

**Phytochemical, Antimicrobial and Gc/Ms Analysis of the Root of *Stachytarpheta Cayennensis* (L. Vahl) Grown in Eastern Nigeria****Iwu Irenus Chinonye<sup>1</sup>, Onu Uchenna Lynda<sup>1</sup>, Ukaoma Adanna A<sup>3</sup>, Oze Rita .N<sup>1</sup>**<sup>1</sup>Department of Chemistry, Federal University of Technology Owerri Nigeria<sup>3</sup>Department of Biological Sciences Federal University of Technology Owerri Nig

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**ABSTRACT:** *Stachytarpheta cayennensis* is a plant full of phytonutrient, it has been applied by many traditional healers to treat host of diseases. Its full constituents have not been fully documented. The phytochemical screening of the root of this plant revealed the presence of alkaloids, tannins, saponins, glycosides, steroids and phenols. The antimicrobial analysis on selected human pathogens; *Streptococcus specie*, *Staphylococcus aureus*, *Klebsiella*, *Proteus specie* and *Pseudomonas specie* showed that the extract was only sensitive to *Proteus specie* having a diameter of inhibition of 4mm at concentration of 240mg/cm<sup>3</sup> and minimum inhibition concentration of 120mg/cm<sup>3</sup>. The spectrum obtained from the GC/MS analysis showed ten peaks at M/z 128, 220, 242, 256, 252, 270, 256, 296, 282 and 281 corresponding to molecular formulas of C<sub>10</sub>H<sub>8</sub> for Cyclopentyl cycloheptene, C<sub>15</sub>H<sub>24</sub> for Butylated hydroxyl toluene, C<sub>16</sub>H<sub>32</sub>O for Hexadecanoic acid, C<sub>16</sub>H<sub>28</sub>O<sub>2</sub> for 11-tetradecyl-1-ol acetate, C<sub>17</sub>H<sub>38</sub>O<sub>2</sub> for Hexadecanoic acid methyl ester, C<sub>16</sub>H<sub>32</sub>O<sub>2</sub> for n-hexadecanoic acid C<sub>19</sub>H<sub>34</sub>O<sub>2</sub> for 9-octadecenoic acid methyl ester, C<sub>18</sub>H<sub>34</sub>O<sub>2</sub> for octadec-9-enoic acid and C<sub>18</sub>H<sub>35</sub>NO for 9-octadecenamide respectively. 9-octadecenamide (Oleamide) is useful for the treatment of insomnia. Oleamide accumulates in the cerebrospinal fluid during sleep deprivation and thus induces sleep in animals. It may be a potential medicinal treatment for mood and sleep disorders

**KEYWORDS:** *Stachytarpheta Cayennensis*, Phytochemicals, Phytonutrients, Human Pathogens

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**INTRODUCTION**

Medicinal plant abounds in the tropical areas of Nigeria. Most of these plants have been neglected for years as no adequate research into their chemical constituents and usefulness had been carried out. Our team has therefore decided to investigate and elucidate the chemical component and usefulness of most of these plants. *Stachytarpheta cayennensis* is a common plant growing in the rain forest area of Nigeria, it belong to the family Verbenaceae. Its common names include; Gervao, Brazilian tea, Rooter comb, Blue porters weed, Snake weed etc. Siju et al (2016). It is among the species known as snake weeds, Verbenaceae. This plant is rich in phytonutrients and has been implicated in the treatment of inflammation. Its pharmacological properties includes antinflammatory, antihelmintic, diuretic, lazative, lactogogue, purgative, sedative, spasmogenic, vomifuge, vasodilatory, vulnerary and cooling tonic for stomach. Okwu and Ohehen (2010). Other members of the Verbenaceae family include *stachytarpheta jamaicensis* and *stachytarpheta australis*. These plants invade disturbed areas such drainage channels, roadsides, overgrazed areas and monsoon forest. It is a perennial clumping shrub that grows to 2m with tough stems and woody rootstock. Shallow tooted leaves with hairy underside flowers which can range from purple to violet, dark or pale blue to almost

white with a long stiff snakelike stem. Seeds are dark brown or black and measure up to 5 mm long. The snake weed family looks alike but Idu et al (2007) had differentiated between *S. Cayennensis* and *S. jamaicensis*. The leaves of *S. cayennensis* have angular stem and pubescence leaves but *S. jamaicensis* has smooth circular stem glabrous leaves. There is the presence of trichomes in *S. cayennensis* but absent in *S. jamaicensis*, the medicinal applications of *S. cayennensis* are numerous

