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ANTIBACTERIAL ACTIVITIES OF LEAVES EXTRACTS OF *X. AETHIOPICA* AGAINST SOME ENTEROBACTERIACEAE AND GC-MS ANALYSIS OF PHYTOCONSTITUENTS

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ABSTRACT

Xylopia aetthiopica has varying pharmacological potencies as well as antibacterial activities against bacterial pathogens including *Escherichia coli*, *Salmonella* spp and *Klebsiella pnuemoniae*. This present study was conducted to determine the antibacterial activities of leaves extracts of *X. aethiopica* against some Enterobacteriaceae species and identify bioactive constituents with therapeutic potentials present in the plant extract. Standard techniques were used in crude extraction followed by phytochemical screening. Findings revealed that *Xylopia aethiopica* leaves was found to contain flavonoids, tannins, saponins, alkaloids and cardiac glycosides. Antibacterial activities showed that ethanol and aqueous leaves extracts possessed inhibitory effects on *Salmonella* spp, *E. coli* and *K. pneumoniae* that ranged 12.5±0.5 mm to 15.0±0.0 mm, 8.0±0.0 mm to 16.5±0.5 mm and 10.0±0.0 mm to 13.0±0.0 mm respectively and 10.0±0.0 mm to 15.0±0.0 mm, 8.0±0.0 mm to 16.5±0.5 mm and 10.0±0.0 mm to 20.0±0.0 mm respectively. Resistance with no zone of inhibition was observed with *Salmonella* spp and *E. coli* at 62.5 mg/mL in ethanol leaves extracts. The bioactive compounds in ethanol leaves extract of *X. aethiopica* determined using GC-MS revealed the presence of 106 compounds including their corresponding percentage concentrations in a chromatogram. The major phytoconstituents are 6-(1'-Oxo-2'-propenyl)-1,3-cis,cis –cyclooctadiene, 2,6,11-Dodecatrienal, 2,6-dimethyl-10-methylene-, 3-Tetradecen-5-yne, (Z)-, Z,Z-3,11-Octadecadien-1-ol acetate. The results obtained are promising and a pointer to the possible use of *X. aethiopica* as antibacterial remedies.

KEYWORDS: GC-MS, *Xylopia aethiopica Salmonella* spp, *E. coli* and *K. pneumonia*.

INTRODUCTION

Treatment of microbial diseases has been threatened by the emergence and spread of multidrug resistant (MDR) bacteria, which has significant public health concerns. Consequently, there are fewer or no effective antimicrobial agents available for the treatment of these infections caused by MDR bacteria.[1] Antibiotic resistance is described as the ability of bacteria to change in response to the use of antibiotics that would resist the efficacy of the antibiotics.[2] There is therefore, urgent need to find new antimicrobial agents as alternative therapy. The use of medicinal plants for treatment of human diseases is as old as creation, and they are known to contain phytochemical compounds that have therapeutic values.[3] Thus, using medicinal plants as alternative therapy for microbial infections is necessary. Besides medicinal plants having antimicrobial potencies, they also have anti-inflammatory, mollusicidal, insecticidal, antioxidant, anti-diabetic, anti-carcinogenic, anti-allergic and hepato-protective properties.[4] Plants are rich in a variety of secondary metabolites, which include but not limited to: alkaloids, tannins, triterpenoids, saponins, phenols, steroids, cardiac glycosides, carotenoids, flavonoids and anthraquinone derivatives etc.[5] These phytochemical compounds which are present in any part of a plant such as leaves, roots, fruits, seeds, stem barks and flowers[6], have been reported to have antimicrobial properties *in vitro*.[5] These phytochemical compounds are produced by plants as means of survival in a hostile environment[4], for growth, defense against competitors, predators and pathogens.[7] Phytochemical compounds confer high medicinal potency on plants enabling them to be used as a source of raw materials for the production of many drugs.[8] Ajuru *et al*.[4] demonstrated that the mechanism of action of phytochemical compounds in plants is similar to the mechanism of action of chemical compounds in conventional drugs. Furthermore, Monon *et al*.[9] reported that secondary metabolites of medicinal

plants could be responsible for their therapeutic activity. However, harmful side effects such as mutagenic and cytotoxic effects of some medicinal plants is a major challenge and caution should be considered when administering for therapeutic use.[10]

