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Advanced phytochemistry and chemo-metric profiling of the bioactive medicinal components of n-hexane seed extract of *Xylopia aethiopica* using FTIR and GC-MS techniques

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Abstract

The use of herbal medicine has achieved global success as an ethno-medicine, yet there are lots of drawbacks in their commercial value and wide acceptance due to poor regulation and standardization. Present study aims to chemo-metrically characterize the phyto-chemical principles present in the n-hexane seed extracts of *Xylopia aethiopica* using chromatographic and spectroscopic methods. The crude n-hexane extracts were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) model 8400S and GC-MS (Model-QP 2010 plus Spec). The identification of compounds was done using NIST ver.2.0-year 2005 Library. The biological activity are based on Dr. Duke's phytochemical and ethno-botanical databases. The FTIR revealed functional groups such as alkenes, alkanes, alcohols, aromatic rings as well as fatty acids. The GC-MS revealed phyto-compounds such as alpha-Terpineol, alpha-cubabene, pinocarvone, copaene and alpha-muurolene. The presence of these vital phytochemicals with excellent pharmacological activity may explain their usage in traditional medicine.

Keywords: *Xylopia aethiopica*; GC-MS.; FTIR; Phytochemical database; Spectroscopy; Herbal medicine

1. Introduction

Phytochemicals, are produced as secondary metabolites in plants that have beneficial effects on health when they are consumed as nutrients. They have an effective role in the formation of the color, smell and taste of the plants [1]

Some activities of phytochemicals includes antioxidant activity, antioxidants are substances that may protect the cells against free radicals, which may play a role in heart disease, cancer and other underlying diseases. Free radicals are molecules produced when your body breaks down food or when an individual is exposed to tobacco, smoke or radiation.

Phytochemicals have potential anti-oxidant activity and are able to scavenge hydroxyl radicals, capture peroxyl radicals, inhibits hydrogen peroxide, and quench reactive oxygen species. Some phytochemicals have proven to show higher activities than vitamin C and Vitamin E. Phytochemicals with anti-oxidant activity include: Allyl sulfides (onions, leeks, and garlic), carotenoids (fruits, carrots), flavonoid (fruits, vegetables), Polyphenols (tea, grapes) [2].

Uda fruits popularly known as *Xylopia aethiopica* has shown to possess varied and different classes of compounds such as: octatriene, cyclohexanemethanol, kaurene, octadecadienoic acid, cyclodecadiene, cyclohexane, copaene and silane.

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The chemical and bioactive components and mineral constituents of *Xylopia aethiopica*, which is valued as a spice in Nigeria, were determined along with the physicochemical characteristics of the seed oil. The seeds has the following chemical components: moisture, ash, crude lipids, crude proteins, fatty acids, crude fiber, carbohydrates, and starch. Calcium and potassium are the major minerals in the seed. The extracts of lipids and fatty acids is highly examined for nutritional health benefits. Linoleic and oleic acids were the predominant unsaturated fatty acids, while palmitic acid is the major saturated acid. Xylopia contains a varied amounts of secondary metabolites which accounts for its different chemical class and its diverse biological activities. Such biological activities include anti-fertility, anti-malaria, cardio-protective, useful in haemorhagic conditons, anticancer, anti-convulsant, and anti-ulcer and a host of curative supplements [3].

The seeds and fruits of *Xylopia* contains a host of active medicinal ingredients and essential oils such as pinene, terpinene, trans-pinocarcavone, and p-cymene. Major constituents of the oil are pinene, cadinol, and cineole.

Xylopia aethiopica or Ethiopian pepper is a plant that thrives in the evergreen rain forests of tropical and subtropical Africa, and it is currently grown most prominently in Ghana as a crop. Almost all parts of *Xylopia aethiopica* possess great medicinal values in traditional medicine. In most parts of Africa, it is used in the treatment of cough, rheumatism, dysentery, malaria, uterine fibroid, boils.

Figure 1 *Xylopia aethiopica* tree

Figure 2 (*Xylopia aethiopica*) seeds

Herbal remedy have been known to be source of bioactive green chemicals which can be used to prevent and manage various human health challenges whether chronic or acute diseases [4]. About 80 % of the population in the developing countries depend on herbal medicine for their primary health needs [5].