*Stachytarpheta cayennensis* has shown antiplasmodial activities against *Plasmodium berghei berghei*. Ezeayi et al. Studies by Penido et al (2006) revealed the anti inflammatory and ulcerogenic properties of *S. cayennensis*. They were able to provide evidence for the anti inflammatory and gastriprotective properties of the plant which includes its anti ulcerogenic properties. Okoye et al (2010) reported the anti microbial and antispasmodic activities of the leaf of the plant. Their work seems to provide evidence for the use of the plant in wound healing and gastrointestinal ulceration treatment. Olayiwole and Ibikunle (2013) reported the antipsychotic effect of the leaves of *S. cayennensis*, their result gives credence to the use of the extract in treatment of mental illness. Ramanuj et al (2014) had reported the anti tyrosinase activities of *S. cayennensis* and showed that the plant can be used as a skin whitening and anti-ageing agent. Most plants found in Africa have one or more anti malaria properties. Talkmore et al (2015) studied the use of twenty different plant samples in the treatment of malaria in Zimbabwe.

Other members of the Verbenaceae family are receiving wide reaching attention. Ezeabara and Ezeh (2015) studied different parts of *stachytarpheta augustifolia* and concluded that the plant is full of phytonutrients. Dickson et al had also proven that apart from *S. cayennensis*, *margaritaria discoidea* is useful in wound healing. Bangou et al (2012) studied the antioxidant and antibacterial activities of five verbenaceae species and concluded that they had excellent antimicrobial properties. Souza silva et al (2012) studied the hexane extract of *S. gensnerioides* and reported that the specie had good antimicrobial potentials Alkaloid, phenolic compounds, tannins, saponins, flavonoids has been reported in *S. jamaicensis* by Silvaranjanl et al (2013)

## MATERIALS AND METHODS

**Sample Collection** the root of *Stachytarpheta cayennensis* were obtained in Eziobodo community in Owerri West L.G.A of Imo state, Nigeria. The roots were chopped into small pieces and washed with tap water to get rid of sand particles. They were then room dried for a period of one month before milled into powder using a milling machine. The milled sample was then stored in airtight bottles till required for analysis Iwu et al (2018)

### Frothing test for Saponins

This test is based on the ability of the saponins to produce froth in aqueous solution. 5g of the plant extract was weighed into a test tube and 50cm<sup>3</sup> of water was added and extracted after two hours. The water extract was shaken vigorously in a conical flask. The production of a stable froth indicates the presence of saponins in the sample

**Test for Flavonoids**

5g of the sample was soaked with 20cm<sup>3</sup> of water and then filtered and to the filtrate drops of ammonia and 3cm<sup>3</sup> of concentrated H<sub>2</sub>SO<sub>4</sub> was added. A yellow precipitate which disappears on storage indicates the presence of flavonoids.

**Test for Alkaloids**

5g of the sample was extracted using 20% acetic acid in ethanol .5cm<sup>3</sup> of the extract was treated with Wagner's reagent (iodine crystals and KI). A yellowish brown precipitate indicates the presence of alkaloids.

**Test for Tannins**

5g of the root sample was weighed into a beaker and 50cm<sup>3</sup> of water was added and allowed to soak properly for two hours and extracted. Te extract was treated with drops of ferric chloride. A blue-black precipitate indicates the presence of tannins.

**Test for Steroids**

5mcm<sup>3</sup> of the water extract was treated with concentrated H<sub>2</sub>SO<sub>4</sub> in acetic anhydride. The formation of a blue-green color indicates the presence of steroids.