Over 80% of human populations in developing countries depend on plant-derived medicine for their health related needs[8,11] because they are safe, non-toxic, environmental friendly, affordable and easily available.[7] This is due to the fact that many synthetic and conventional drugs are not easily available, ineffectiveness and lack of potency as a result of emergence of multi-drug resistance microorganisms to these antimicrobial agents.[12] Also, increased poverty in some developing countries resulting in inability to afford conventional chemotherapeutic agents, pay hospital bills as well as economic recession experienced by some patients are some of the reasons why people depend on plant-derived medicine for their health needs, and these plants are easily accessible. Medicinal plants are plants that are used in human disease treatment, because they contain compounds that possess therapeutic values.[3]

Xylopia aethiopica is an aromatic angiosperm that belongs to the family, Annonaceae. It thrives in the evergreen rain forest of tropical and subtropical Africa. The plant matures as a slim, tall tree growing up to 30 m high with a straight stem having a slightly stripped or smooth bark.[13] Its fruits are odoriferous, with slender pods and slightly curved, also, it has a small twisted bean shaped pods and are characterized by deep brown colour.[7] *Xylopia aethiopica* is coined from a Greek word "*Xylon pikron"*, which mean "bitter wood", and the second part of botanic name, *aethiopica* refers to its origin, Ethiopia.[11] The plant is commonly called "African pepper, Negro pepper or Ethiopian pepper" in English Language. However, it is called "Ata" by the Ibibio and Efik tribes. It produces an attractive spicy flavour when the fruit is smoked during the drying process. Fresh and dried fruits, leaves, stem bark and root bark and essential oils showed various degrees of activities against Gram positive bacteria, such as *Bacillus subtilis* and *Staphylococcus aureus*, Gram negative bacteria, such as *Pseudomonas aeruginosa* and the yeast-like fungus, *Candida albicans*, using the cup plate method.[14] Almost every part of the plant is used in traditional medicine for managing various ailments including skin infections, candidiasis, dyspepsia, cough and fever.[14] In a study, Fleischer *et al*.[14] reported that all the morphological parts of the plant (fruit, leaf, stem bark and root bark) yielded varying amounts of essential oils, with the fresh fruits and the leaves providing the highest and the lowest yields respectively.

The analysis of the chemical constituents of medicinal plants would be necessary to determine and isolate the bioactive constituents of these plants and hence, making way for the assessment of their biological activities. Presently, there are lots of techniques that can be employed to identify these constituents of plants such as chromatographic and spectroscopic techniques. A combination of gas chromatography (GC) and mass spectrometry (MS) has over the years proven to be one of the best techniques in the identification of the chemical constituents of plants. While gas chromatography separates the constituents, mass spectrometry determines the molecular weight of these compounds. The GC also separates volatile components in a sample while MS fragments the components and identifies them on the basis of their mass. Separation in GC is based on their boiling point where the substances with higher boiling point come out later and those with lower boiling point come out first. When they are out, they go into the MS which identifies them using their mass to charge ratio.[15] Therefore, this study was designed to determine the antibacterial efficacy of leaf extracts of *X. aethiopica* and identify the bioactive constituents contained in the plant using GC-MS.

MATERIALS AND METHODS

Collection and Authentication of Plant Materials

Fresh leaves of *Xylopia aethiopica* were obtained from the botanical farm of University of Uyo, Uyo in Akwa Ibom State, Nigeria. The plant was identified, authenticated and deposited in the herbarium unit of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

Preparation of Plant Materials

The leaves of the plant were washed thoroughly 2-3 times with running tap, then air dried at ambient temperature for one month, after which the plant was pulverized using laboratory pestle and mortar. The coarse materials were further reduced to fine powder with the aid of electric blender. The fine powder was stored in air-tight well labelled small plastic containers for further analysis.

