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Chemical Constituents of Methanol Fruit Extract of *Xylopia aethiopica* by GC-MS and FT-IR Spectroscopy

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Abstract: *Xylopia aethiopica possesses great nutritional and medicinal values in traditional medicine Almost all parts of Xylopia aethiopica are very useful medicinally, but the fruits are most commonly used for therapeutic purposes. Extracts of the fruits are used in the treatment of cough, biliousness, bronchitis, rheumatism, dysentery, malaria, uterine fibroid and amenorrhea The bioactive compounds in methanol extract of Xylopia aethiopica fruits were determined using a combination of gas chromatography -mass spectrometry (GC-MS) and Fourier Transform Infra-red Spectroscopy.The compound identification was based on the molecular structure, molecular mass and calculated fragments and their functional group. The GC-MS revealed the presence of 26 compounds among them are; Mannosamine; 1-Methyl-4-[nitromethyl]-4-piperidinol; Paromomycin; N-[2-[[2-Pyridylmethyl]amino]ethyl]aziridine; N-[3-[N-Aziridyl]propylidene]-2-[2-pyridyl]ethylamine; 2-Oxatricyclo[4.3.1.0(3,8)] decane; 2-Oxaadamantane; exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol; Isopulegol; Paromomycin; Acetic acid, (3-nitro-2-pyridyl) thio-; 5-Pyrrolidin-2-ylidenemethyl-3,4-dihydropyrrol-2-one; 1-(3,6-Dimethyl-2-pyrazinyl) propanone; N,N'-Bis (Carbobenzyloxy)-lysine methyl(ester.The FT-IR analysis revealed the presence of Hydroxyl (O-H) stretching at 3671.5cm^{-1}, Aliphatic amine(N-H) at 3322.9cm^{-1} Carbonyl (C=O) at 1701.5 cm^{-1} , Aliphatic stretching (C-H) at 2937.1 cm^{-1} and 2830.9 cm^{-1} .*

Keywords: *Herbal medicine, GC-MS, Xylopia aethiopica , ethno pharmacological and FT-IR*

1. INTRODUCTION

Dietary spices contain a wide variety of volatile and nonvolatile chemicals obtained from parts of plants such as the fruits, berries, roots, pods, and the barks, majority of which are used in herbal medicine for the treatment of diseases [1]The World Health Organization (WHO) gave a list of about 21,000 plants used for medicinal purposes around the world (2). Plants have the ability to synthesize a wide variety of chemical compounds that possess therapeutic properties and defend against attack from predators such as insects, fungi and herbivorous animals [3, 4]. About 12,000 of such compounds, representing less than 10 % of the total, have been extracted, isolated and characterized. These compounds have been found to exert their effects on the human body through mechanisms that are similar to those already established in conventional drugs revealing that herbal medicines may be as effective as the latter [3,5]. Furthermore, the plant kingdom is believed to hold many new drug templates; hence, the continued investigation into ethno-medicinal plants [6].

Herbal medicine or phytomedicine is acknowledged as the most common form of alternative medicine [7]. Long in the creation of mankind, plants have been used medicinally [8]. The World Health Organization (WHO) estimates that about 80% of the world's population relies on these unconventional plant-based medicines as their primary medical intervention especially in the developing countries of the world where medical facilities/modern medicines are largely inadequate[9]. Scientific evaluation of ethno pharmacological information from medicinal plants is necessary for the development of accessible, affordable and high safety herbal therapies [10]. One of such commonly used medicinal plants is *Xylopia aethiopica* (Annonaceae).*Xylopia aethiopica* is a slim, tall, evergreen aromatic tree which grows up to 15–30 m high and about 60–70 cm in diameter. It is native to the lowland rainforest and moist fringe forest in the savanna zones of Africa, but largely found in West, Central and Southern Africa. These trees are widely distributed in the humid forest zones especially along rivers in the drier area of the region [11]. *Xylopia* is a Greek word

(_xylonpikron‘) for _bitter wood‘, while aethiopica refers to its Ethiopian origin (Ethiopia). Its common names include; African pepper, Guinea pepper, spice tree, negro pepper, West African pepper and Senegal pepper [12]. An attractive spicy flavor is obtained after Negro pepper is smoked during the drying process.

Xylopia aethiopica leaves are simple, alternate, oblong, and elliptic to ovate. Its flowers are bisexual, solitary or in 3-5 flowered fasicles or in strange, sinuous, branched spikes, or cymes, up to 5.5 by 0.4 cm and creamy-green. Fruits of *Xylopia aethiopica* look like small, twisted bean-pods which are dark brown, cylindrical, 2.5 to 5 cm long and 4 to 6 mm thick. Each pod houses about 5 to 8 kidney-shaped seeds grains of approximately 5 mm length [11].

