBIAL PROPERTIES

TABLE3 RESULT OF ANTIMICROBIAL ANALYSIS

Conc	<i>Streptococcus specie</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumonia</i>	<i>Pseudomonas aeruginosa</i>	<i>Aspergillus niger</i>	<i>Mucor specie</i>
Stock	R	20	22	18	24	9
1/10	R	14	18	13	17	R
1/20	R	4	6	3.5	5	R

1/40	R	4	6	3.5	R	R
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Zone of inhibition in (mm). R = resistance

The antimicrobial properties of *Rauwolfia vomitoria* are contained in table 3 above

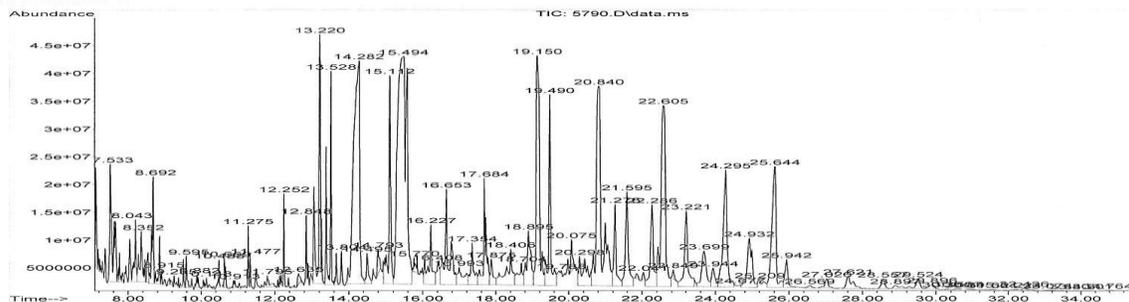
The plant extract showed marked inhibitory potentials against certain selected human pathogens, the main stock inhibited the growth of *Staphylococcus aureus* by a diameter of 20 mm, similarly, other micro-organisms were not left out with inhibitions recorded against *Klebsiella* specie, 22mm, *Pseudomonas aeruginosa* 18mm, *Aspergillus niger* 24mm and *Mucor* specie 9 mm but could not inhibit the growth of *Streptococcus* specie. Most of these pathogens have been implicated to be the main causes of some human ailments. *Staphylococcus aureus* is a gram positive coccus that causes skin infection such as; pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, abscesses, pneumonia, toxic shock syndrome, bacteremia and sepsis. [12]. *Pseudomonas aeruginosa* is a gram negative gamma-proteobacteria which belongs to the family *Pseudomonaceae*. It causes bacteremia, pneumonia, folliculitis, swimmer ear which is an ear infection accompanied with swelling, ear pus. Itching, discharge and difficulty in hearing, eye inflammation with associated pains, pus, swelling redness and impaired vision. *Klebsiella*, a non-motile gram negative, oxidase rod shaped bacteria which causes infectious wounds, pneumonia, blood stream infections and urinary tract infections[21, 12]

GC-MS ANALYSIS

The results obtained in the GC-MS analysis of the plant extract are contained in fig (1) below and interpreted in table 4. Similarly the structures of the compounds are also enlisted in fig 2

Fig 1. GC/MS spectrum of the leaf extract of *Rauwolfia vomitoria*.

File :C:\msdchem\1\data\AUGUST2019\080819\5790.D
 Operator : PRINCE USIN
 Acquired : 8 Aug 2019 16:59 using AcqMethod PHYTOCHEMICAL ANALYSIS.M
 Instrument : 5975 7890
 Sample Name: GPE 19359-03
 Misc Info : PLANT EXTRACT
 Vial Number: 8