Extraction of Plant Materials

The method as described by Ajuru *et al*.[4] was adopted for the extraction. About 213.5 g of *X. aethiopica* pulverised leaves was weighed into 1500 mL flask of 70% ethanol; equally, 210 g of *X. aethiopica* pulverised leaves was weighed into 1500 mL flask containing water; The leaves were macerated for 72 h thereafter filtered through Whatman No. 1 filter paper and then through cotton wool. The extracts were evaporated to dryness using a hot water bath at 50 ^{O}C for 72 h and then stored in the refrigerator at 4 ^{O}C till further use.

The percentage yield of extraction by different plant extracts will be calculated using the formula.

$$Percentage\ yield(\%) = \frac{Dry\ weight\ of\ extract}{Dry\ weight\ of\ plant\ material} X\ 100$$

Qualitative Phytochemical Screening of the Extracts

The ethanol and aqueous plant extracts was subjected to phytochemical screening using standard method.[16,17]

Sterility Testing of the Extracts

Approximately 1 mL of each of the extracts was introduced into 5 mL of nutrient broth and incubated at 37 ^{O}C for 24 h. The absence of turbidity or clearness of the broth after incubation indicates that the extracts are sterile.

Preparation of Different Concentrations of Extracts

Ethanol and aqueous extracts of *X. aethiopica* were reconstituted using 10% Dimethyl Sulfoxide (DMSO) and water respectively to make concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL solutions.

Collection and Confirmation of Bacterial Isolates

Stock bacterial cultures of *Escherichia coli, Salmonella* spps and *Klebsiella pneumoniae* previously isolated from stool specimens of diarrhoeal patients were obtained from microbiology laboratory of University of Calabar Teaching Hospital (UCTH), Calabar. The bacterial cultures were sub-cultured using MacConkey agar and incubated aerobically at 37 ^{O}C for 18-24 h. Discrete colonies were identified and confirmed using standard microbiological procedures.[18] Thereafter, confirmed using Analytical Profile Identification (API) system for Enterobacteriaceae.

Standardization of Inoculum

The confirmed bacterial cultures were separately inoculated into test tubes containing sterile nutrient broth and incubated at 37 ^{O}C for 24 h. After incubation, the overnight broth cultures were diluted accordingly and matched with 0.5 MacFarland turbidity standard, which is approximately 1.5×10^{8} cfu/mL using a spectrophotometer.[19]

Antibacterial Activity Spectra of the Extracts

Agar well diffusion method was used to determine the antibacterial activities of the extracts according to the method adopted by Gonelimali *et al*.[20]. About 100 µL of the standardized bacterial suspensions were spread on the surface of dry Mueller-Hinton agar plates and allowed to seed. A sterile 6 mm cork borer was used to bore four equidistant wells on the seeded agar plates, while the fifth well was used as negative control. Micropipette was used to transfer 0.1 mL of the various concentrations of the extracts (500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL) into the wells. The fifth well will contain negative control (10% DMSO). The seeded plates were left at room temperature for 15 min to allow for proper diffusion of the extracts into the agar and thereafter incubated at 37 ^{O}C for 18- 24 h. The antibacterial assay was performed in duplicate. Antibacterial assay was determined by measuring the diameter of zone of inhibition to the nearest millimeter with a ruler. The measured diameter of zones of inhibition will be interpreted using templates from CLSI.[21]