Its parts are used in ethno-medicine to treat various ailments including skin infections, candid as is, dyspepsia, cough, dysentery, biliousness, bronchitis, rheumatism, malaria, uterine fibroid, amenorrhea, boil, sore, fever, respiratory ailments, lumbago and neuralgia. It is also used as a mouthwash to treat toothaches [12-13]. The powdered root is used as a dressing for sores and to rub on gums for pyorrhoea and in local treatment of cancer in Nigeria. Mixture of *Xylopia aethiopica* with salt servesas a cure for constipation. Its decoction is used in Gabon against rheumatism and as an emetic and the leaf-sap mixed with kola nut is given to treat epileptic fits [14]. It is taken to encourage fertility and to ease childbirth. When crushed, *Xylopia aethiopica* is rubbed on the forehead to treat headache and neuralgia. An extract of the seeds is also used as a vermifuge for roundworms [15]. In negro pepper fruits, the essential oil (2 to 4.5%) has been found to contain β-pinene, 1,8-cineol, α-terpineol, terpinene-4-ol, paradol, bisabolene and other terpenes. In other work, linalool (E)-β ocimene, α-farnesene, β-pinene, α-pinene, myrtenol and β-phellandrene were found [16] Among the non-volatile constituents, tetracyclic diterpenes of the kaurane type have been identified [17]. The bark oil has abundance of pinene, trans-pinocarveol, verbenone and myrtenol. However, the leaf oil is rich in spathulenol, cryptone, beta-caryophyllene and limonene [18]. The plant is said to contain anonaceine, whichis an alkaloid resembling morphine. The fruit contains volatile aromatic oil, a fixed oil and rutin [19]. The dark brown, small and twisted bean-podslike fruits are an important item of trade and the most widely used of its various morphological parts. [12]

1.1. Botanical Classification

Xylopia aethiopica belongs to the following category;

Kingdom: Plantae

Order: Magnoliids

Family: Annonaceae

Genus: *Xylopia*

Specie: *Xylopia aethiopica*

2. Materials and Methods

2.1. Collection and Identification of Plant Material

The fruits were collected from a farm land in of okobe, in Obi Ngwa Local Government of Abia State, Nigeria. The fruits were authenticated by a taxonomist Dr E.O. Emmanuel of Plant Science and Biotechnology Abia State University. Specimen was deposited at the Herbarium of Department of Plant Science and Biotechnology, Faculty of Biological Sciences, Abia State University, Uturu. Nigeria under the reference number ABXA 6926.

2.2. Preparation of Plant Materials

The fruits were air-dried for 15 days at room temperature (25-30 ºC). The sample was milled using and electric blender to coarse powder and powdered sample was kept in a clean closed container pending extraction. 50 g of pulverized dried fruit material was mixed with 150 ml of solvent (95 % methanol) and kept in rotary shaker at 100 rpm overnight and filtered with What man No.1 filter paper The extract was concentrated under reduced pressure using Digital Heidolph Rotary evaporator (4000 series) and the supernatant fruit extract was decanted after complete removal of the solvent

Fig1a. *Dried fruits of Xylopia aethiopica*

Fig1b. *Fresh fruits, stems and leaves Xylopia aethiopica*

2.3. GC-MS Analysis

The GC-MS analysis of the methanol fruit extract of *Xylopia aethiopica* was carried out using a HP 7890 GC instrument integrated with an Agilent 5975C MSD mass spectrometer (Aligent, Santa Clara, CA, USA). The capillary column was an Agilent HP-5MS (30.m x 0.25 mm i.d. x 0.25 NM film thickness), helium (Purity > 99.999 %) was used as the carrier gas, and the flow rate was 1 mL/min. The injector temperature was 250 ^{0}C, and the injection mode was split less. The G.C oven temperature was held at 50 ^{0}C for 5 min, which was increased to 210 ^{0}C at a rate of 3 ^{0}C/min, maintained at 210 ^{0}C for 3 min, and finally increased to 230 ^{0}C at 150 C/min. The mass spectrometer conditions were as follow: ionization energy, 70 Ev; ion Source temperature, 230 ^{0}C; quadruple temperature, 150 ^{0}C; quadruple mass spectrometer scan range 30 – 500 atomic mass units (amu); solvent delay time 2.8 min.

2.4. Identification of Compounds

The components of the methanol extract of *Xylopia aethiopica* was identified by matching the peaks with computer Wiley Ms. libraries and confirmed by comparing mass spectra of the peaks and those from literature [20].