Gas Chromatography- Mass Spectrometry (GC-MS) is an analytical method that combines or encompasses the features of both gas-chromatography and mass spectrometry to identify different substances within a test sample, like liquid chromatography-mass spectrometry , it allows analysis and detection even of tiny amounts of a substance [6]. The GC-MS is composed of two major building blocks; the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column whose properties regarding molecule separation depends on the column's dimension (length, diameter, and film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane) [7]. The

difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column.

In this present research, chemo-metric evaluation of *Xylopia aethiopica* n-hexane seed extract using advanced instrumentation method such as GC-MS, FTIR and UV spectroscopy were carried out to evaluate the phyto-chemical components thereby justifying their ethno-medicinal usage.

2. Material and methods

2.1. Chemicals

All chemicals used including n-hexane were of analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA)

2.2. Plant Materials and Extraction

The seeds of *Xylopia* was cut and grinded into tiny pieces after collection and carefully dried under the shade to remove moisture this was carried out for 72 hours. The dried seeds were pulverized with the aid of a mechanical mill or grinder. A 1,950 g of the pulverized seed powder were macerated using one liter of n-hexane for three days with intermittent agitation and shaking. The extract were obtained by filtration using a filter paper. The filtrate obtained were concentrated under vacuum and reduced pressure (BUCHI, Rotavapourator R – 205, (BUCHI Labortechnik, AG CH-9230, Flamil, Switzerland) at 40 ^{o}C. The dried extract was carefully scrapped and placed into the sample bottles and refrigerated till use.

2.3. Preparation of extracts for GC-MS analysis

The different dried extracts (n-hexane) were re-dissolved in their respective solvents, vortexed and filtered through 0.45 mm syringe filter. 1 mm aliquot solution of the sample was injected into the GC-MS equipment.

2.4. Instrumentation and chromatographic conditions

GC-MS analysis was carried out on a GC-MS (model-QP2010 plus Shimadzu Tokyo, japan) which comprises of AOC-20i auto-sampler and gas-chromatography hyphenated to a mass spectrometer (GC-MS) instrument equipped with a VF 5 ms fused silica capillary column of 30m length, 0.25 mm diameter and 0.25 micro-metre film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was applied. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.58 m/min. The injector and mass transfer line temperature were set at 250 to 200^{o}C respectively, and an injection volume of 1micro liter was employed (split ratio 10:1). The oven temperature was programmed from 80 degree Celsius (isothermal for 1 minute). with an increase of 10 ^{o}C per minute to 200^{o}C for 4 minutes at 10 ^{o}C per minute to 280^{o}C The MS operating parameters were as follows; ionization energy, 70eV; ion source temperature, 200 degree Celsius, solvent cut time, 2.5 minutes, relative detector gain mode, scan speed 1666 mps; scan range 40-800 micro-meter, the interface temperature is 250^{o}C. The total running time of GC-MS was 30 minutes. The relative percentage of the extract was expressed as percentage with peak area normalization [9].

2.5. Identification of components

The relative percentage amount of each phyto-component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standard and Technology) Ver. 2.2 Year 2005 library. The compound's biological activity prediction is based on Dr. Duke's J. phytochemical and Ethno-botanical Databases by Dr. Duke J. of the Agricultural Research Service/USDA [8]. Interpretation of GC-MS was conducted using the database of NIST having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name and molecular weight of the phyto-components of the test materials were ascertained [10].

2.6. Fourier Transform Infrared Spectroscopy Analysis

This is an analytical technique used to identify organic, polymeric and inorganic materials. FTIR uses fourier transform (a mathematical process to convert the raw data into actual spectrum. An FTIR spectrometer collects high resolution spectral data over a wide range [11]. The FTIR spectrometer sends infrared radiation of about 10,000 to 100 per centimeter through a sample, with some radiation absorbed by the sample. The absorbed radiation is converted into rotational and/or vibrational energy by the sample molecules. The radiation is modified to contain a different combination of frequency. The radiation is generated by starting with a broad light source which contains the full spectrum of wavelength to be measured. The light shines into an interferometer which is a configuration of mirrors. As

the mirror moves, each wavelength in the beam is periodically blocked, transmitted, blocked, transmitted, by an interferometer, due to wave interference, Computer processing is required to turn the raw data (light absorption for each mirror position) into the desired result (light absorption for each wavelength) [12].