The GC-MS analyses of the spectra of *Rauwolfia vomitoria* revealed 34 absorption Peaks .Peak 1 occurred at m/z 132 with the molecular formula $C_{10}H_{12}$ and identified as 1- phenyl-1-butene. Peak 2 occurred at m/z 188 with the molecular formula $C_{12}H_{10}O_2$ and identified as 2-Naphthalenol,1,2-dihydro,-acetate. Similarly, other peaks are interpreted as follows; peak 3, m/z 190, molecular formula $C_{12}H_{10}O_2$ and named Oxacyclotetradeca-4,11-diyne. Peak 4 , m/z 146 , molecular formula of $C_{13}H_{18}O$ and name, 1H- indene,-2,3-dihydro-4,7-dimethyl. Peak 5 , m/z 142, molecular formula, $C_{11}H_{10}$,name, Naphthalene ,2-methyl . Peak 6 , m/z 196, molecular formula $C_{14}H_{30}$ name, 7-tetradecane Peak 7 , m/z 198 , molecular formula, $C_{14}H_{30}$ name ,Tetradecane. Peak 8 , m/z 240 , molecular formula , $C_{17}H_{36}$ name, Tetradecane,2,6,10,-trimethyl. Peak 9 , m/z 206 , molecular formula $C_{14}H_{34}O$, name, Phenol,2,4-bis(1,1-dimethyl). Peak 10 m/z 242 , molecular formula $C_{16}H_{34}O$ name,1-hexadecanol. Peak 11, m/z 226 with the molecular formula $C_{16}H_{34}$, name, Hexadecane. Peak 12, m/z 194 , molecular formula $C_{12}H_{18}O_2$ name,5-isopropenyloxymethyl-3,3-dimethyl-cyclohexane. Peak 13, m/z 302, molecular formula $C_{16}H_{18}N_2O_2S$ name,1(1-phenylsifonylminoethyl). Peak 14 , m/z 278 , molecular formula, $C_{20}H_{38}$, name, Decane,5,6,-bis(2,2-dimethylpropylidene)

Peak 15 , m/z 266 , molecular formula, $C_{19}H_{28}$,name,1-nonadene. Peak 16 , m/z 217 , molecular formula, $C_{14}H_{28}O$ name Tetradecanal. Peak 17 , m/z 296 , molecular formula, $C_{20}H_{40}O$, name, 3,7,11,15-tetramethyl-2-hexadecan-1-ol. Peak 18 , m/z 256, molecular formula $C_{16}H_{32}O_2$ name, n-hexadecanoic acid. Peak 19 , m/z 296 , molecular formula $C_{20}H_{40}O$ name,phytol. Peak 20, m/z 282, molecular formula $C_{18}H_{34}O_2$ name, Cis-vaccenic acid. Peak 21, m/z 312 ,molecular formula, $C_{20}H_{40}O_2$, name, Eicosanoic acid. Peak 22 , m/z 390, molecular formula $C_{24}H_{38}O_4$, name, 1,2-benzene,carbonxylic acid. Peak 23 m/z 268, molecular formular, $C_{16}H_{28}O_3$, name. Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate. Peak 24 , m/z 165 , molecular formula, $C_{30}H_{50}$, name, squalene. Peak 25 ,m/z 394 ,molecular formula, $C_{28}H_{58}$,name, octacosane. Peak 26 , m/z 430 , molecular formula, $C_{29}H_{50}O_2$,name, vitamin E. Peak 27, m/z 380 , molecular formula $C_{27}H_{56}$, name, heptacosane. Peak 28 , m/z 400 , molecular formula $C_{28}H_{48}O$, name, campesterol. Peak 29, m/z 414, molecular formula, $C_{29}H_{50}O$,name γ -sitosterol. Peak 30, m/z 424 , molecular formula, $C_{30}H_{48}O$, name, 4,4,6 α ,6 β ,7,8,8 β ,9,10,11,12,12 α ,14,14 α ,14 β -octadecanol. Peak 31, m/z 426, molecular formula, $C_{30}H_{50}O$, name, lupeol. Peak 32, m/z 468, molecular formula $C_{32}H_{52}O_2$, name,12-oleanen-3-ylacetate-3(α). Peak 33 ,m/z 468 , molecular formula, $C_{32}H_{52}O_2$,name, Lup-20(29)-en-3-ol,acetate-3(β) Peak 34 ,m/z 312 , molecular formula, $C_{20}H_{40}O_2$,name, Ethanol,2-(9-octadecenloxy),(Z)

Table 4: compounds obtained from fig1

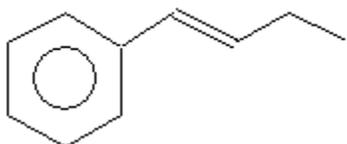
Compound	Chemical name	Molecular formular	Molecular weight
1	1-phenyl-1-butene	$C_{10}H_{12}$	132
2	2-Naphthalenol,1,2-dihydro,-acetate	$C_{12}H_{10}O_2$	188