2.5. FTIR Spectroscopic Analysis

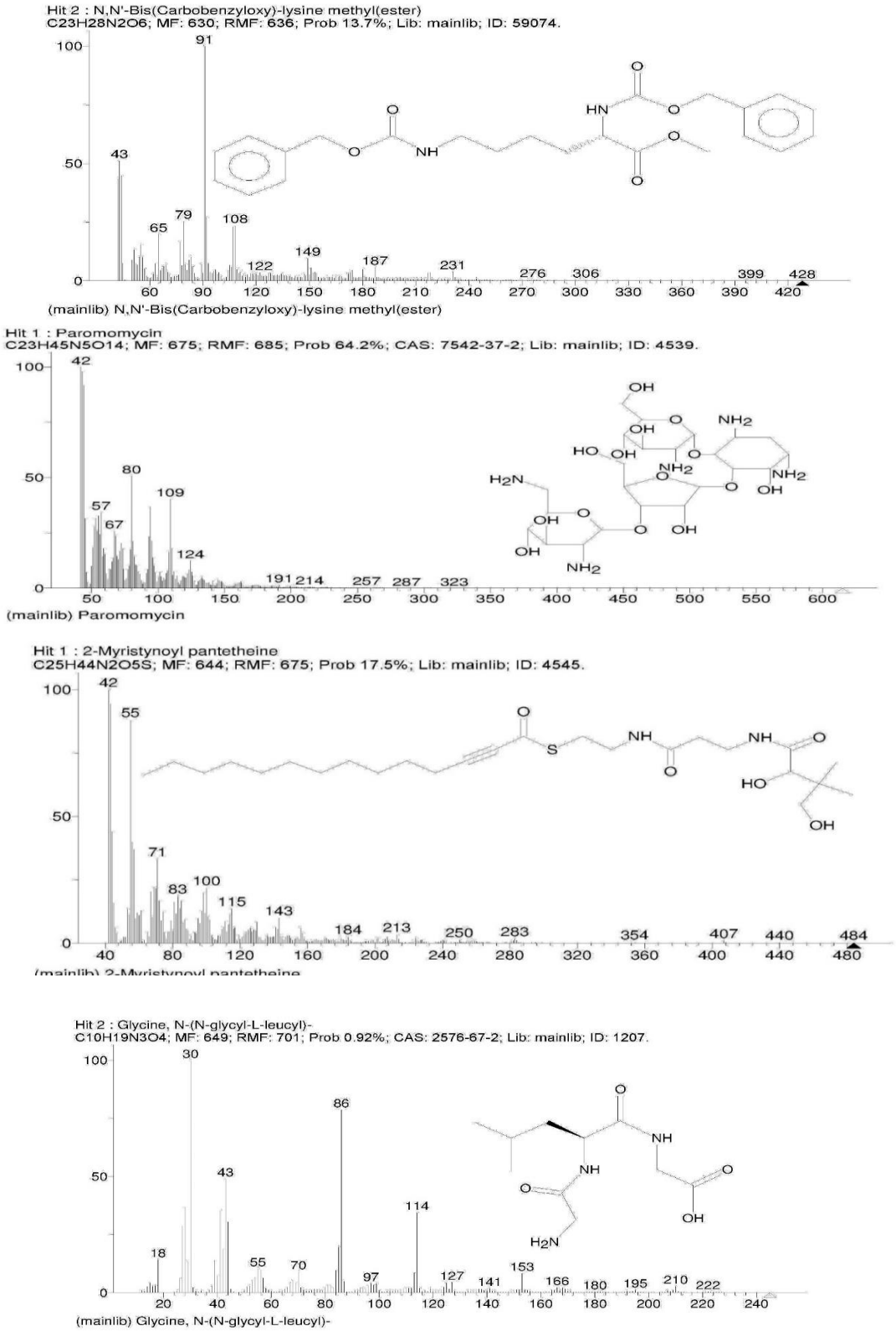
FTIR analysis was performed using Perkin Elmer spectrophotometer system, which was used to detect the characteristic peaks and their functional groups using ATR (Attenuated Total Reflectance) accessory. The IR scan was performed in the wave number region of 4000-550 cm-1 (mid- infrared range).

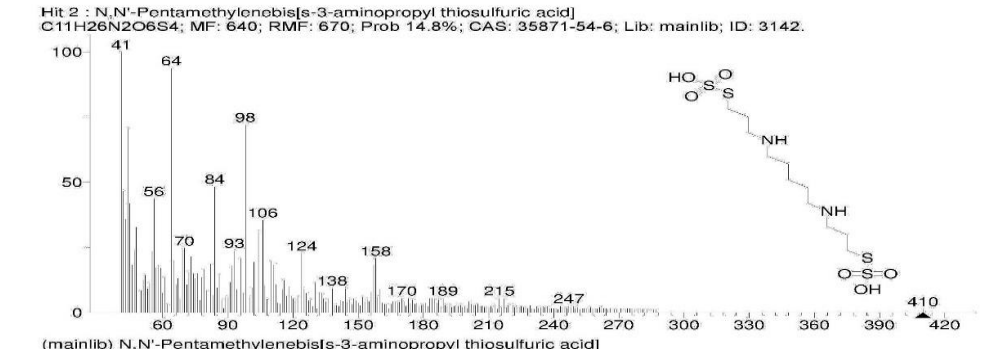
3. DISCUSSION

Medicinal plants have been used for centuries for the treatment of several diseases and to promote good health [21]. People that use spices in their diets are known to have low incidence of chronic diseases [22],. One benefit of spices and herbs is that they contain bioactive components such as polyphenols that can reduce oxidative stress and modulate harmful biological pathways. Several polyphenols have been shown to have scavenging activity as well as inhibiting amylase and glucosidase activities [23]. *Xylopia aethiopica* commonly known as “African guinea pepper” or “Ethiopian pepper” has several medicinal properties; it is anti-tumour, anti-asthmatic, anti-inflammatory and antimicrobial. It has been used for the treatment of hypotension and coronary vasodilatory conditions [24]. It has traditionally been used for the treatment of cough, dysentery, boils and sores [14]. The fruits of *Xylopia aethiopica* is used as a carminative, and as a post-partum tonic. Other medicinal uses are for stomach ache, treatment of bronchitis, biliousness and dysentery [25]. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in medicinal plants and traditional medicines. In recent years GC-MS studies have been increasingly applied for theanalysis of medicinal plants as this technique has proved to be a valuable method for the analysis of nonpolar components and volatile essential oil, fatty acids, lipids and alkaloids. . FTIR is the most powerful tool for identifying the functional groups present in compounds [26]. The presented study is carried out on the

bioactive compounds present in the *Xylopia aethiopica* fruits by the use of GC-MS and FT-IR techniques.