10 mg of the individual extract was diluted with 50 ml of the corresponding solvent, centrifuged at 3000rpm for 10min and filtered using What-mann No 4 filter paper using vacuum pump. A further 1:10 dilution of the centrifuged solution was made with the solvent. The extract obtained was scanned in the wavelength ranging from 200-800 rpm using the UV-2500 PC series Ver. 2.30 spectrophotometer and the characteristic peaks were noted. The diluted extract above was used in carrying out the FTIR analysis using FTIR-8400S spectrometer system. The characteristic peaks were also recorded.

3. Results

Table 1 FTIR peak Values (cm^{-1}) and Functional Groups of Different Phyto-components Identified in the n-hexane and Ethanol Extract of *Xylopia aethiopica* Dunal A. Rich (Annonaceae)

Peak values	Description/ intensity	Likely functional groups present	Inference
3100-3700	Sharp, Broad Strong	-OH -COOH C=O Carbonyl aldehyde	CH CH3
1722	Sharp, Strong	Ketone Carboxylic acid	-CH C=O
2900	Sharp, Strong	Esters, Amides	Aromatic Compounds
1600	Weak	Alkenes(double bond)	C=C

3.1. Some Mass - Spectral Peaks Observed/NIST Library Comparison

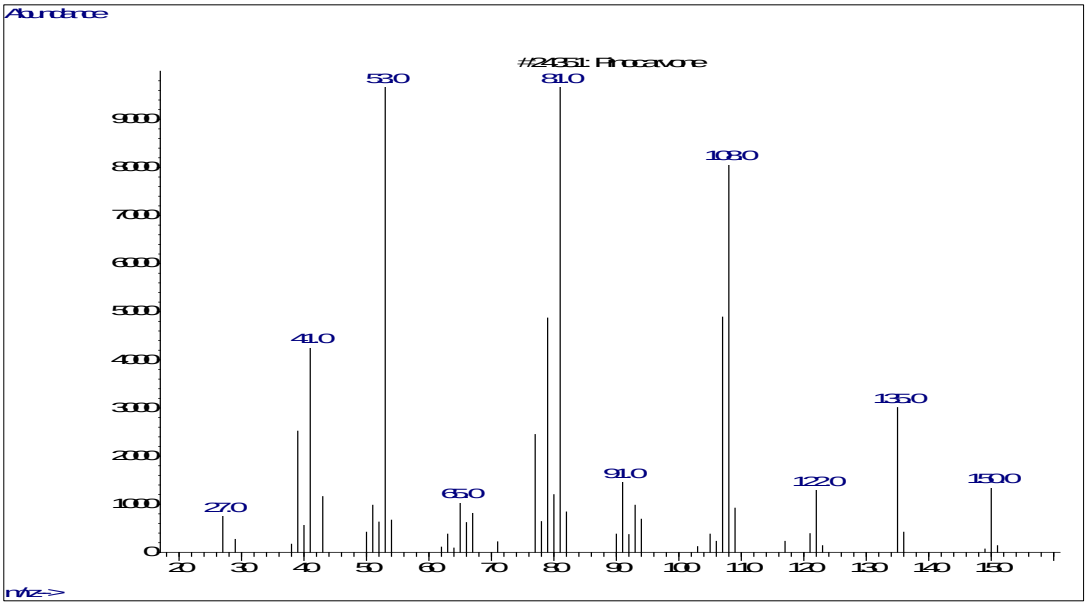

Figure 3 Observed Mass spectral data for Pinocarvone

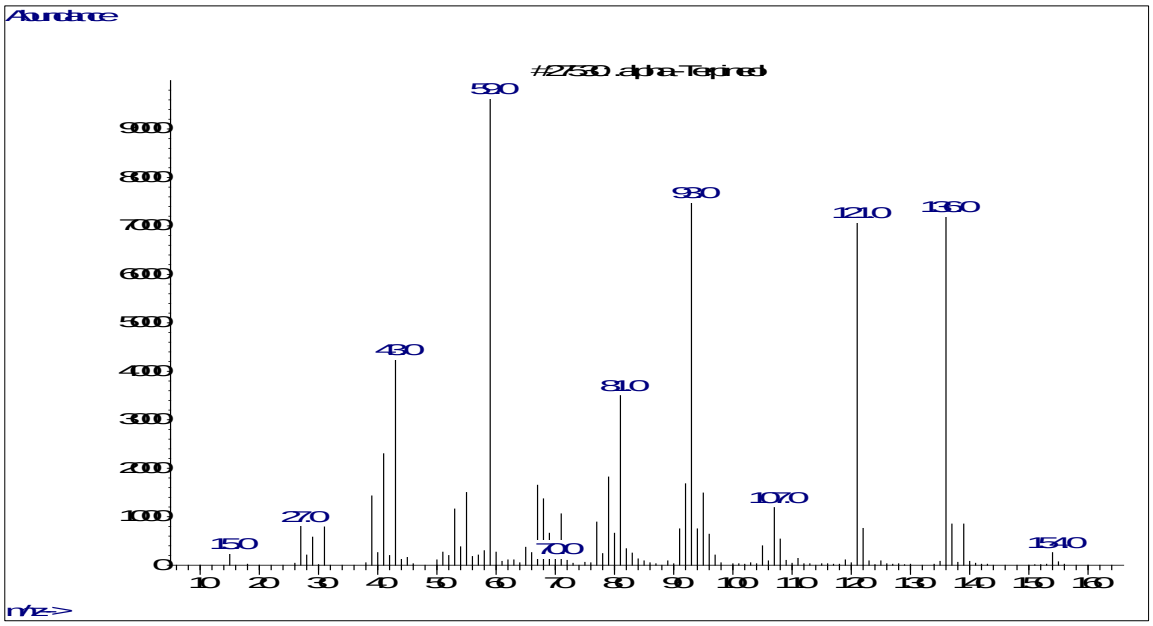

Figure 4 Observed Mass spectral data for alpha-Terpineol

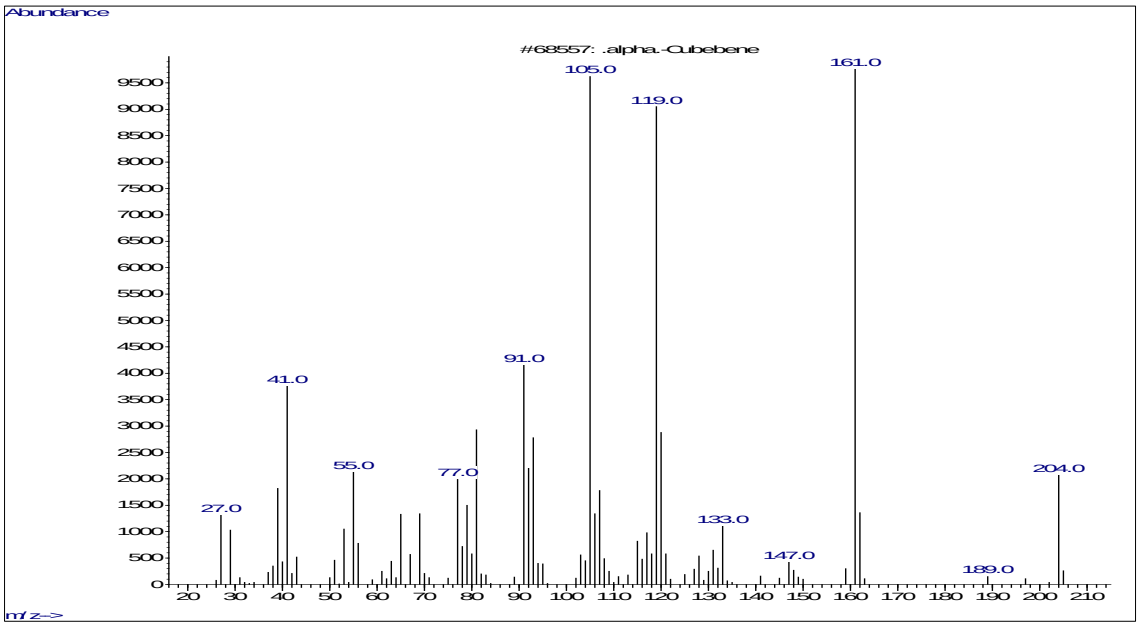

Figure 5 Observed Mass spectral data for alpha-Cubebene