**Test for Phenols**

20cm<sup>3</sup> of the water extract was treated with 5cm<sup>3</sup> of concentrated sulphuric acid and drops of sodium nitrate (NaNO<sub>3</sub>). 2cm<sup>3</sup> of sodium hydroxide was added to the mixture. A blue precipitate indicated the presence of phenols.

**Test for Glycosides**

20cm<sup>3</sup> 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.

**Preparation of Samples for GC-MS Analysis**

Two hundred grams of sample was soaked in ethanol for 48 hours and then extracted. The extract was 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-MS analysis.

**GC-MS Experimental Procedures**

GC-MS 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 cm<sup>3</sup>/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 50m/min. The eluents

were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5kv and sampling rate of 0.2 seconds. 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 Iwu et al (2016, 2018)

**Antimicrobial Analysis** The microorganisms; *Staphylococcus aureus*, *Streptococcus spp*, *Klebsiella spp*, *Proteus spp* and *Pseudomonas spp* were used for the analysis. They are clinical isolates of human pathogens obtained from the Federal Medical Centre Umuahia and were brought to the laboratory and resuscitated in buffered peptone broth (Secharian chemie) and thereafter into nutrient agar medium and incubated at 37°C for 24 hrs. Iwu et al (2018)

### **Antibacterial Assay**

The test solution of each extract was prepared by dissolving 0.1 g of the plant extract separately in 1.0cm<sup>3</sup> of dimethyl sulphoxide (DMSO) to get a concentration of 100mg/cm<sup>3</sup>. The antibacterial activity was performed by filter paper disc diffusion technique. Filter paper disc (Whatman No 1.6 mm diameter) were placed in glass petridishes and sterilized in hot air oven. Iwu and Onu (2018) the media (10g nutrient Agar in 200cm<sup>3</sup> distilled water, autoclaved at 115°C for 30 minutes) was cooled to 50°C. The sterile nutrient Agar media were poured into the sterile petridish and allowed to solidify. The bacteria were swabbed with a sterile wire loop. Each disc was impregnated with 0.2cm<sup>3</sup> of plant extract. Standard antibiotic Ciprofloxacin was used as a control on a disc with DMSO 100mg/cm<sup>3</sup>. The discs were used after drying them in an incubator at 40°C to remove any trace of solvent. Discs were introduced into the surface of the medium. The plates were microbated at 37°C for 24 hours to obtain zones of inhibition. The experiments were repeated three times for each extract and twice for reference antibiotics to minimize error and the average of these values were recorded.

### **Minimum Inhibitory Concentration (MIC).**

The minimum inhibitory concentration of the extract was determined by incorporating constant volume 0.2cm<sup>3</sup> of each diluents of the extract into the perforated disc on a seeded nutrient agar plate as described in the anti microbial susceptibility test section . 0.1g of each extract was dissolved in 1cm<sup>3</sup> of DMSO to obtain 100mg/cm<sup>3</sup>. This concentration of DMSO was then doubled to obtain 50mg/CM<sup>3</sup> then doubled again to obtain 12.5mg/cm<sup>3</sup> and again to obtain 6.25mg/cm<sup>3</sup>. Each concentration was then used in the method earlier described to obtain zone of inhibition. The least concentration that showed inhibitory zones was taken as the MIC.

**RESULT AND DISCUSSION**

The results of the phytochemical screening of the plant are presented below.

**Table 1. phytochemical screening of *Stachytarpheta Cayennensis***

Phytochemical	Inference
Alkaloid	+
Saponis	+
Flavonoids	-
Tannins	+
Steroids	+
Glycosides	+
Phenols	+

Results of phytochemical screening (Table 1) of *Stachytarpheta cayennensis* revealed the presence of important bioactive plant secondary metabolites. Alkaloids, tannins, flavonoids, steroids, glycosides and phenols. Flavonoid test was negative. The contribution of these compounds to health of humans cannot be overstated. The root of *Stachytarpheta cayennensis* contained alkaloids. Alkaloids rank among the most efficient therapeutically significant plant substance. Pure isolated alkaloids and their synthetic derivatives are used by ethnomedicinal practitioners for their analgesic, antispasmodic and bactericidal effects. They exhibit marked physiological activity when administered to animals; the high alkaloid content of *Stachytarpheta cayennensis* may be the reason for its use in the treatment of cough, wounds, and malaria, rheumatism and skin infections. Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever Iwu et al (2016)