The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentration (peak area %) are presented in Fig.2, which shows the presence of 26 bioactive phytochemical compounds in the methanol extract of *Xylopia aethiopica* . The Fourier Transform Infrared Spectroscopy was used in identification of functional groups as presented in figure 3 and table 1

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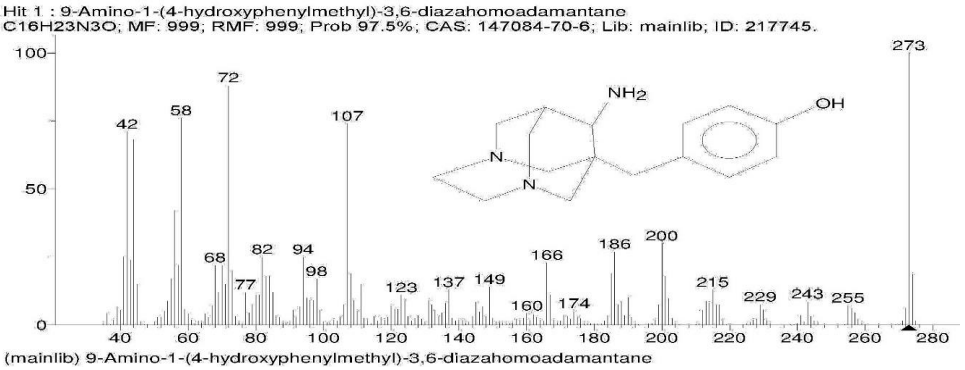
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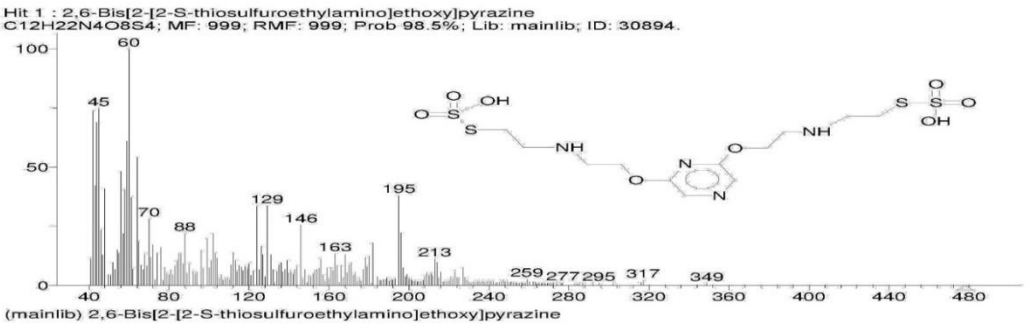
C16H23N3O; MF: 999; RMF: 999; Prob 97.5%; CAS: 147084-70-6; Lib: mainlib; ID: 217745.
(mainlib) 9-Amino-1-(4-hydroxyphenylmethyl)-3,6-diazahomoadamantane


Hit 1 : 2,6-Bis[2-[2-S-thiosulfuroethylamino]ethoxy]pyrazine
C12H22N4O8S4; MF: 999; RMF: 999; Prob 98.5%; Lib: mainlib; ID: 30894.
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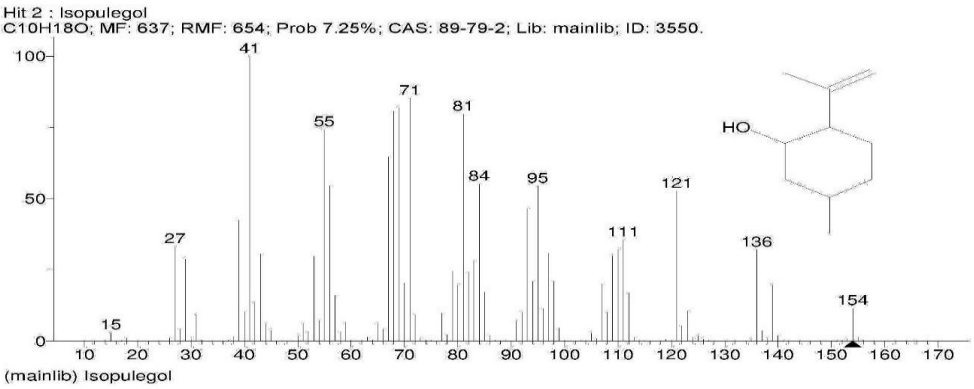
Hit 2 : Isopulegol
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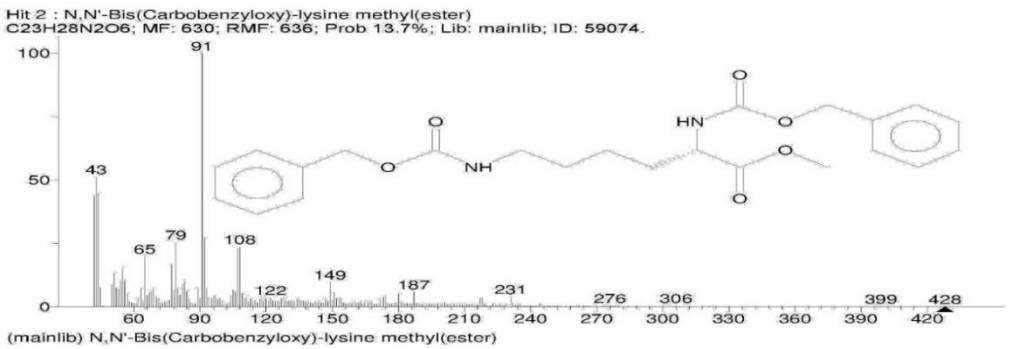
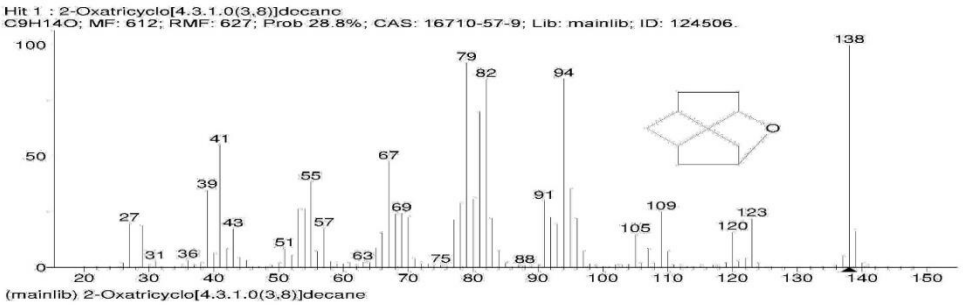

