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GC-MS Profile, Spectrophotometric Determination of Antioxidant Activity, Total Phenolic and Flavonoid Contents of Leaves of *Cochlospermum Planchonii*

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ABSTRACT

Cochlospermum planchonii is a multipurpose traditional medicinal plant that is found in most tropical regions of the world. The objectives of this research were to identify the class and type of phytochemicals present, quantify the total phenolic and flavonoid contents, and determine the antibacterial and antioxidant activity of the plant leaves extract. This was achieved by extracting with two solvents of very different polarity (n-hexane and methanol). Harborne's method was used for the identification of the class of phytochemicals while GC-MS was used to identify the type of phytochemicals. Folin-Ciocalteau method was used to determine the total phenolic content while aluminum colorimetric assay was used to determine the total flavonoid content. Kirby-Bauer disc diffusion method was used for the antibacterial assay while DPPH was used to evaluate the antioxidant activity. The phytochemical screening showed presence of flavonoids, saponins, tannins, terpenoids and glycosides while terpenoids, alkaloids and anthraquinones were absent in the methanol extract. Steroids and terpenoids were present in the n-hexane extract while saponins, alkaloids, anthraquinones and glycosides were absent. The total phenolic and flavonoid contents obtained were 97.48 ± 0.42 mg Gallic acid equivalent/g extract and 161.85 ± 0.37 mg quercetin equivalent/ g extract respectively. Several phytochemicals were identified using GC-MS. The most abundant in the methanol and n-hexane extracts was n-hexadecanoic acid (9.90%) and octacosane (24.24%) respectively. The observed DPPH scavenging activity was 66.73 ± 0.26% while extract reduced Fe^{3+} ferricyanide complex to the ferrous form (Fe^{2+}). The methanol extract was active against *Staphylococcus aureus*, *Salmonella typii*, *Streptococcus pyogenes*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The leaves of *Cochlospermum planchonii* is rich in phenolics and flavonoids and possess high antioxidant activity. It also exhibits antibacterial activity against some human pathogens.

Keywords: *Cochlospermum planchonii*, Phenolics, flavonoids, Antioxidant, antibacterial, GC-MS

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INTRODUCTION

Humans have always relied on plants for the maintenance of health, relief of symptoms and treatment of diseases. Many authors have published research works on the efficacy of traditional medicinal plants in treating infections and diseases and as such are considered as the bedrock for modern medicine (Evans et al., 2002)[1]. Despite great advancements in modern medicine, medicinal plants still play major roles in primary healthcare programs of many nations, for example, China and India have well developed traditional medicinal practices. Examination of phytochemical constituents present in medicinal plants would aid in the determination of the biological activities of these plants. Some medicinal plants have antioxidant property which contribute to their use in the prevention and protection against diseases. Some particular phytochemicals present in a medicinal plant possess the ability to scavenge free radicals and exhibit antimicrobial activity. The increase of free radicals at cellular level leads to DNA damage, protein oxidation and lipid peroxidation resulting in cell death via apoptosis or necrosis. The antioxidants have the ability to donate hydrogen atom to a free radical and also chelate metal ions such as iron and copper which is an important pharmacological activity. They can also upregulate antioxidant enzymes. Govid, (2015)[2] proposed that the use of medicinal plants with high level of antioxidant constituents will be effective in the treatment of hepatic damages. Most synthetic antimicrobials are considered as almost ineffective and have various side effects. The emergence and spread of multidrug resistant microbes is a huge threat in the treatment of microbial infections. About 700,000 lives are lost annually due to antimicrobial-resistant infections. The impact of Antimicrobial resistance is worse in most under developed nations (the review). Many countries have developed programs with the aim of developing new effective and safe antimicrobials (Rocal et al., 2015)[3]. Medicinal plants are sources of pharmaceutical substances which can serve as lead compounds (Lifongo et al., 2014)[4] and only a small percent has been systematically studied for their antimicrobial use out of about 4,000,000 plant species that has been recorded (Selvamohan et al., 2012)[5].