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of the extracts

Gas Chromatography-Mass Spectroscopy analysis was performed to separate, identify the molecular structure and quantify the bioactive compounds present in the plant extracts. An Agilent 8860 GC system connected to a Mass Spectrometer Detector was used. One microlitre of the sample was injected in the pulsed spitless mode onto a 30m x 0.25mm ID DB 5MS coated fused silica column with a film thickness of 0.15 micrometre. Helium gas was used as a carrier gas, while hydrogen and compressed air was used as the ignition gas. The column head pressure was maintained at 20 psi to give a constant of 1ml/min. Other operating conditions were preset. The column temperature was initially held at 55^{o}C for 0.4min, increased to 200^{o}C at a rate of 25^{o}C/mins, then to 280^{o}C at a rate of 8^{o}C/mins and to a final temperature of 300^{o}C at a rate of 25^{o}C/mins, held for 2mins. The identification time was based on retention time, and components with lower retention time elute first before those with higher retention time. 50ml sample was added to 10ml of the solvent mix (1:1 - Hexane: Dichloromethane) was measured and poured into the beaker flask to dissolve and homogenize the extract. The homogenized extract was cleaned up with 100 -200 mm mesh silica gel and 3 g of anhydrous sodium sulfate in a well-packed column, conditioned with hexane to form a slurry. One microlitre of the sample was injected through the injection port into the GC-MS. The identification of compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds available in the database of National Institute of Standard and Technology (NIST MS 2.0) and the name, molecular weight and structure determined.

RESULTS

Percentage yield results of X. aethiopica leaves

Results of percentage yield of *X. aethiopica* is presented on Table 1. It showed that aqueous extract yielded more extract than ethanol extract.

Table 1: Percentage yield of ethanol and aqueous extracts of *X. aethiopica* leaves.

Plant extract	Percentage Yield (%)	
	Ethanol extract	Aqueous extract
X. aethiopica leaves	8.61	8.86

***Phytochemical screening results of X. aethiopica* leaves**
The results of phytochemical screening of *X. aethiopica* leaves is presented on Table 2. The preliminary screening of phytochemical constituents of the leaves of *X. aethiopica* showed the presence of flavonoids, saponins, tannins and cardiac glycosides and alkaloids. Other phytochemical compounds were not screened.

Table 2: Phytochemical screening of ethanol and aqueous crude extracts of *X. aethiopica* leaves.

Bioactive components of the plant	*X. aethiopica* leaves	
	Ethanol	Aqueous
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Cardiac glycosides	+	+

Antibacterial activity results of the extracts
Table 3 shows the antibacterial activity spectra of ethanol and aqueous leave extracts of *X. aethiopica* at different concentrations. The ethanol and aqueous extracts of the plant showed inhibitory effects against *Salmonella* spp and *E. coli* at 500 mg/mL to 125 mg/mL. In contrast, the aqueous extract showed inhibitory effects against *K. pneumoniae* at 500 mg/mL to 62.5 mg/mL. However, the least concentration of the extracts (62.5 mg/mL) had no inhibitory effect against *E. coli* and *Salmonella* spp. The positive control (ciprofloxacin) was observed to be effective on the test bacteria.

Identification of chemical composition of the extract
Identification of chemical compounds in *X. aethiopica* leaves was done using Gas Chromatography-Mass Spectrometry (GC-MS) to obtain the peak spectra of compounds. Gas chromatogram pattern of leaves of *X. aethiopica* can be observed on Figure 1. Furthermore, the chemical components of leaves of *X. aethiopica* can be observed on Table 4. Chromatogram pattern of 1,3-cis,cis –cyclooctadiene from *X. aethiopica* leaves was further analysed using mass spectrometry and the results can be seen in Figure 2.

Figure 1: Gas chromatogram pattern of leaves of *X. aethiopica.*

Table 3: Antibacterial activities of ethanol and aqueous leave extracts of *X. aethiopica* and standard antibiotics against bacterial isolates.

	Concentrations of ethanol leave extracts (mg/mL), antibiotics (µg) and mean zones of inhibitions (mm)								
	X. aethiopica								
	Ethanol				Aqueous				CPX
	500	250	125	62.5	500	250	125	62.5	10
Salmonella spp	15.0±0.0	13.5±0.5	12.5±0.5	NZI	15.0±0.0	12.0±0.0	10.0±0.0	NZI	45.0±0.0
E. coli	16.5±0.5	13.0±0.0	8.0±0.0	NZI	16.5±0.5	13.0±0.0	8.0±0.0	NZI	42.0±0.0
K. pneumoniae	13.0±1.0	11.5±0.5	10.±0.0	NZI	20.0±0.0	14.5±0.5	11.0±0.0	10.±0.0	39.0±0.0

Table 4: Chemical components of *X. aethiopica* leaves.