Hit 2 : N,N'-Bis(Carbobenzyloxy)-lysine methyl(ester)
C23H28N2O6; MF: 630; RMF: 636; Prob 13.7%; Lib: mainlib; ID: 59074.
(mainlib) N,N'-Bis(Carbobenzyloxy)-lysine methyl(ester)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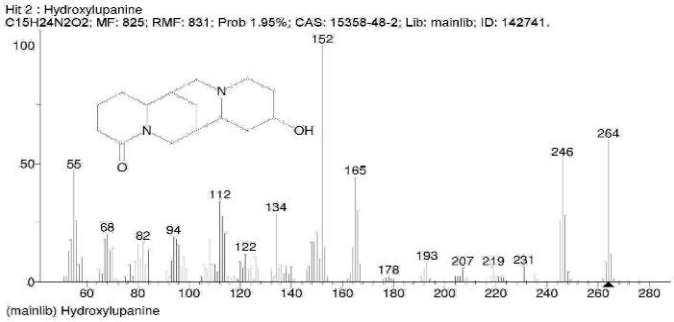
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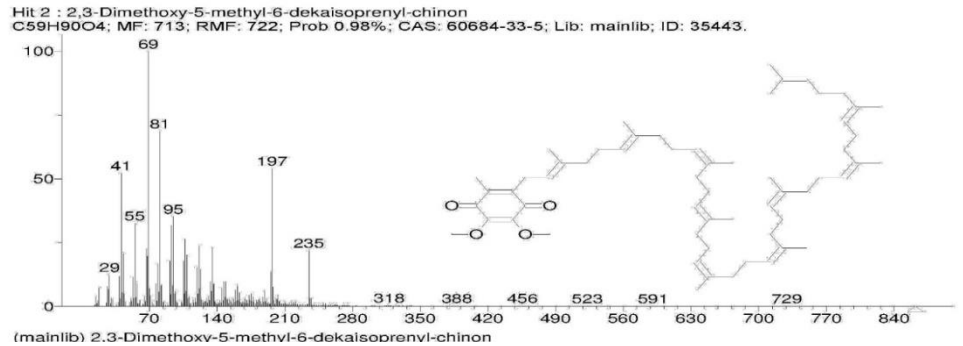
(mainlib) Hydroxylupanine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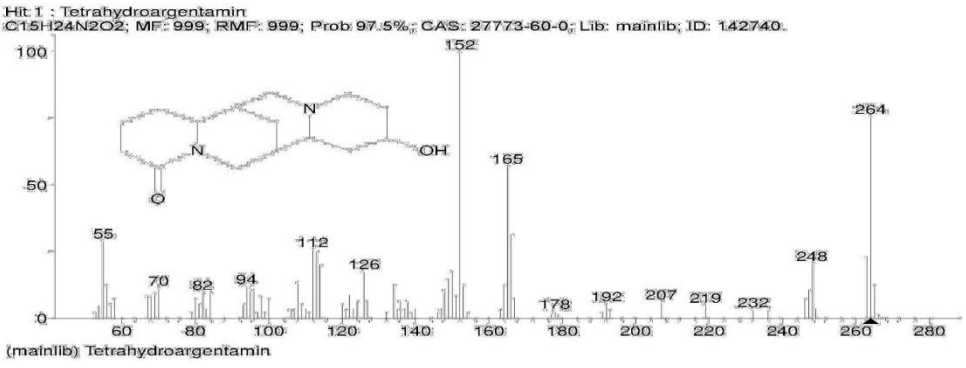
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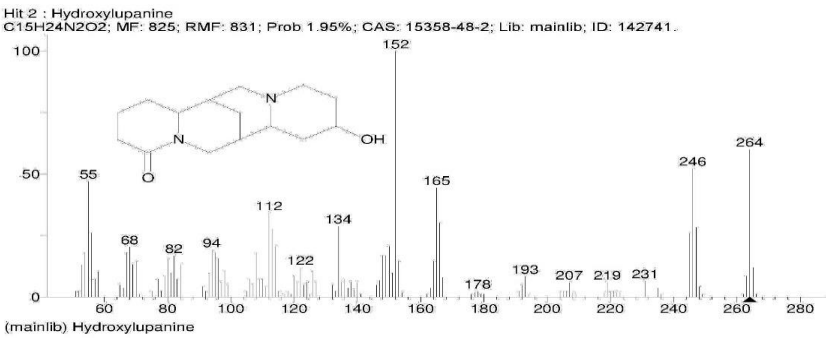