Cochlospermum planchonii is a member of Cochlospermaceae. It is found in most tropical regions of the world. It has very bright yellow flowers that are about 50 cm in height. This plant is used in the treatment of infertility, diabetes, premenstrual pain, management of jaundice (Igoli et al., 2005)[6], elimination of worms, bilharzia, hepatitis (Blench, 2007)[13]; malaria (Banoit-Vical, 2003)[7]; CNS depressant, analgesic and anti-inflammatory (Arul et al., 2009)[8]. The objectives of this research were (i) to identify the phytochemicals present, (ii) to quantify the total phenolic and

flavonoid contents, (iii) to estimate the antibacterial activity against some human pathogens and (iv) to investigate the antioxidant capacity of the leaves of *Cochlospermum planchonii*.

MATERIALS AND METHOD

Preparation of plant material and extracts

The Whole plant was identified by a traditional health practitioner in Omu aran by its local name. Then this name was located in the book Yoruba names of Nigeria plants by written Shola and Gbile, (2002). The plant leaves were taken to Landmark University, Omu aran and a voucher specimen deposited at the department of biological sciences. The leaves were dried under laboratory conditions for two weeks. The dried leaves were pulverized into fine powder using a food blender. 50 g of the powdered leaves was extracted with n-hexane and methanol using a Soxhlet extractor. The extracts were concentrated by distilling off the solvent and finally evaporated to dryness under vacuum. The extracts were stored at 4 ^{o}C until analysis.

Phytochemical analysis

Phytochemical screening

The plant extracts were screened for presence of flavonoids, saponins, steroids, terpenoids, alkaloids, glycosides, tannins and anthraquinones using the method described by Harborne, (2005).[11]

GC-MS analysis

The GC- MS analysis of the extracts was performed on GC–MS (7890A/5975C) Agilent, USA. The equipment has a triple axis detector coupled with an auto injector. The operation conditions for the gas chromatograph are as follows: capillary (Agilent 19091J-433:3516.15684) column length – 30 m, internal diameter of column – 0.25µm, thickness – 350 µm, ion source temperature – 250 ^{o}C, interface temperature - 300 ^{o}C, pressure – 1.342 psi, injector size 1 µL which is in splitless mode, injection temperature – 300 ^{o}C. The column temperature was gradually increased from 35 ^{o}C to 150 ^{o}C at a rate of 4 ^{o}C/ min for 2 min and further increased to 250 ^{o}C at a rate of 20 ^{o}C/min for 5 mins. The total run time was 45.75 mins. The MS scan was from low mass of 50 to high mass of 750.

Total flavonoid content

To 1 ml of a dilute solution of the methanol extract (1mg/ml) was added 0.7 ml of 5 % (w/w) sodium nitrite and 10 ml of 30% (v/v) ethanol and stirred for 5 mins. Then 0.7 ml of 10% Aluminum chloride (w/w) was added stirred for 6 mins and 5 ml of 1M sodium hydroxide added to it. The mixture was later diluted with 25 ml of 30% (v/v) ethanol, stirred properly and allowed to stand for 10 mins prior to measurement. The absorbance was measured at 500 nm using a

spectrophotometer (Genseys 10S vl.200 217H 311 008). The total flavonoid content was calculated from a calibration curve and the result expressed as quercetin equivalent per g extract. The samples was analyzed in triplicates.

Total phenolic content

5 ml of 50% methanol was added to 5 mg of the methanol extract and mixed properly using a vortex mixer. 0.5 ml of this solution was pipetted into a test tube and 3.5 ml distilled water and 0.25 ml of Folin-Ciocalteau reagent were added to it. The mixture was incubated for 8 mins and room temperature. Then 0.75 ml of 20% sodium carbonate was added and left to incubate for 2 h. The absorbance was measured at 765 nm against a reagent blank using a spectrophotometer (Genseys 10S vl.200 217H 311 008). The total phenolic content was expressed as mg Gallic acid equivalent/g extract from a calibration curve with Gallic acid. The analysis was carried in triplicates.