S/NO	List of Compounds	Concentration (%)
1	Ethane, 1-bromo-2-chloro-	5.83
2	Propanoic acid, 2-chloro-, ethyl ester	0.56
3	Propanoic acid, 2-chloro-, methyl ester, (S)-	0.83
4	1-Bromo-1-chloroethane	0.09
5	Propanoic acid, 2-chloro-, methyl ester	0.88
6	Ethanesulfonyl chloride, 2-chloro-	0.34
7	1-Pentanol, 5-cyclopropylidene-	0.42
8	Santolina triene	0.67
9	Preg-4-en-3-one, 17.alpha.-hydroxy -17.beta.-cyano-	0.10
10	6-Hydroxybenzofuran-3-one	0.85
11	2-Methoxy-4-vinylphenol	0.90
12	Ethanone, 1-(2-hydroxy-5-methylphe nyl)-	0.04
13	3-Pyridinecarboxylic acid, 6-amino	0.45
14	cis-muurola-3,5-diene	0.44
15	1,5-Decadiyne	0.35
16	Acetonitrile, (3,5,5-trimethyl-2-cyclohexen-1-ylidene)-, (Z)-	0.81
17	2,2-Dimethyl-4-(1-hydroxy-3,5-hexadienyl)-1,3-dioxolane	0.11
18	7-Octen-2-ol, 2-methyl-6-methylene	0.21
19	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	0.21
20	3H-Naphth[1,8a-b]oxiren-2(1aH)-one, hexahydro-	0.80
21	3-Octyne, 7-methyl-	0.55
22	2-Heptanol, 2-methyl-	0.39
23	7-Octen-2-ol, 2-methyl-6-methylene	0.84
24	Succinic acid, tridec-2-yn-1-yl 2-methoxyethyl ester	0.71
25	5-Azulenemethanol, 1,2,3,4,5,6,7,8 -octahydro-.alpha.,.alpha.,3,8-tetramethyl-	0.63
26	7-Octen-2-ol, 2-methyl-6-methylene	0.29
27	Silane, (chloromethyl)dimethyl-	0.33
28	Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-	0.62
29	1,7-Octadiene, 2,7-dimethyl-3,6-bis(methylene)-	0.35
30	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-carbomethoxy, trans-	0.18
31	Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)-	0.09
32	Carbonic acid, 2,3-dichlorophenyl 2-methoxyethyl ester	0.23
33	Butanoic acid, 2-hydroxy-, ethyl ester	0.43
34	1,1'-Oxalylbis-3-thiosemicarbazide	0.57
35	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, .alpha.,.alpha.,6,8-tetr amethyl-, stereoisomer	0.88
36	Decaethylene glycol, monomethyl ether, TBDMS derivative	0.18
37	Anthracene, tetradecahydro-, (4a.alpha.,8a.alpha.,9a.alpha.,10a.alpha.)-	0.77
38	1,4-Benzoxazepin-3(2H)-one, 9-amino-4,5-dihydro-7-methyl-	0.85
39	Benzamide, 3-methoxy-N-propyl-	0.82
40	2-Undecyne	0.87
41	1,5,7-Octatrien-3-ol, 2,6-dimethyl	0.77