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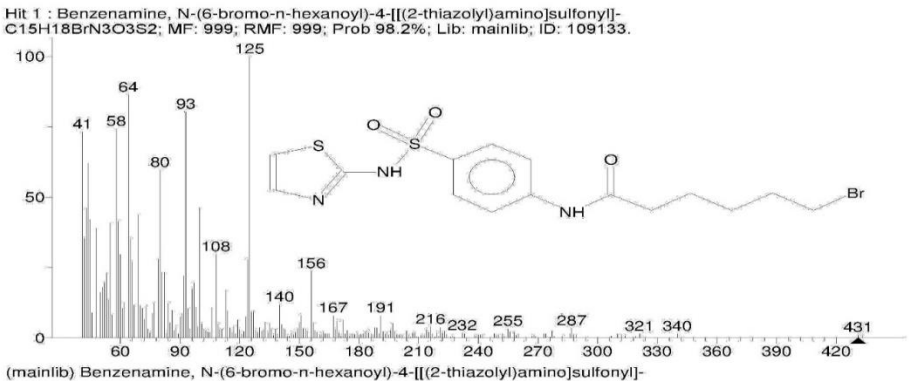
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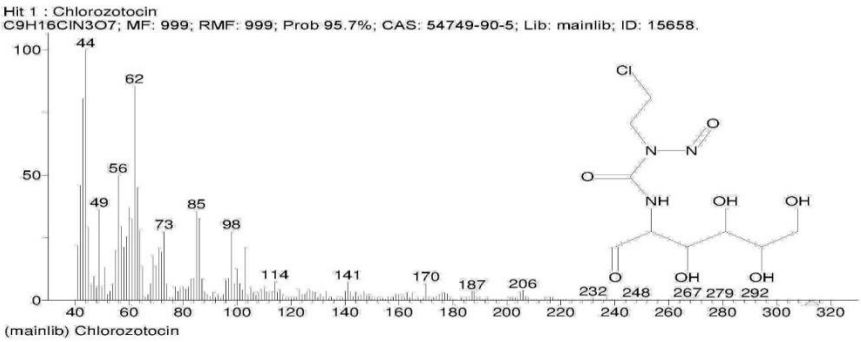
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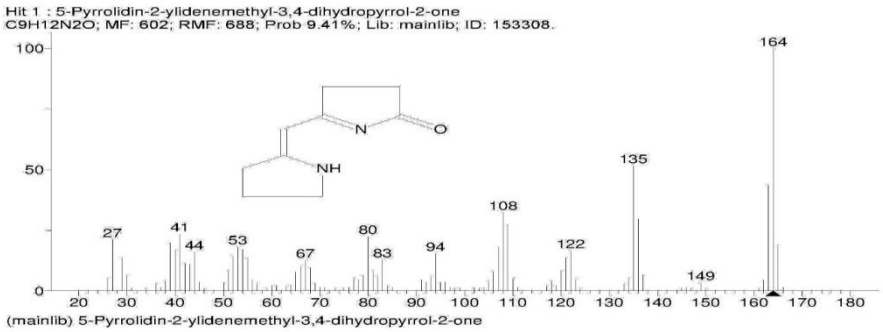
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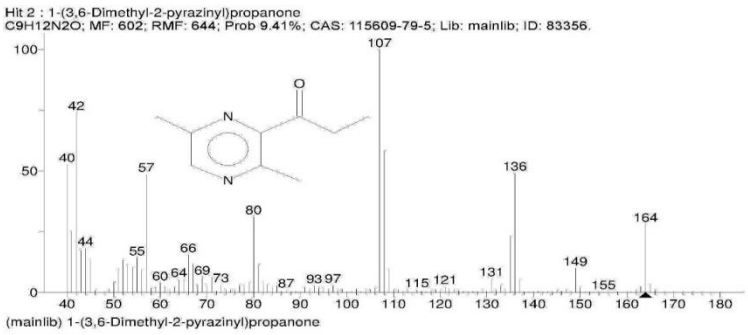
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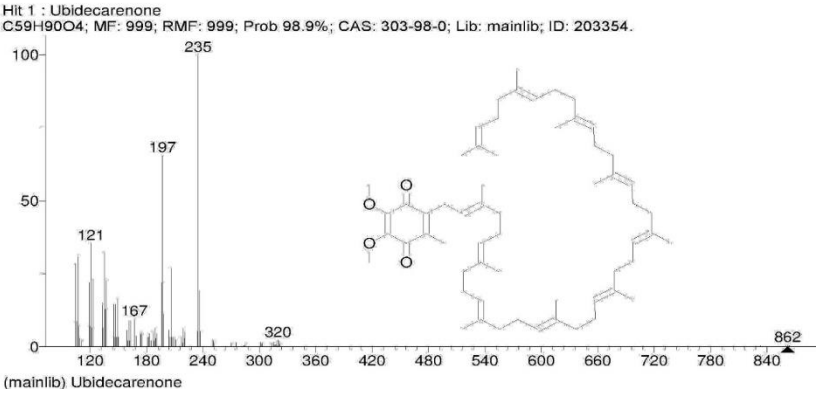