Antioxidant activity

DPPH assay

5 mg of the methanol extract was dissolved in 5 ml of methanol using a vortex mixer. Different dilutions of the sample were prepared with methanol to obtain concentrations of 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml. To each of these dilutions was added 2 ml of DPPH solution (prepared by dissolving 4mg of DPPH in 100 ml methanol) and left in the dark for 20 mins for colour development. The control contained methanol and 2 ml DPPH. The absorbance was measured at 517 nm against a reagent blank. Ascorbic acid was used as the standard or positive control. The ability of the extract to scavenge DPPH radical was determined from the equation:

$$\text{DPPH scavenging activity} = \frac{A_c - A_e}{A_c} \times 100$$

Where A_c – Absorbance of control, A_e – Absorbance of extract

Ferric reducing power

5 mg of the methanol extract was mixed with 5 ml of methanol to prepare a sample concentration of 1mg/ml. Different dilutions of this solution were prepared (100 -250 µg/ml) and mixed with 2.5 ml of 200 mmol/L phosphate buffer (pH =6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated for 20 mins at 50 ^{o}C for 20 mins. Then 2.5 ml of 10% trichoroacetic acid was added and centrifuged at 765 rpm for 10 mins. 5 ml of the upper layer was collected and mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. Ascorbic acid was used as the standard and the analysis was done in triplicates.

Antibacterial activity

Bacteria used

The bacteria used are *Staphylococcus* aureus, *Kliebsella pneumoniae*, *Salmonella typii*, *Escherichia coli*, *Shigella dysenteric*, *Streptococcus pyogenes*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. These bacteria were clinical isolates collected from Bowen teaching hospital, Ogbomoso, Oyo State, Nigeria.

Preparation of antibacterial medium

100 ml of distilled water was added to 2.8 g of nutrient agar and stirred for 5 mins. The dissolved nutrient agar was sterilized by placing it in an autoclave at a temperature of 121 ^{o}C for 15 mins. It was then cooled to 45 ^{o}C and poured into sterile petri dishes to solidify.

Preparation of test samples

1 mg of the extract was dissolved in 1 ml of dimethylsulphoxide. Then different dilutions (100 - 250 µg/ml) were prepared from this concentration and used for the analysis.

Disc diffusion test

Disc diffusion method by Bauer et al, 1966 was used for the analysis. Sterile Whatman № 1 filter paper was impregnated with the extract and placed on the surface of the nutrient agar plates which had been inoculated with the test bacteria. The plates were then incubated at 37^{o}C. The zones of inhibition of the test organisms were measured after 24 h. Streptomycin was used as the standard antibiotic. All analysis were carried out in triplicates.

Statistical analysis

Statistical analysis was performed on Microsoft excel 2013. The results are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Phytochemical screening of plant extracts is important in order to identify the class of compounds in which each phytocomponent belongs. This will also aid in eliminating certain tests or reactions that would have been carried out in determining bioactive components thereby saving time, cost of chemicals and energy. The result of the phytochemical screening of the methanol and n-hexane leaf extracts is presented in Table 1. The pharmacological effects of the plant leaves maybe due to the presence of the identified class of phytochemicals. These secondary metabolites possess multiple biological functions such as antioxidant, anti-inflammatory, antibacterial, etc. properties. According to Bamisaye et al, 2017 alkaloids were identified in their extract but is absent in ours. This could be as a result of environmental factors. Our data as presented in Table 2 showed that the methanol extract of *C. Planchoni* contains lots of flavonoids and other polyphenols which may be important oxidative stress mechanism inhibitors. The phenol content of a plant depends on a

number of intrinsic and extrinsic factors (Dratianni et al., 2007). The antioxidant property of the plant maybe due to more than one compound and it is difficult to measure the antioxidant capacity of each compound separately. Most methods developed to estimate the antioxidant capacity of different plant materials measure the ability to scavenge specific radicals by inhibiting lipid peroxidation or chelating metals (Swapana et al., 2013)[9]. The antioxidant capacity of the methanol extract was determined using DPPH and ferric reducing ability. The DPPH scavenging activity as presented in Table 2 could be as a result of the high content of flavonoids and polyphenols in the plant leaves. This result is in agreement with that of Mansouri et al., (2005)[10] who linked the antioxidant activity of Algerian ripe date palm fruit to the high phenolic content. The ferric reducing power of the methanolic extract as presented in Figure 1 shows that there is an increase in absorbance as the concentration increased. The extract exhibited reducing power by causing a reduction of Fe^{3+}/ferricyanide to Fe^{2+}. Its ferric reducing ability is comparable to that of ascorbic acid. The reducing ability of a compound or extract may serve as an important pointer to its potential antioxidant capacity.