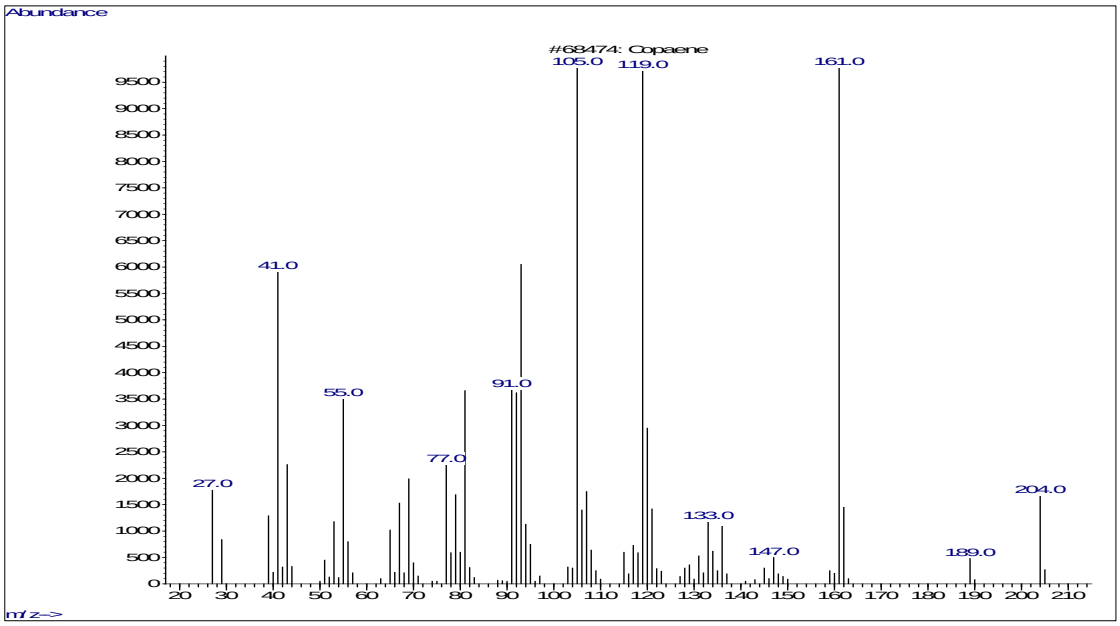

Figure 6 Observed Mass spectral data for Copaene

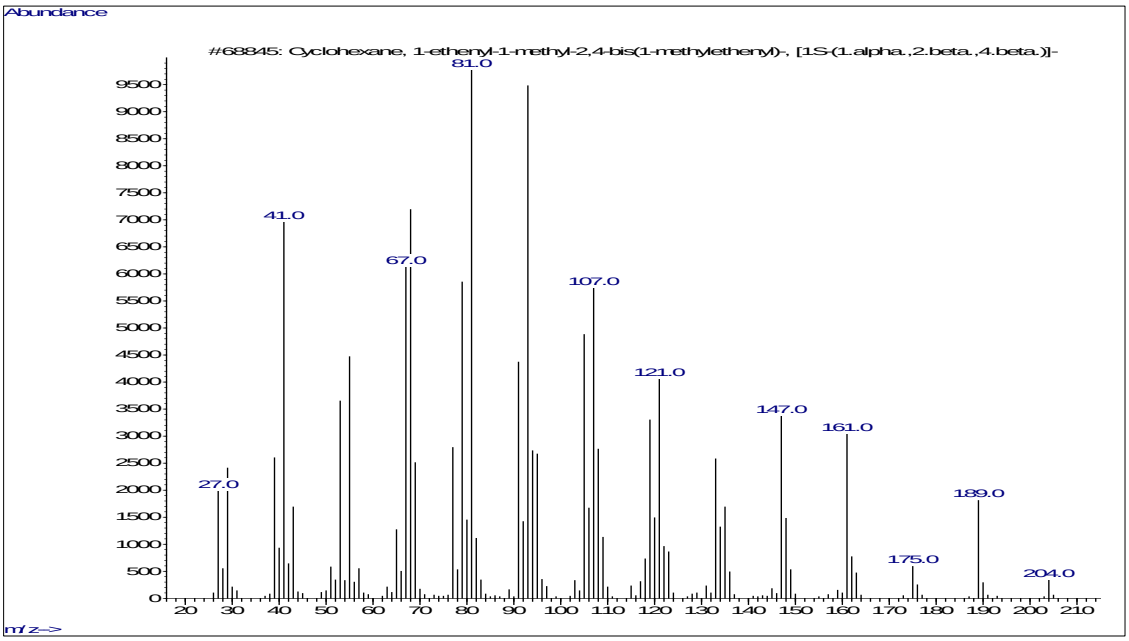

Figure 7 Observed Mass spectral data for Cyclohexane derivative

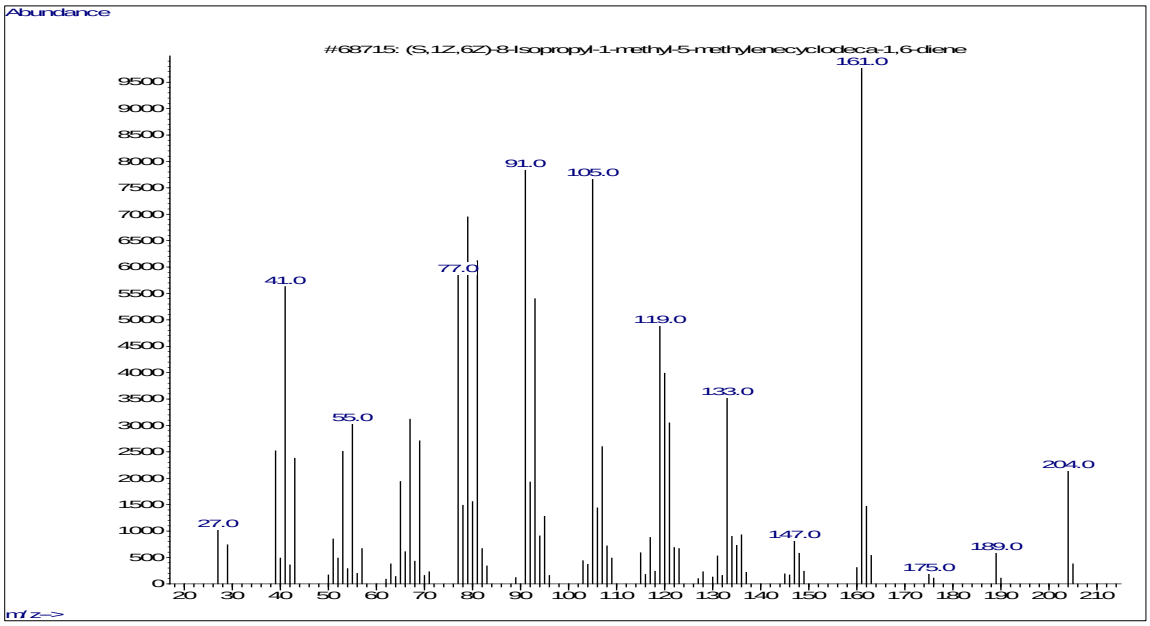

Figure 8 Observed Mass spectral data for Isopropyl derivative

Table 2 Biological Activities of Some bioactive compounds Present in the Different Extracts of Xylopia aethiopica Dunal .A. rich (Annonaceae)