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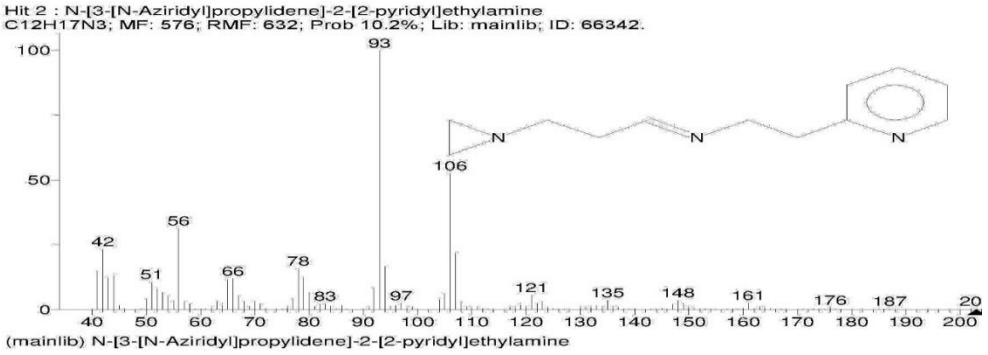
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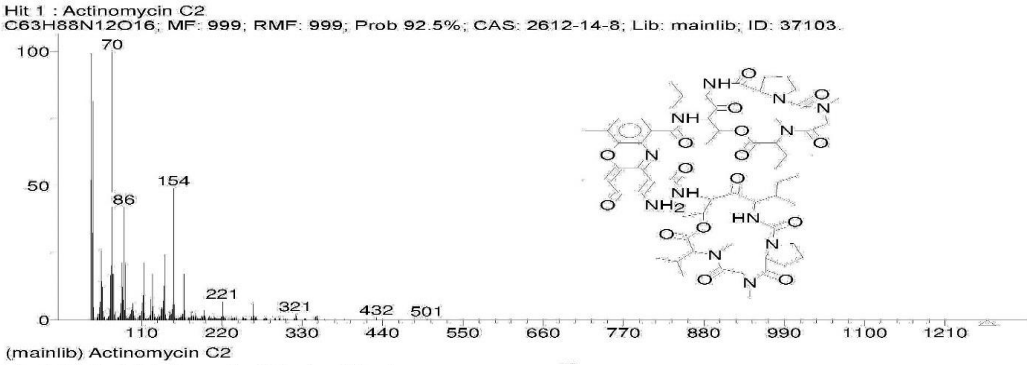
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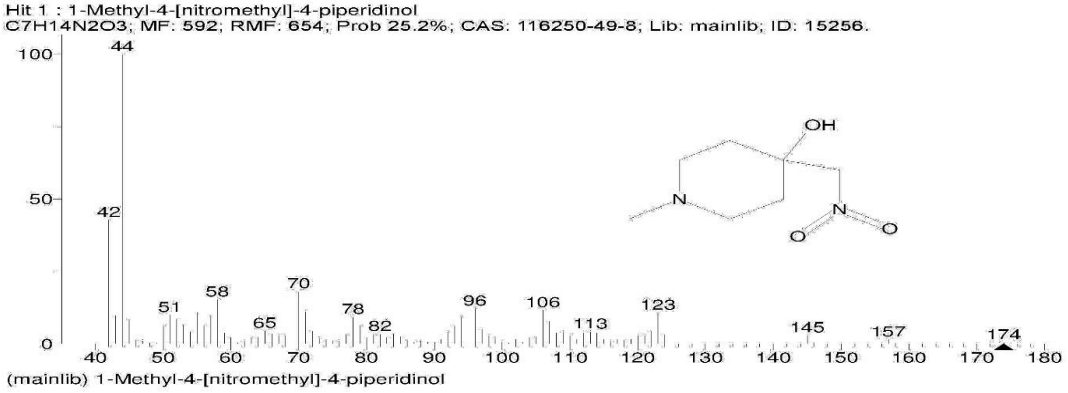
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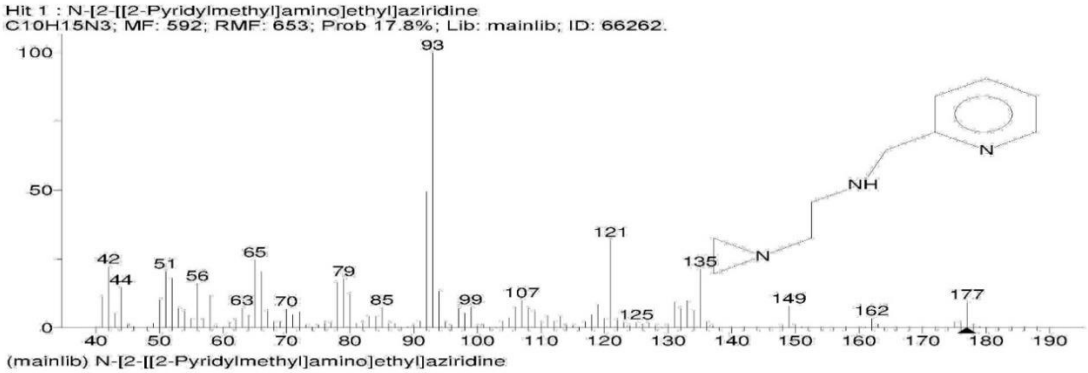
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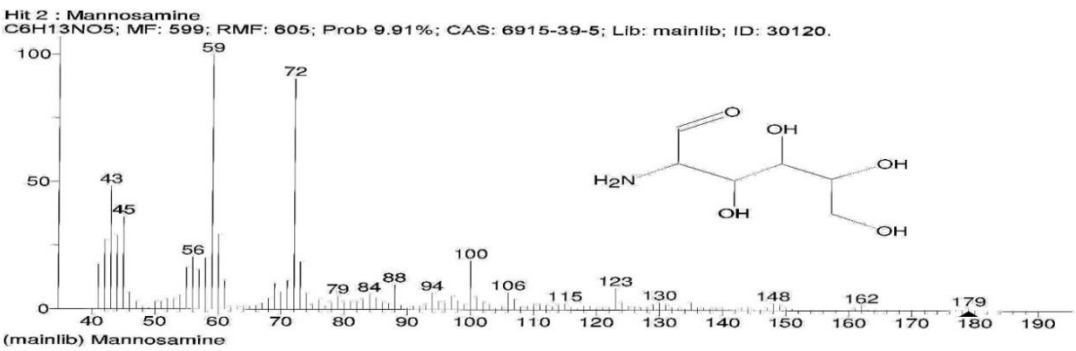
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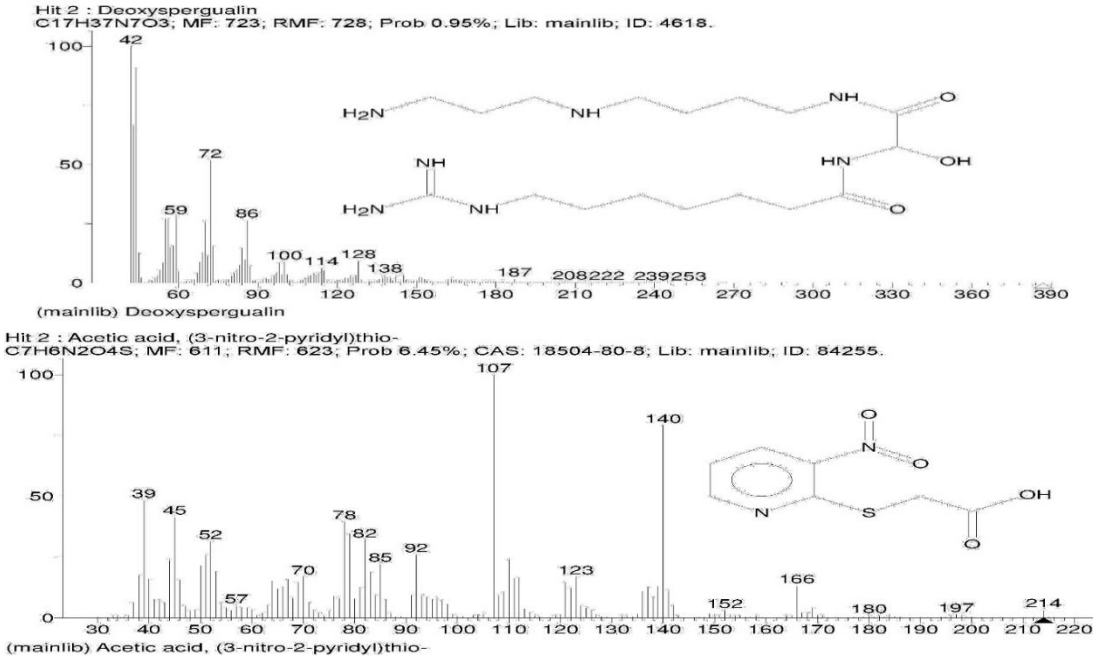