42	R(-)3,7-Dimethyl-1,6-octadiene	0.24
43	Undecanoic acid, 10-bromo-	0.35
44	n-Hexadecanoic acid	0.54
45	n-Decanoic acid	0.49
46	Pentadecanoic acid, ethyl ester	0.12
47	Ethyl 13-methyl-tetradecanoate	1.90
48	Dodecanoic acid, ethyl ester	3.09
49	1H-3a,7-Methanoazulene, octahydro- 1,4,9,9-tetramethyl-	0.34
50	Tricyclo[3.2.2.0]nonane-2-carboxylic acid	0.98
51	2,6,11-Dodecatrienal, 2,6-dimethyl-10-methylene-	9.22
52	1,E-8,Z-10-Tetradecatriene	2.09
53	(Z,Z)-3,6-Nonadienal	0.39
54	4-Decyne	3.89
55	Cycloheptane, 1-bromo-3-iodo-	0.04
56	Cyclopentanecarboxylic acid, 2-methyl-3-methylene-, methyl ester	3.09
57	Phenylacetic acid, 2-methylcyclohex-2-enyl ester	4.90
58	9,12-Octadecadienoyl chloride, (Z, Z)-	0.61
59	1-Pentadecyne	0.23
60	11,14-Eicosadienoic acid, methyl ester	0.36
61	Z,Z-3,11-Octadecadien-1-ol acetate	7.12
62	9,12-Octadecadienoic acid, ethyl ester	2.09
63	9-Oxabicyclo[6.1.0]nonane, cis-	1.90
64	E-9-Tetradecenoic acid	1.12
65	9,12,15-Octadecatrienoic acid, (Z, Z,Z)-	2.11
66	3-Undecen-1-yne, (E)-	3.81
67	(3E,6E)-Nona-3,6-dienyl 2,2,2-trifluoroacetate	0.46
68	Bicyclo[3.1.0]hexane, 6-methylene-	0.24
69	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	1.53
70	3-Tetradecen-5-yne, (Z)-	9.02
71	Doconexent	2.35
72	6-(1'-Oxo-2'-propenyl)-1,3-cis,cis -cyclooctadiene	9.35
73	Costunolide	0.35
74	2(3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-(3a.alpha.,6.alpha.,7.beta.,7a.beta.)]-	1.35
75	Methyl 4,7,10,13-hexadecatetraenoate	1.90
76	Acetic acid, 2-methyl-2-phenyl-, hydrazide	0.48
77	Phenol, 2-methoxy-4-(2-propenyl)-,acetate	0.04
78	Cyclododecyne	0.28
79	8-Methylene-3-oxatricyclo[5.2.0.0(2,4)]nonane	0.72
80	3-Methylene-1,6-heptadiene	0.22
81	Hexane, 1-chloro-5-methyl-	0.91
82	Methyl 4,7,10,13,16,19-docosahexaenoate	0.53
83	(3E,6E)-Nona-3,6-dienyl 2,2,3,3,3- pentafluoropropanoate	0.27
84	1H-3a,7-Methanoazulene, octahydro- 1,4,9,9-tetramethyl-	0.98
85	1,5-Hexadiene, 2,5-dimethyl-3-methylene-	0.36
86	Methyl (Z)-5,11,14,17-eicosatetraenoate	0.37
87	Hexane, 1-chloro-5-methyl-	0.87
88	5,8,11,14,17-Eicosapentaenoic acid , methyl ester, (all-Z)-	0.02
89	Bicyclo[10.1.0]tridec-1-ene	0.08
90	1H-3a,7-Methanoazulene, octahydro- 1,4,9,9-tetramethyl-	0.29
91	Bicyclo[10.1.0]tridec-1-ene	2.78
92	Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.,5.alpha.)-	2.089
93	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	1.97
94	Methyl 4,7,10,13,16,19-docosahexaenoate	0.67
95	cis-5,8,11,14,17-Eicosapentaenoic acid	0.77
96	3-Adamantan-1-yl-butan-2-one	1.77
97	Andrographolide	0.92

98	p-Camphorene	0.29
99	Isopimara-9(11),15-diene	2.09
100	Santolina triene	1.00
101	Phytol	0.72
102	Isophytol	0.88
103	Linoleic acid ethyl ester	0.38
104	Oleic Acid	0.81
105	Caryophyllene oxide	0.29
106	Terpineol	0.44