Phytocomponent	**Nature of compounds**	**Biological activities**
α-TERPINEOL	Methane monoterpenoids	Anti-nociceptive, Anti-convulsants, sedative and hypnotic activities, Anti-bronchitis, Insecticidal actions,cardiovascular actions, Anti-hypertensive, and anti-oxidant
PINOCARVONE	Monoterpenoids	Calming and sedative, mucolytic, expectorant, Analgesic, digestive, wound healing
α-CUBEBENE	Tricyclic sesquiterpenoids	Anti-oxidant, anti-proliferative, anti-genotoxic, anti-tumor
COPAENE	Tricyclic sesquiterpenoids	Anti-pyretic, Anti-fungal, demulcent, cathartic activity, laxatives
NAPHTHALENE	Polycylic Aromatic molecule	Insecticides, dyes, deodorants, anti-neurodegenerative, anti-psychotic, anti-cancer, anti-microbial, anti-diabetic, anti-inflammatory
α-MUUROLENE	Sesquiterpenoids	Anti-diabetic, anti-emetic actions, anti-inflammatory, anti-hypertensives, anti-diuretics
α-FARNESENE	Cyclic sesquiterpenoids	Anti-pyretic, analgesic, anti-inflammatory, anti-congesants
γ-ELENIENE	Sesqquiterpenoids	Anti-cancer, Anti-tumor, Anti-inflammation
ISOPATHULENOL	Saturated hydrocarbon	Anti-oxidant, anti-inflammatory, anti-congesant Anti-tumor actions, cosmetics uses/actions

Table 3 Phytocomponents identified in the n-hexane seed extracts of X*ylopia aethiopica* dunal a. rich (annonaceae) by GC-MS, FTIR analysis

Sn	Retention time(sec)	Name of compounds	Molecular formula	Molecular weight g/mol	Peak area %	% composition
1	5.9168	α-TERPINEOL	$C_{10}H_{18}O$	154.25	0.8823	35
2	5.3312	PINOCARVONE	$C_{10}H_{14}O$	150.2176	0.138	87
3	9.404	α-CUBEBENE	$C_{15}H_{24}$	204.357	0.333	93
4	10.0304	COPAENE	$C_{15}H_{24}$	204.36	0.8491	96
5	12.4727	NAPHTHALENE	$C_{10}H_8$	128.1705	0.519	98
6	13.0565	α-MUUROLENE	$C_{15}H_{24}$	204.35	0.4743	95
7	14.242	α-FARNESENE	$C_{15}H_{24}$	204.35	0.3995	80
8	14.3488	γ-ELENIENE	$C_{15}H_{24}$	204.357	1.0818	95
9	16.1234	ISOPATHULENOL	C_5H_{12}	72.15	4.2289	91
10	22.5148	HEXADECANOIC ACID	$C_{16}H_{32}O_2$	256.4	0.4329	93
11	24.5679	KAUR-1,6-ENE	$C_{20}H_{32}$	272.5	1.8854	86
12	27.4534	HEXADECANOIC ACID	$C_{16}H_{32}O_2$	256.4	1.4156	99
13	37.7659	OLEIC ACID	$C_{18}H_{34}O_2$	282.47	0.4235	90
14	37.522	CYCLOHEXADIENE	C_6H_8	80.13	0.52	95
15	13.607	ISOPROPYL	C_3H_8O	60.1	0.90	96
16	25.757	OCTADECADIENOIC	$C_{18}H_{32}O_2$	280.447	0.40	99
17	22.517	PENTADECANOIC ACID	$C_{15}H_{30}O_2$	242.397	0.36	98