Saponins were found to be available in the crude extracts; the saponin content fortifies the use of the extract from this plant in the treatment of wounds. Some of the general characteristic of saponins includes formation of foams in aqueous solutions, hemolytic activity and cholesterol binding properties .Iwu et al (2016) .Saponin has the natural tendency to ward off microbes and this makes them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotic, helping the body to fight infections and microbial invasion.

The roots of *Stachytarpheta cayennensis* also contained an appreciable amount of phenolic compounds. There is a growing interest in polyphenolic compounds as therapeutic agents against many diseases such as cardiac and cerebral disorders, arteriosclerosis and rheumatic or pulmonary diseases. The activated phagocytic cells are known to produce potentially destructive oxygen species like super oxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and Hypochloric acid (HOCl) during chronic inflammatory disorder. Many polyphenolics are known to exhibit antioxidant properties, they are free radicals scavengers. Phenolic flavonoids are also excellent hydroxyl scavengers. These properties promotes health and prevents certain chronic disorders such as cancer, cardiovascular diseases, diabetics and arthritis .The presence of phenols means that these extracts could act as anti-inflammatory, anti-clotting, antioxidants, immune system enhancers and hormone modulators. Phenols have been the subject of extensive research as disease preventives. They have the ability to block specific enzymes that causes inflammations. They modify the prostaglandin pathways and thereby protect platelets from clogging.

The crude extracts of the root of *Starchytarpheta cayennensis* was found to contain tannins. Tannins have astringent and antimicrobial properties, hastening the healing of wounds and inflamed mucous membrane. The presence of Tannins in these samples supports their use in treating wounds, various ulcers, hemorrhoids, frost bites and burns in herbal medicine.

Steroids were also found present in the phytochemical analysis of the root extracts. Steroids have cholesterol nucleus and are associated with reproductive hormone. Okwu and Ohehen (2010) isolated steroidal glycosides from the leaves of *stachytarohete jamaicensis*. Glycosides were also present in the extracts. Most glycosides are stored in plants as inactive chemicals and can be activated by enzyme hydrolysis which breaks off the sugar part thus releasing the chemical for use by the plant. Poisons are usually bound to glycosides as part of their elimination from the body. Glycosides have sugar part (glycone) bonded to a non-sugar (aglycone) part. Alcoholic glycosides have analgesic, antipyretic, and anti-inflammatory effects. Anthraquinone glycosides act as a laxative. Also flavonoid glycosides are known for their antioxidant effects and decrease capillary fragility. Phenolic glycosides are known to have urinary antiseptic effect while steroidal glycosides are used to treat heart diseases. Thioglycosides as the name implies contain sulphur.

**Table 2 Results of the antimicrobial activities of *Starchytarpheta cayennensis*.**

Sample	Concentration of root extract (mg/cm <sup>3</sup> )	Diameter of zone of inhibition (mm)				
		<i>Staphylococcus aureus</i>	<i>Streptococcus</i> species	<i>Klebsiella</i> species	<i>Proteus</i> species	<i>Pseudomonas</i> species
Ethanollic extract	240	-	-	-	4	-
Ethanollic extract	120	-	-	-	2	-
Ethanollic extract	60	-	-	-	-	-
Ethanollic extract	30	-	-	-	-	-
LEV	0.02	20	12	20	10	8
S	0.03	8	4	20	8	6

LEV= Levofloxacin, S = Streptomycin

The root extract showed inhibition of the growth of gram negative *Proteus* species. At 240mg/cm<sup>3</sup> the positive *Staphylococcus aureus*, gram negative *Streptococcus* species, gram negative *Klebsiella* species and gram positive *Streptococcus* species were not inhibited. Standard drugs were used as control. Streptomycin and Levofloxacin inhibited all the