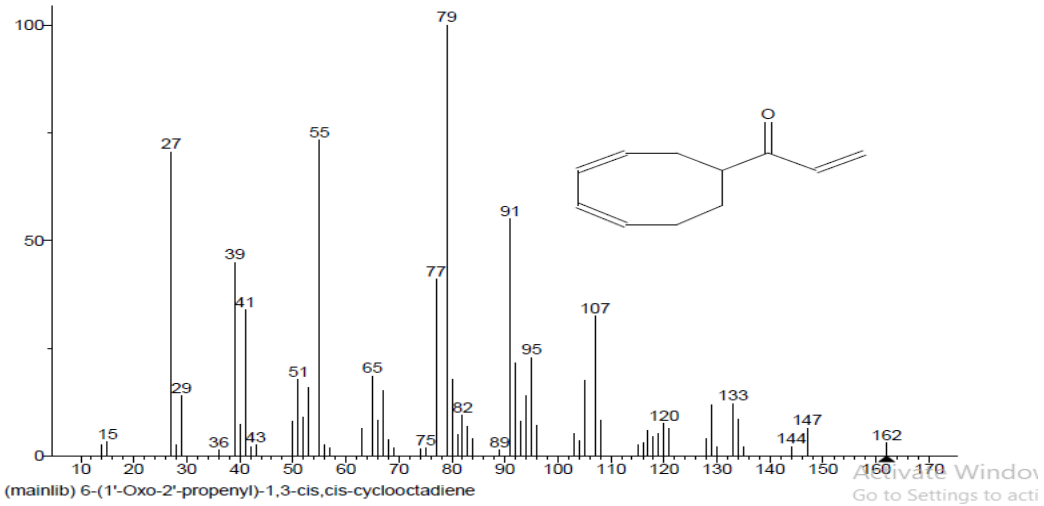

Figure 2: Mass spectrometry chromatogram pattern of 1,3-cis,cis –cyclooctadiene compound from X. aethiopica leaves.

DISCUSSION

The results obtained from this study revealed that the leaves of the plant, *X. aethiopica* possess some bioactive substances. These bioactive substances are alkaloids, saponins, tannins, flavonoids and cardiac glycosides. This result confirms reports of Alagbe *et al.*[7] and Aguoru *et al.*[22] who reported presence of these phytochemical substances as observed in this study. However, in a study carried out by Ilusanya *et al.*[23] using *X. aethiopica* fruits, it was observed that alkaloids were absent. These bioactive compounds have different functions and mechanisms of action. For instance, tannins in plants are complex mixtures of organic compounds used as astringent as they precipitate tissue protein to form vascular plugs.[7] They are known to prevent the synthesis of bacterial cell proteins by forming complexes which are irreversible with proline rich protein in bacteria. They are used in treating diarrhoea and dysentery[7,23] and are known to show curative activity against several pathogens.[23] Flavonoids are known to have antioxidant activities against free radicals, inflammation allergies, cellular signaling, platelet aggregation.[7] Flavonoids are reported to reduce the risk of estrogen-induced cancer by interfering with the enzymes that synthesizes estrogen.[12] Moreover, they disrupt microbial cell membranes by forming complexes with the extracellular soluble proteins.[12] Plants containing cardiac glycosides can be used as cardiac drugs as the phytochemical compound is useful in heart pumping, furthermore, alkaloids have a wide range of pharmacological activities including antibacterial, anticancer and anti-asthmatic activities and plants with rich alkaloids have bitter taste.[7,12] The phytochemical constituents of medicinal plants may vary widely depending on the parts used, extraction method, stage and age of maturity, geographical location/ origin, storage conditions and harvesting seasons.[24] The bioactive constituents present in this medicinal plant could be responsible for the therapeutic activity attributed to *X. aethiopica*.[12,24] The presence of these bioactive compounds suggests great potential of *X. aethiopica* as a source of useful phytomedicine.

The results obtained in this study showed that the ethanol and aqueous extracts of *X. aethiopica* have inhibitory effects on the test isolates at varying concentrations. This indicates that the plant possesses some bioactive compounds that can inhibit or kill the growth of these bacteria; and this supports the use of the plant by folkloric medicine practitioners in the treatment of various diseases such as urinary tract infection, cough, skin infections.[13,14]