Table 1: Phytochemical screening of extracts

	Flav	Ster	Sap	Alk	Tan	Anthr	Gly	Ter
Met	+	-	+	-	+	-	+	+
n-hex	-	+	-	-	-	-	-	+

Note: Flav- flavonoids; Ster-Steroids; Sap- Saponins; Alk-Alkaloids; Tan- Tannins; Anthr-Anthraquinones; Gly- Glycosides; Ter- Terpenoids; + - present; - - absent

Table 2: Total flavonoid, phenolic contents and antioxidant activity of methanolic extract

Parameters	Total flavonoid content(mg quercetin equivalent/ g extract)	Total phenolic content(mg Gallic acid equivalent/g extract)	DPPH Scavenging activity (%)
Values	161.85 ± 0.37	97.48 ± 0.42	66.73 ± 0.26

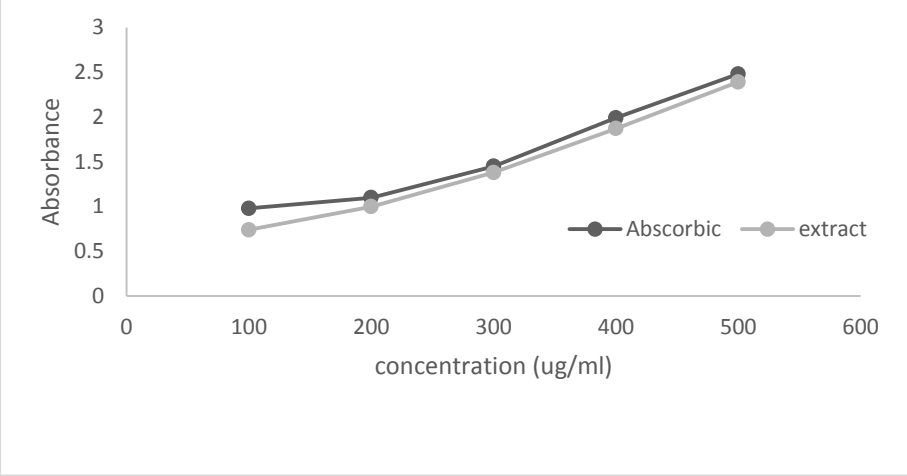

Figure 1: Ferrous reducing ability of the methanolic extract of *Cochlospermum planchonii*

GC-MS analysis was carried out to further identify individual phytochemicals as shown in Tables 3 and 4. The separated compounds as presented in Fig. 2 were identified based on their mass spectra which were compared with those of standard mass spectra from NIST (National Institute of Standards and Technology) library, (2011) incorporated into the operating system of the GC-MS equipment. Some of the identified compounds were polyphenols, alcohols, carbonyls, esters and fatty acid esters in the methanol extract while straight chain and cyclic hydrocarbons, alcohols and esters with long straight chain ends were identified in the n-hexane extract (Tables 3 and 4). The most abundant phytochemicals identified in the methanol extract is n-hexadecanoic acid (9.90%) and octacosane (24.24%) in the n-hexane extract. Another compound with appreciable quantity in the n-hexane extract is heptacosane (17.00%).