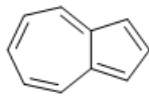
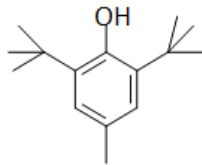

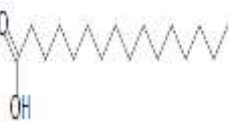
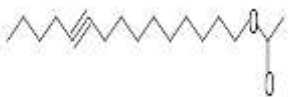
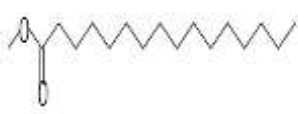



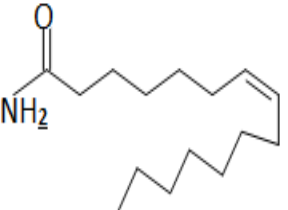
pathogens at very low concentrations of  $0.03\text{mg}/\text{cm}^3$  and  $0.02\text{mg}/\text{cm}^3$ . The minimum inhibition concentration (MIC) of the extract was  $120\text{mg}/\text{cm}^3$ . Though the root extract was not so effective with these pathogens, but it may still have marked effects on other pathogens not enlisted in this study

**Table 3 Sensitivity test for standard antibiotic drugs against pathogens used to evaluate the inhibitory properties of *Starchytarpheta cayennensis***

Antibiotics	Staphylococcus aureus	Streptococcus species	Klebsiella species	Proteus species	Pseudomonas species
CPX	+++	++	++	++	+
NB	+++	-	+	+++	-
GN	++	-	+++	++	-
AMX	-	-	++	++	-
S	+++	+	+++	++	++
RD	+	+	+++	+	++
E	++	-	-	+++	+
CH	-	-	+++	++	+++
APX	-	-	+++	-	-
LEV	+++	+++	+++	+++	+++

CPX = Ciproflox; NB = Norfloxacin; GN = Gentamycin; AMX = Amoxil; S = Streptomycin; RD = Rifampicin; E = Ethromycin; CH = Chloramphenicol; APX = Ampiclox; LEV = Levofloxacin

**Table 4.3: Sensitivity test for standard antibiotics on test pathogens.**

Chromatographic peak	Name of compound	Molecular formula	Molecular weight(g)	Retention time (s)	Structure
1	Cyclopentacyclopentene	$C_{10}H_8$	128	10.125	
2	Butylated Hydroxy Toluene	$C_{15}H_{24}O$	220	15.292	
3	Methyl Tetradecanoate	$C_{15}H_{30}O_2$	242	19.017	
4	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.833	
5	11-Tetradecyn-1-ol acetate	$C_{16}H_{28}O_2$	252	20.292	
6	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	21.183	
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.783	
8	9-Octadecenoic acid methyl ester	$C_{19}H_{34}O_2$	296	22.592	
9	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	23.133	
10	9-Octadecenamide	$C_{18}H_{35}NO$	281	24.525	