Reports on the ethnopharmacological uses of *X. aethiopica* by authors globally[13,14,23] supports the

findings of this study. This is attributable to the significant anounts of bioactive compounds of therapeutic benefits contained in the plants.[23] From the results, it is deduced that the antibacterial activities of the extracts correspondingly increased as the concentration of the extracts increase. Ilusanya *et al*.[23] reported no inhibitory effect against *E.coli* and *K. pneumoniae* at all concentrations using *X. aethiopica* leaves. In contrast, in this study, both solvents showed inhibitory effects against *E.coli* and *K. pneumoniae.* It was also observed in the study that the bioactive compounds were present in ethanol and aqueous extracts. This shows that ethanol and aqueous extracted the bioactive constituents equally and that both solvents are good extraction solvent for the extraction of *X. aethiopica* leaves. However, Ilusanya *et al*.[23], reported that ethanol extracted the bioactive constituents better than aqueous.

The bioactive compounds of *X. aethiopica* leaf extracts by GC-MS is presented on Table 4. One hundred and six (106) compounds were identified. The Major compounds greater than 1 % were; Ethane, 1-bromo-2-chloro- (5.83 %), Ethyl 13-methyl-tetradecanoate (1.90 %), Dodecanoic acid, ethyl ester (3.09%), 2,6,11-Dodecatrienal, 2,6-dimethyl-10-methylene- (9.22 %), 1,E-8,Z-10-Tetradecatriene (2.09%), 4-Decyne (3.89 %), Cyclopentanecarboxylic acid, 2-methyl-3-methylene-, methyl ester (3.09 %), Phenylacetic acid, 2-methylcyclohex-2-enyl ester (4.90 %), Z,Z-3,11-Octadecadien-1-ol acetate (7.12 %), 9,12-Octadecadienoic acid, ethyl ester (2.09 %), 9-Oxabicyclo[6.1.0] nonane, cis- (1.90 %), E-9-Tetradecenoic acid (1.12 %), 9,12,15-Octadecatrienoic acid, (Z, Z,Z)- (2.11 %), 3-Undecen-1-yne, (E)- (3.81 %), 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)- (1.53 %), 3-Tetradecen-5-yne, (Z)- (9.02 %), Doconexent (2.35 %), 6-(1'-Oxo-2'-propenyl)-1,3-cis,cis –cyclooctadiene (9.35 %), 2(3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-(3a.alpha.,6.alpha.,7.beta.,7a.beta.)]-(1.35 %), Methyl 4,7,10,13-hexadecatetraenoate (1.90 %), Bicyclo[10.1.0]tridec-1-ene (2.78 %), Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.,5.alpha.)- (2.089 %), 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)- (1.97 %), 3-Adamantan-1-yl-butan-2-one (1.77 %), Isopimara-9(11),15-diene (2.09 %), Santolina triene (1.00 %) while the remaining compounds are less than 1 % however, they all have a marked therapeutic properties. Among the identified compounds in this study, are fatty acids such as n-hexadecanoic acid and 9, 12-octadecadienoic acid, which, may be associated with the reported antioxidant activity of the plant[15] and a lot of other constituents. Terpineol, n-hexadecanoic acid and 9,12-octadecadienoic acid were also reported to be found in *X. aethiopica* fruits[15,7], however, terpineol and n-hexadecanoic acid are less than 1 % in this study. *Xylopia aethiopica* has been used as spice in food; and could be as a result of the compound, terpineol, which is a citrus essential oil. The result obtained in this study contained some compounds, though at different concentrations, which, are in agreement with previous findings.[7,15,25] This dissimilarity could be attributable to extraction procedures, parts of plant used, species, geographical location, age of plant and method of harvesting.[7]

CONCLUSION

Having identified many bioactive constituents in ethanol leaf extract of *Xylopia aethiopica* in the present study, it is evident that *X. aethiopica* has many bioactive constituents that possess a wide range of therapeutic activities including: antibacterial, antimalarial, antiprotozoal, anti-carcinogenic, cytotoxic, antioxidant, anti-inflammatory etc., and would be of great pharmaceutical benefits if harnessed. It is therefore recommended that the active constituents are isolated and further studies carried out to ascertain their efficacy in the prevention and treatment of various diseased conditions both *in vivo* and *in vitro*.

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