Table 3: Identified compounds from GC-MS analysis of methanol extract of *Cochlospermum planchonii*

Peaks	Retention time	Name of compound	% composition	Molecular formula	Molecular weight
1	21.58	catechol	3.93	$C_6H_6O_2$	110
2	22.01	2, 3-dihydrobenzofuran	4.05	C_8H_8O	120
3	25.05	2-methoxy-4-vinylphenol	3.94	$C_9H_{10}O_2$	150
4	26.30	2,6-dimethoxyphenol	0.93	$C_8H_{10}O_3$	154
5	29.35	(Z)-2-methoxy-4-(1-propenyl)phenol	0.75	$C_{10}H_{12}O_2$	180
6	29.24	3-acetylcoumarin	0.40	$C_{11}H_8O_3$	188
7	32.90	Caffeic acid	2.18	$C_9H_8O_4$	180
8	36.65	11-hexadecyn-1-ol		$C_{16}H_{30}O$	238
9	36.85	2,6-dimethoxy-4-(2-propenyl)phenol	2.57	$C_{11}H_{14}O_3$	194
10	38.24	4-methoxyphenol acetate	2.79	$C_9H_{10}O_3$	166
11	38.43	(Z)- 2-(9-octadecenyloxy) ethanol	0.47	$C_{20}H_{40}O_2$	312
12	38.68	Phytol acetate	3.86	$C_{22}H_{42}O_2$	338
13	38.73	6,10,14-trimethyl-2-pentadecanone	0.59	$C_{18}H_{36}O$	268
14	38.90	Not identified	2.08	-	-
15	38.98	Not identified	4.76	-	-
16	39.06	3,7,11,15-tetramethyl-2-hexadecen-1-ol	3.23	$C_{20}H_{40}O$	296
17	39.15	2-methyl-1-hexadecanol	0.64	$C_{17}H_{36}O$	256
18	39.23	9-decahexadecanoic acid	1.24	$C_{16}H_{30}O_2$	254
19	39.38	Not identified	-	-	-
20	39.42	Not identified	-	-	-
21		Not identified	-	-	-
22	39.82	n-hexadecanoic acid	9.90	$C_{16}H_{32}O_2$	256
23	40.25	Not identified	-	-	-
24	40.47	Not identified	-	-	-
25	40.66	Trans-13 octadecenoic acid	3.74	$C_{18}H_{34}O_2$	282
26	40.95	Octadecanoic acid	3.23	$C_{18}H_{36}O_2$	284
27	40.99	Methyl, trans2-octadecenoate	0.54	$C_{19}H_{36}O_2$	296
28	41.18	Oleic acid	1.47	$C_{18}H_{34}O_2$	282
29	41.49	Methyl stearate	0.95	$C_{19}H_{38}O_2$	298

30	41.60	Lupeol	0.62	$C_{30}H_{50}O$	426
31	42.14	Not identified	-	-	-
32	43.10	Not identied	-	-	-
33	43.40	Not identified	-	-	-
34	43.76	3',8,8'-trimethoxy-3-piperidyl-2,2'-binaphthene-1,1',4,4'-tetrone	1.13	$C_{26}H_{20}O_7$	444
35	45.62	Not identified	-	-	-