Staphylococcus aureus was resistant to

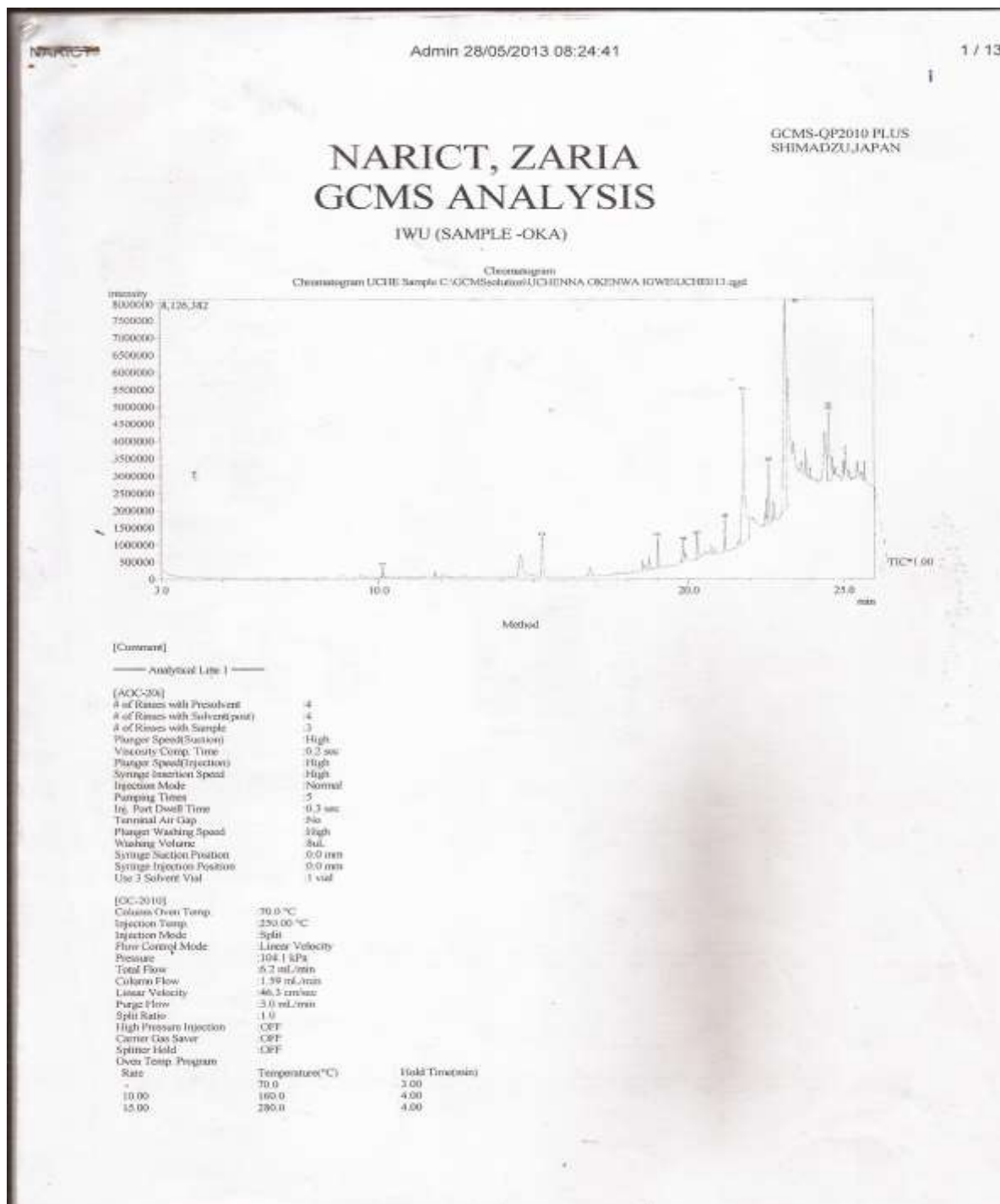


An antibiotic sensitivity test is usually done to help choose the antibiotic that will be most effective against specific types of bacteria or fungus infecting an individual. Standard drugs were used to inhibit all the pathogens in table 4.3 above and some were found to be very effective in inhibiting all strains of the bacteria amongst which were Ciproflox, Amoxil, Rifampicin and Levofloxacin. Thus were used as control in the antimicrobial test carried out.

The antimicrobial sensitivity test table 3 revealed the resistance of the selected pathogens against some common antimicrobial drugs. Just like the extracts most of his pathogens were not sensitive to the antimicrobial drugs.the pathogens Streptococcus and Klebsiella had grown resistant to Ethromycin. Similarly drugs such as Norfloxan,Gentamycin,Amoxilampiclox and Chloramphenicol were of no value in the situation of infections streptococcus species our extract tend to be a better drug when compared to Ampiclox in the treatment of infections resulting from Proteus specie.