3	Oxacyclotetradeca-4,11-diyne	$C_{13}H_{18}O$	190
4	1H-indene,-2,3-dihydro-4,7-dimethyl	$C_{11}H_{14}$	146
5	Naphthalene ,2-methyl	$C_{11}H_{10}$	142
6	7-tetradecane	$C_{14}H_{28}$	196
7	Tetradecane	$C_{14}H_{30}$	198
8	Tetradecane,2,6,10,-trimethyl	$C_{17}H_{36}$	240
9	Phenol,2,4-bis(1,1-dimethyl)	$C_{14}H_{34}O$	206
10	1-hexadecanol	$C_{16}H_{34}O$	242
11	Hexadecane	$C_{16}H_{34}$	226
12	5-isopropenyloxy methyl-3,3-dimethyl-	$C_{12}H_{18}O_2$	194

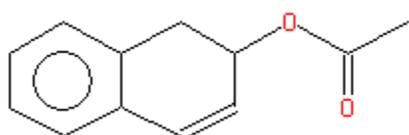
	cyclohexane		
13	1-(1-phenylsifonylaminoethyl)	C₁₆H₁₈N₂O₂S	302
14	Decane,5,6,-bis(2,2-dimethylpropylene)	C₂₀H₃₈	278
15	1-nonadene	C₁₉H₂₈	266
16	Tetradecanal	C₁₄H₂₈O	212
17	3,7,11,15-tetramethyl-2-hexadecan-1-ol	C₂₀H₄₀O	296
18	n-hexadecanoic acid	C₁₆H₃₂O₂	256
19	Phytol	C₂₀H₄₀O	296
20	Cis-vaccenic acid	C₁₈H₃₄O₂	282
21	Eicosanoic acid	C₂₀H₄₀O₂	312
22	1,2-benzene,carboxylic acid	C₂₄H₃₈O₄	390
23	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C₁₆H₂₈O₃	268

24	Squalene	$C_{30} H_{50}$	165
25	Octacosane	$C_{28} H_{58}$	647
26	Vitamin E	$C_{29} H_{50} O_2$	430
27	Heptacosane	$C_{27} H_{56}$	380
28	Campesterol	$C_{28} H_{48} O$	400
29	γ -sitosterol	$C_{29} H_{50} O$	414
30	4,4,6 α ,6 β ,7,8,8 β ,9,10,11,12,12 α ,14,14 α ,14 β – octadecanol	$C_{30} H_{48} O$	424
31	Lupeol	$C_{30} H_{50} O$	426
32	12-oleanen-3- ylacetate,3(α)	$C_{32} H_{52} O_2$	468
33	Lup-20(29)-en- 3-ol,acetate,3 β	$C_{32} H_{52} O_2$	468
34	Ethanol,2-(9- octadecenloxy), (Z)	$C_{20} H_{40} O_2$	312

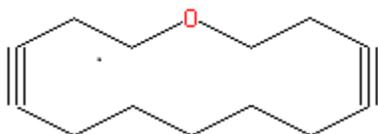
Fig 2. Structure of listed chemicals above in table