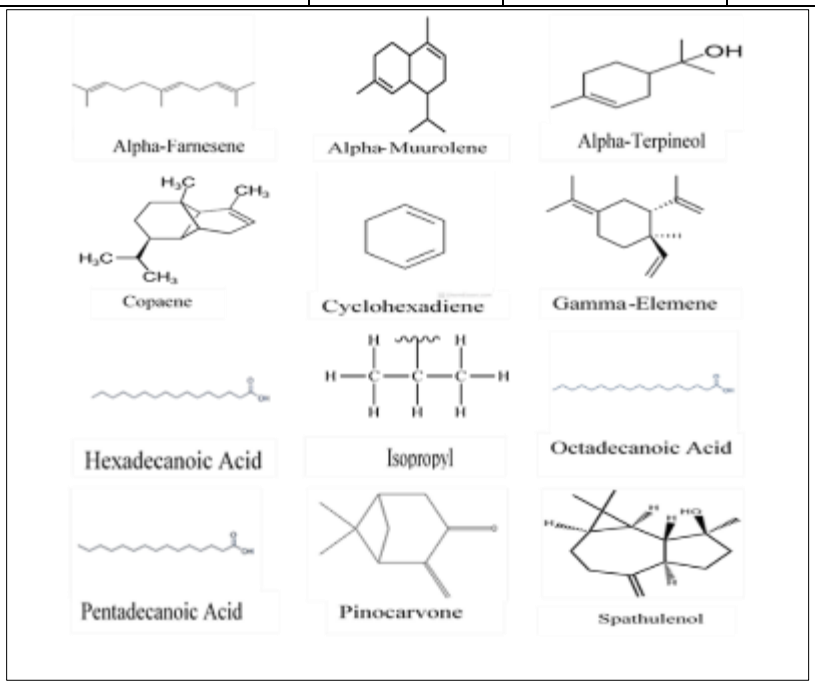

Figure 9 Chemical structures of bioactive compounds present in n-hexane seed extracts of X*ylopia aethiopica* dunal a. rich (annonaceae) by GC-MS

4. Discussion

Some of the Identified Compounds which have been Reported to Exhibit Varied Biological Activities include: Alpha-terpineol, this is a methane monoterpenoid compound with the chemical formula $C_{10}H_{18}O$, this bio-active compound has the following biological activities ranging from -anti-nociceptive, anti-convulsants, sedative, hypnotic activities, anti-bronchitis, Insecticidal actions, cardiovascular actions, anti-hypertensive, and anti-oxidant. Pinocarvone, is a monoterpenoid compound with chemical formula $C_{10}H_{14}O$, its biological activities ranges from; calming and sedative effects, mucolytic actions, expectorant activities, digestive supplements and wound healing. Alpha- cubebene, is a carbon-tricyclic sesquiterpenoids with chemical formula $C_{15}H_{24}$, its biological activities ranges from post-natal dressing, anti-tumor, anti-genotoxic, anti-oxidant, anti-proliferative actions. Naphthalene is an Aromatic polycylic molecule with chemical formula $C_{10}H_8$, its biological activities ranges from anti-cancer, anti-microbial, anti-inflammatory, and anti-diabetic, anti-neurodegenerative, Insecticides, deodorants and anti-psychotic dyes [13].

Alpha-muurolene is a sesquiterpenoid compound with the chemical formula, $C_{15}H_{24}$ its biological activity ranges from anti-tumor, laxatives, carthartic activities, neuromuscular agents, anti-oxidants [6]. Alpha-farnesene is a cyclic sesquiterpenoids with chemical formula $C_{15}H_{24,}$ its biological activities ranges from anti-pyretic, analgesic, anti-inflammatory, anti-diabetic actions. Gamma-Elemene is sesquiterpenoid with chemical formula $C_{15}H_{24}$, its biological activities ranges from anti-cancer, anti-inflammatory,anti-oxidants, lipid lowering activities, anti-hypertensives, anti-diabetic. Isopathulenol is saturated hydrocarbon with chemical formula C_5H_{12}, its biological activities ranges from anti-oxidants, anti-inflammatory, anti-congesants, demulcents, laxatives, sedative actions [14]. Hexadecanoic acid is a saturated fatty acid with chemical formula $C_{16}H_{32}O_2$, it possesses a wide variety of biological activities ranging from Purgatives, laxatives, anti-microbial, cosmetics, make-up actions. Kaur-16-ene is a diterpenoids with the chemical formula $C_{20}H_{32}$, its biological activities ranges from anti-cancer, purgatives, anti-diabetic, bone healing, sedatives, and analgesics [15].

Octadecadienoic acid is an unsaturated fatty acid, with the chemical formula $C_{18}H_{32}O_2$ has a wide range of biological activities ranges from anti-lipidemia, anti-cholesterol, anti-fungal, bacterioicidal actions, and sedatives [16 -20]. Pentadecanoic acid is a saturated fatty acid ,with the chemical formula $C_{15}H_{30}O_2$ its biological actions ranging from anti-thrombosis, flavoring agent, anti-bacterial, cosmetics, anti-bacterial.

5. Conclusion

This research could provide a chemo-metric characterization for the proper identification of n-hexane extract of *Xylopia aethiopica* which may suggest some evidence for their ethno-medicinal uses in different parts of Nigeria. Efforts should also be made to use other spectroscopical methods to analyse the polar fraction so as to capture all the possible molecules present in this rare medicinal plant.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest

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