Figure2. *The Mass spectra of methanol fruit extract of Xylopia aethiopica*

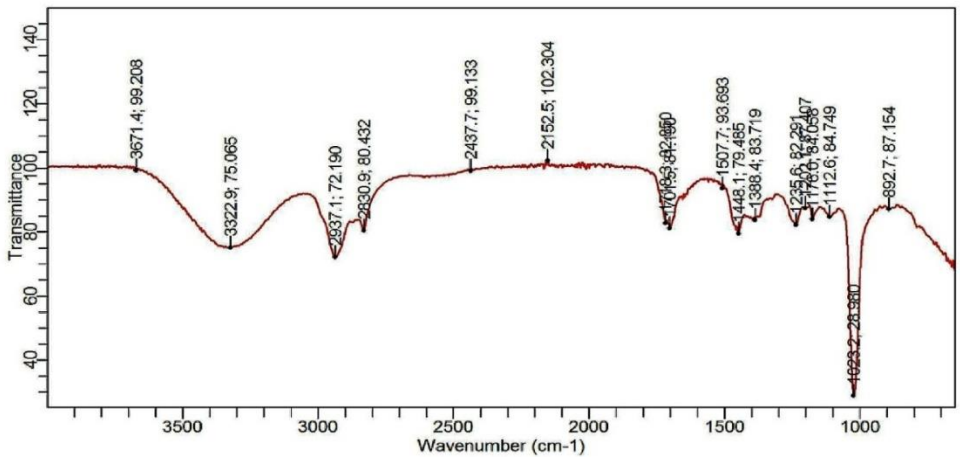

Figure3. *FTIR Spectrum of methanol fruit extract of Xylopia aethiopica*

Table1. *FTIR Analysis of Xylopia aethiopica*

Absorption frequency (CM^{-1})	Functional Group	Stretching/bending Vibration
3671.5	Hydroxyl	O-H
3322.9	Aliphatic amine	N-H
2937.1	Alkanes	C-H
2830.9	Alkanes	C-H
1701.5	Carbonyl	C=O
1507.7	Alkene	C=C bending
1448.1	Hydroxyl	O-H Bending
1176.0	Alcohol/Esther	C-O bending

4. CONCLUSION

At this moment, there is a growing demand globally in herbal medicines associated with improved laboratory investigation into the pharmacological properties of the bioactivities from natural source

and their ability to manage an assortment of diseases. Exploration of herbal medicines had created an avenue for several conventional and alternative therapies.The present study identified 26 bioactive compounds in the methanol extract of *Xylopia aethiopica* fruits using gas chromatography-mass spectrometry (GC-MS) and Fourier Transform Infra-red spectroscopy revealed the functional groups. This plant has many bioactive compounds that possess antioxidant, anti-inflammatory, anti-microbial and anticancer properties. This showed that the plant extract should be of great pharmaceutical interest. Haven identified many bioactive compounds in methanol extract of *Xylopia aethiopica* fruits in the present study, and it is recommended that the active ingredients are isolated and subjected to further tests to compare their usefulness in the prevention and treatment of various ailments.

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