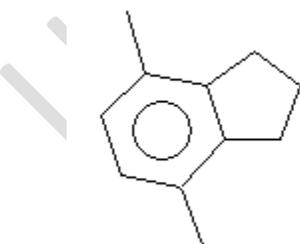
1. 1-phenyl-1-butene



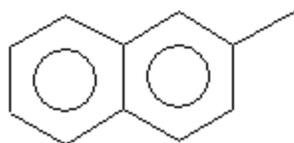
2. 2-Naphthalenol,1,2-dihydro,-acetate



3. Oxacyclotetradeca-4,11-diyne



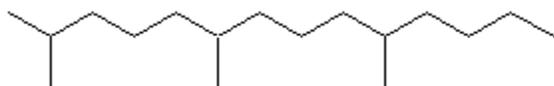
4. 1H-indene,-2,3-dihydro-4,7-dimethyl



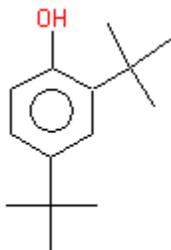
5. Naphthalene ,2-methyl



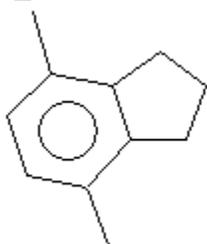
6. 7-tetradecene



7. Tetradecane



8. Tetradecane,2,6,10,-trimethyl,



9. Phenol,2,4-bis(1,1-dimethyl),

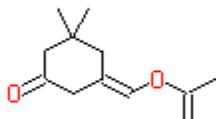


10. 1-hexadecanol

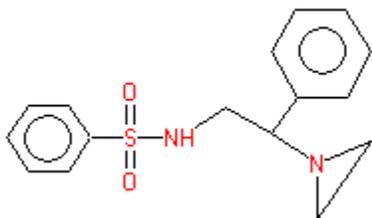


71

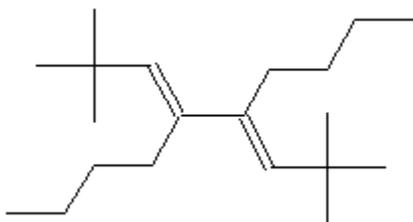
11. Hexadecane,



12. 5-isopropenyloxymethyl-3,3-dimethyl-cyclohexane



13. 1-(1-phenylisfonylaminoethyl)



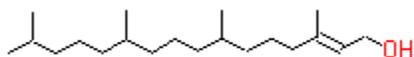
14. Decane,5,6,-bis(2,2-dimethylpropylidene)



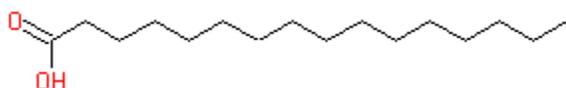
15. 1-nonadene



16. Tetradecanal



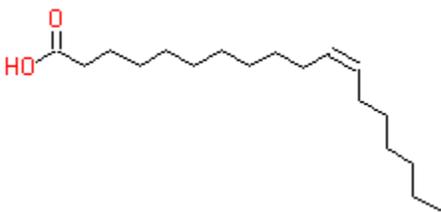
17. 3,7,11,15-tetramethyl-2-hexadecan-1-ol



18. n-hexadecanoic acid



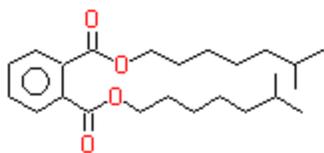
19 . Phytol



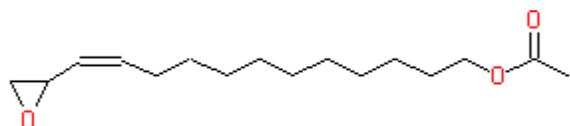
20. Cis-vaccenic acid



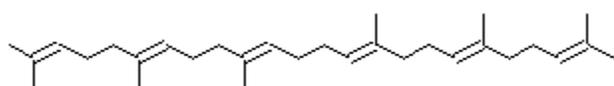
21. Eicosanoic acid



22. 1,2-benzene,carboxylic acid



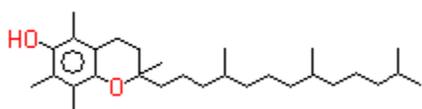
23. Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate



24. squalene



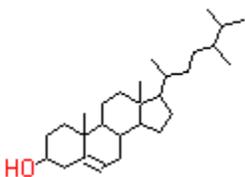
25. octacosane



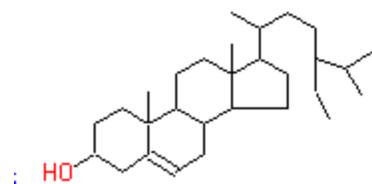
26. Vitamin E



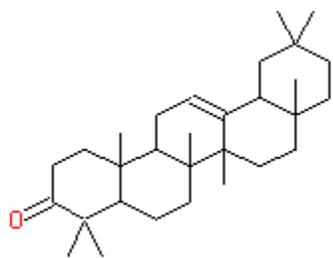
27. Hectacosane



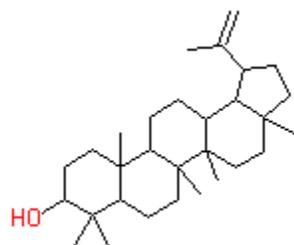
28. campesterol



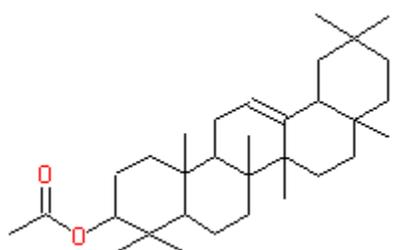
29. γ -sitosterol



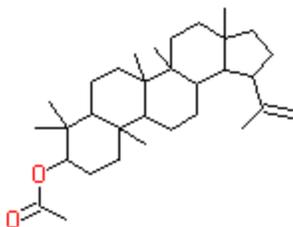
30. 4,4,6 α ,6 β ,7,8,8 β ,9,10,11,12,12 α ,14,14 α ,14 β -octadecanol



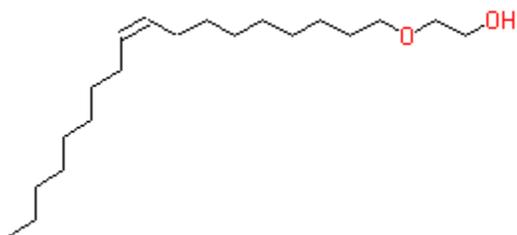
31. lupeol



32. 12-oleanen-3-ylacetate,3(α)



33. Lup-20(29)-en-3-ol, acetate, 3 (β)



34. 2-(9-octadecenloxy), (Z)

Most of these compounds found in *Rauwolfia vomitoria* have many pharmacological properties. lupeol, is a triterpenoid with strong anti-inflammatory and anti-cancer activity, the compound is also known as fagarsterol. its presence justifies the use of this plant in the treatment of cancer and inflammations [22] Squalene is a natural product that reduces skin damage by ultra violet radiation, it also reduces the cholesterol level in the blood and prevents cardiovascular diseases, having antitumor, anticancer against ovarian, breast, lungs and colon cancer. Phytol is a precursor of vitamin K1 and E. It is a schistosomicide drug. it is used in making synthetic vitamin E and K. It has antimicrobial, antioxidant anti-inflammatory cytotoxic effects. Vitamin E was detected in the extract. vitamin E is a group of eight fat soluble compounds that include four tocopherols and four tocotrienols.. Vitamin E functions for strong immunity and healthy skin and eyes. it is an antioxidant is believed to help in healing and reduction of scarring when applied to the skin. it is a lipid soluble component in the cell antioxidant defense system, it can serve to prevent ageing, cancer, arthritis and cataracts, it helps to reduce prostaglandins such as thromboxane

which causes clumping of platelets [23]. Sirosterol is a phytosterol used in lowering cholesterol level and improving benign prostatic hyperplasia, it reduces the risk of cancer and prevent coronary heart disease. Campesterol is a plant steroid that reduces the absorption of cholesterol in the body and balance cholesterol level in the body. It has anti-inflammatory properties making it useful against cardiovascular disease and arthritis as it inhibits pro-inflammatory compounds which are involved in arthritis, it can be used to treat benign prostate hyperplasia, fibromyalgia, allergies and sinusitis. Vaccenic acid is an isomer of oleic acid; it is believed to be useful in the treatment of cardiovascular diseases, cancer, inflammations and to enhance immune functions

CONCLUSION

The probe into the leaf of *Rauwolfia vomitoria* yielded excellent results as 34 compounds were obtained with their molecular masses, molecular weights and structures. The initial phytochemical screening revealed the presence of alkaloids, saponins, flavonoids and cardiac glycosides. The extract showed marked inhibition of the growth of certain human pathogens such as *Staphylococcus aureus*, *Klebsiella* specie, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Mucor* specie but was resistant to *Streptococcus* specie. Most of the compounds identified in this plant has anti-inflammatory, anticancer, antitumor, antioxidant and cardioprotective properties. These properties makes the plant an excellent ingredient for the treatment of cancer, tumor, inflammation, arthritis, benign prostate hyperplasia, fibromyalgia, allergies, coronary heart diseases, wound healing and sinusitis.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers

of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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