The GC/MS analysis of the extract revealed ten chromatographic peaks, these peaks represented ten different compounds as shown in fig 1 belo

**Figure 1: GCMS scan for the chloroform extract of *Starchytarpheta cayennensis* root**



The GCMS analysis on the roots of *Starchytarpheta cayennensis*, revealed ten absorption peaks. These peaks are interpreted in (Table4). Peak 1 occurred at m/z

128 corresponding to the molecular formula  $C_{10}H_8$  and is identified as Azulene, an aromatic. Azulene is an organic compound and also an isomer of naphthalene. Azulene is a dark blue pigment. Also two terpenoids having the azulene skeleton are found in nature as constituents of pigments in mushrooms. Peak 2 appeared at m/z 220 with molecular formula  $C_{15}H_{24}O$  and identified as Butylated hydroxytoluene, a derivative of phenol. It is useful due to its antioxidant properties and is mostly used as food additives, in pharmaceuticals, cosmetics and as fuel additive. Peak 3 appeared at m/z 242 with molecular formula  $C_{15}H_{30}O_2$ , and identified as Methyl tetradecanoate (myristic acid), an ester. Peak 4 occurred at m/z 256 with the formula  $C_{16}H_{32}O_2$ , and identified as Hexadecanoic acid (Palmitic acid), a saturated fatty acid. It has been used extensively in cosmetics, to produce soaps and as a natural additive in organic products. Recently, a long-acting antipsychotic medication, Paliperidone palmitate (marketed as INVEGA Sustena) used in the treatment of schizophrenia has been synthesized using the oily palmitate ester as a long-acting release carrier medium when injected intramuscularly. Peak 5 occurred at m/z 252 with the formula  $C_{16}H_{28}O_2$  and named as 11-tetradecyl-1-ol acetate, a fatty acid. Peak 6 appeared at m/z 270 with formula  $C_{17}H_{34}O_2$  and named Hexadecanoic acid methyl ester also a fatty acid ester. Peak 7 occurred at m/z 256 and its formula is  $C_{16}H_{32}O_2$ , and is named n-Hexadecanoic acid. Peak 8 occurred at m/z 296 with and was identified as 9-Octadecenoic acid methyl ester, which is also an unsaturated fatty acid ester. Peak 9 appeared at m/z 282 with molecular formula  $C_{18}H_{34}O_2$  and named Octadec-9-enoic acid, an unsaturated fatty acid. Peak 10 occurred at m/z 281 with the molecular formula  $C_{18}H_{35}NO$  and was identified as 9-Octadecenamide (Oleamide), an amide. The presence of this compound proves the use of the roots of *Starchytarpheta cayennensis* in the treatment of sleeping disorders. This is so because oleamide accumulates in the cerebrospinal fluid during sleep deprivation and thus induces sleep in animals. It has been studied as a potential medicinal treatment for mood and sleep disorders. Olayiwole et al (2013) The presence of fatty acids, aromatics, ketones and esters boosts the pharmacological properties of this plant. Fatty acid and alcohols in plants react to produce esters. One or both oxygen atoms in the plant can be replaced by sulphur giving a thio acid or dithio acid respectively. Thio acids react readily with alcohols to form thio ester. Thio esters play important role in the break down and synthesis of lipids and steroids in living tissues. Carboxylic acids are transferred from one enzyme reaction to another as thio esters of the complex thiol, Coenzyme A (CoA-SH). The thio esters of benzoic acid with Co-enzyme A is the form in which acetate esters enter the sequence of enzyme catalyzed reaction which results in the synthesis of fatty acids and glycerides.

## CONCLUSION

This work has opened a door way into studies on the root of *stachytarpheta cayennensis*, revealing the phytochemicals present, the antimicrobial properties and the compositions of its essential oils. It is obvious that the root extract has activity against *Proteus* specie, but was not sensitive to the other organism at the concentration used for the analysis; one thing that is certain is that the root extract could have remarkable activities against certain other microbes not studied in this work. The GC/MS result revealed vital chemical substances. One important compound identified is 9-octadecenamide (oleamide) a sleep inducing compound and has anti

epilepsy properties. We could understand why traditional medical healers use the plant extract to treat insomnia and mental disorder this study has thrown more light into the use of different parts of *S. cayennensis* for the treatment of insomnia and mental disorder. It is worthy to note that every part of this plant has a useful pharmacological application.

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