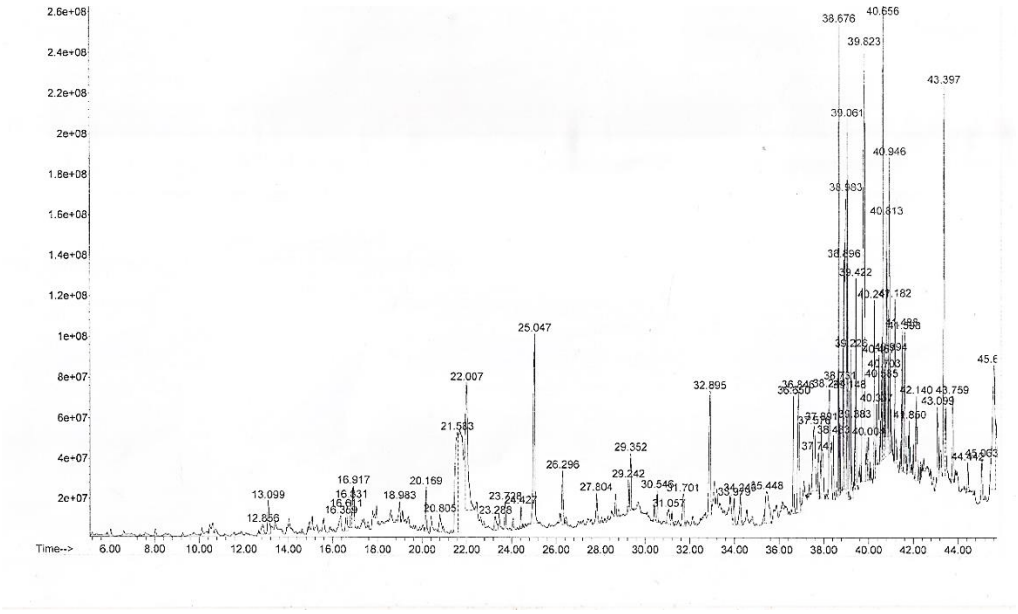

Figure 1: Chromatogram of the methanol extract

Table 4: Identified compounds from GC-MS analysis of n-hexane extract of *Cochlospermum planchonii*

Peaks	Retention time	Name of compound	% composition	Molecular formula	Molecular weight
1	6.045	Ethylcyclohexane	1.50	C_8H_{16}	112
2	6.187	1,1,3-trimethylcyclohexane	3.21	C_9H_{18}	126
3	7.538	P-xylene	1.70	C_8H_{10}	106
4	8.496	1,3-dimethylbenzene	1.39	C_8H_{10}	106
5	8.999	2-isopropyl-5-methyl-1-heptanol	1.12	$C_{11}H_{24}O$	172
6	27.852	Tetradecane	1.06	$C_{14}H_{30}$	198
7	33.845	Hexadecane	1.83	$C_{16}H_{34}$	226
8	36.642	Undecane, 5-cyclohexyl	0.80	$C_{17}H_{34}$	238
9	36.736	Not identified	3.68	-	-
10	38.252	Octadecane	2.52	$C_{18}H_{38}$	254
11	38.433	Hexadecanal	2.03	$C_{16}H_{32}O$	240
12	38.723	6,10,14-trimethyl-2-pentadecanone	1.67	$C_{18}H_{36}O$	268
13	38.943	Phthalic acid, butyltetradecyl ester	1.02	$C_{26}H_{42}O_4$	418
14	39.226	2-heptadecanone	1.09	$C_{17}H_{34}O$	254
15	39.415	Hexadecanoic acid, methyl ester	1.31	$C_{17}H_{34}O_2$	270
16	39.894	17-pentatriacontene	0.82	$C_{35}H_{70}$	490
17	39.933	Eicosane	2.45	$C_{20}H_{42}$	282
18	40.074	(Z)-2-(9-octadecenyloxy) ethanol	1.23	$C_{20}H_{40}O_2$	312

19	40.530	-	1.03	-	-
20	40.562	10-octadecenoic acid, methyl ester	2.38	$C_{19}H_{36}O_2$	296
21	40.703	Heptadecanoic acid, 16-methyl, methyl ester	0.78	$C_{19}H_{38}O_2$	298
22	40.844	Octacosane	24.24	$C_{28}H_{58}$	312
23	41.096	Heptacosane	2.62	$C_{27}H_{56}$	380
24	41.449	2,2,4-trimethyl-3-(3, 8, 12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)cyclohexanol	6.81	$C_{30}H_{52}O$	428
25	41.693	1,54-dibromotetrapentacontane	0.94	$C_{54}H_{108}Br_2$	
26	42.376	Heptacosane	3.50	$C_{27}H_{56}$	380
27	43.075	Heptacosane	17.00	$C_{27}H_{56}$	380
28	43.201	Heptacosane	1.31	$C_{27}H_{56}$	380
29	43.751	9-(2',2'-dimethylpropanoilhydrazono)-3, 6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene	1.06	$C_{29}H_{42}N_4O_3Cl_2$	564
30	44.222	1-chloroheptacosane	4.93	$C_{27}H_{55}Cl$	414
31	45.526	Stigmasterol	3.01	$C_{29}H_{48}O$	412

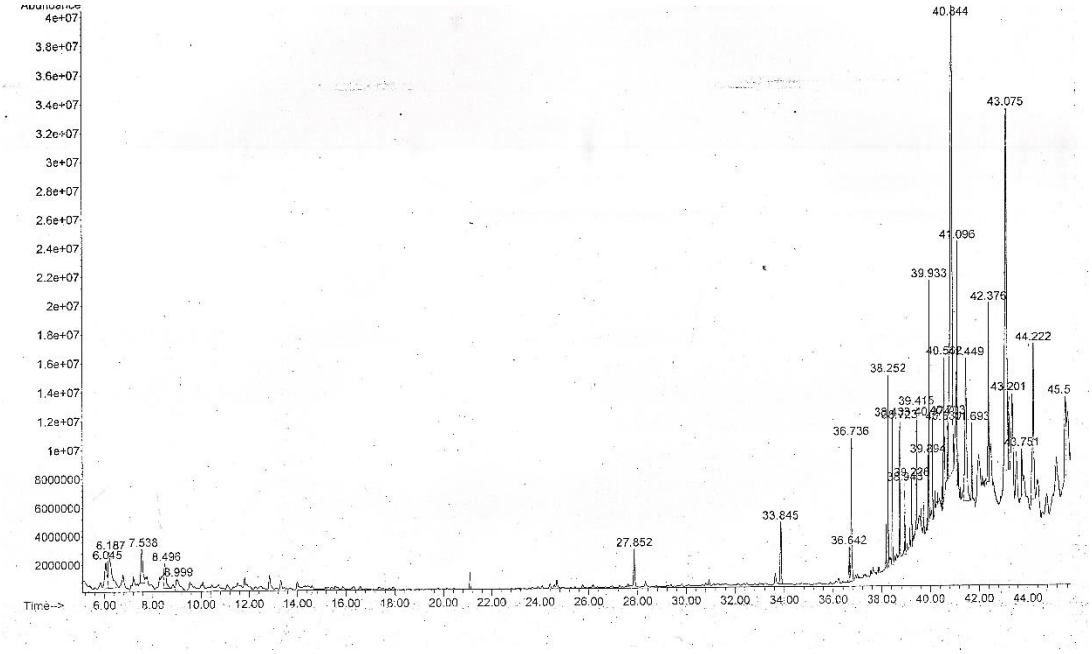

Figure 2: Chromatogram of the n-hexane extract

The plant leaves exhibited high antibacterial activity against *Staphylococcus aureus*, *Salmonella typii*, *Streptococcus pyrogenes*, *Proteus mirabilis* and *Pseudomonas aeruginosa* but inactive against *Escherichia coli* and *Shigella dysenteric* as shown in Table 5. *Salmonella typii* and *Pseudomonas aeruginosa* are bacteria that are resistant to a number of antibiotics and produce toxins that cause many types of enteritis and septicemia. *Staphylococcus aureus* is responsible for certain human diseases such as cholecystitis, urinary tract infection, skin infection, etc. The antibacterial activity of the plant leaves is comparable to that of streptomycin, the standard antibiotic that was used. Phenols have been reported to have a wide spectrum of biological activity

including antithrombotic, cardio-protective, vasodilator and antimicrobial activities (Garcia-Lafuente et al., 2009)[12]. The high content of polyphenols in the plant leaves could be responsible for these antibacterial activities.

Table 5: Antibacterial activity of the methanol leaf extract

Bacteria	Zones of inhibition (mm)	MIC (%)	Streptomycin (zones of inhibition)
Staphylococcus aureus	21.00 ± 0.82	10.00	21.52
Salmonella typii	23.67 ± 1.25	10.00	26.00
Escherichia coli	6.33 ± 1.25	-	-
Shigella dysenteric	5.67 ± 0.47	-	22.55
Streptococcus pyrogenes	21.67 ± 0.94	10.00	21.64
Proteus mirabilis	25.33 ± 0.47	10.00	-
Pseudomonas aeruginosa	22.00 ± 0.82	10.00	30.46

CONCLUSION

Phytochemical screening of the plant leaves revealed presence flavonoids, steroids, saponins, tannins, glycosides and terpenoids. GC-MS analysis showed presence of other phytochemicals such as alcohols, carbonyls, esters and hydrocarbons. The presence of these phytochemicals could be responsible for the observed antioxidant and antibacterial activity of the plant leaves. Further work is needed to determine the exact phytocomponents that are actually involved in these activities.

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