



Analysis of bioactive compounds in ethanolic extract of *Xylopia aethiopica* leaves using gas chromatography and mass spectrometry (GC-MS) technique

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Abstract: This study investigated the bioactive compounds in the ethanolic extract of *Xylopia aethiopica* leaves using gas chromatography and mass spectrometry (GC-MS) technique. The phytoconstituents present in *Xylopia aethiopica* leaves were: flavonoids (951.82 mg/g), tannins (282.70 mg/g), alkaloids (188.47 mg/g), phenols (603.25 mg/g), saponins (11.47 mg/g), steroids (91.20 mg/g), oxalates (190.32 mg/g), and glycosides (190.32 mg/g). A total of 30 bioactive compounds were identified based on their peak areas. The major compounds greater than 1 % were; 13-docosenamamide (21.09 %), terpineol (10.07 %), 1,6-cyclodecadiene (9.37 %), copaene (2.88 %), caryophyllene (8.15 %), β -ocimene (6.05 %), β -myrcene (5.09 %), copaene (2.38 %), 2 - methoxy-4-vinylphenol (1.72 %), β -elemenone (1.31 %), 3,4-dimethylphenyl heptyl ether (1.26 %), ethyl oleate (1.07 %) and γ -elemene (1.27 %) while those less than 1 % (< 1 %) were; 2- methylenebornane (0.66 %), 2-methoxy-2-propenyl (0.72 %), hexadec-7-enal (0.23 %), hexadecanoic acid (0.02 %), didodecyl benzene 1,2 dicarboxylate (0.09 %), methyl stearate (0.16 %), 9,12-octadecadienoic acid (0.08 %), hexadeca-7,10 - dial (0.47 %), 1,1,5 -trimethyl -1,2-dihydronaphthalene (0.01 %), propane, 1,1 - oxybis -3- chloro (0.08 %), 1-trimethylsilylpent-1-en-4-yne (0.03 %), bicyclo[13.1.0] hexadecan-2-one (0.02 %), methyl octadeca-9-yn-11-trans-enoate (0.51 %), cis-linaloxide (0.22 %), tetradecanoic acid, 10,13 -dimethyl ester (0.18 %), didodecyl benzene 1,2 -dicarboxylate (0.47 %) and 2-cyclopentene -1-one, 2 - hydroxy (0.09 %). However, all the compounds have a wide range of pharmacological activities including antimicrobial, antioxidant, anti-malarial, antifungal, anti-arrhythmic, anti-viral, hepato-protective, anti-proliferative, anti-depressant, antipyretic, and antihelminthic.

Keywords: *Xylopia aethiopica*, anti-microbial, phytochemicals, gas chromatography, mass spectrometry, free radicals.

1. Introduction

The demand for herbal medicines has increased globally due to the growing recognition with common consideration that plant-based medicines are safe, non-toxic, environmentally friendly, easily available, and affordable (Alagbe, 2022; Shah et al., 2021). The efficacy of herbs can be linked to the presence of phytochemicals or bioactive compounds that perform therapeutic effects (antioxidants, anti-microbial, hepat-protective, immune-modulatory, hypolipidemic, anti-tumor, antifungal, antiviral, anti-proliferative, antipyretic, anti-depressant, anti-fibrotic, antihelminthic, anti-androgenic, and analgesics) in human beings and animals (Agubosi et al., 2022; Oluwafemi et al., 2020; Sharma et al., 2022). Phytochemicals are generally regarded as chemicals of plant origin used by plants for growth, defense against competitors, predators, and pathogens (Omolere & Alagbe; Shittu & Alagbe, 2020). According to Adewale et al. (2021), there are over 300,000 species of herbal plants with pharmaceutical properties. Among the probable and underutilized herbal plants is *Xylopia aethiopica*.

Xylopia aethiopica (African pepper), belonging to the Annonaceae family, is an aromatic, evergreen tree native to lowland rainforest in the savannah zones of Africa and most parts of Asia (Burkill, 1985; Orwa et al., 2009). The tree can grow up to 15 – 30 meters high and about 60 – 70 cm in diameter. The fruits have small, twisted bean-shaped pods and are characterized by deep brown colour (Soladoye et al., 2012). *Xylopia aethiopica* leaves and seeds have been reported to contain several phytochemicals, such as tannins, alkaloids, saponins,

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flavonoids, anthraquinones, phlobatannins, and glycosides, making them exhibit a wide range of biological effects ([Tan et al., 2010](#); [Tapsell et al., 2006](#)).

Various parts of the *Xylophia aethiopia* plant extracts (seeds, leaves, flowers, fruits, stem bark, and roots) are being employed traditionally for the treatment of gastrointestinal infections, diarrhea, cough, skin infections, respiratory diseases, toothache, sexually transmitted infections, malaria, diabetes, uterine fibroids, hemorrhoids, asthma, rheumatism, and female sterility. A decoction of *Xylophia aethiopia* root and stem bark can be used to treat toothache due to the presence of minerals (copper, zinc, calcium, phosphorus, and potassium) ([Chris-Ozoko et al., 2015](#)) and it has antimicrobial effects on several pathogenic bacteria, including *Bacillus* spp, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella* spp ([Konan et al., 2009](#)). *Xylophia aethiopia* leaves have also been reported to be loaded with vitamins A, B2 (folic acid), B12 (cobalamins), ascorbic acid (vitamin C), and tocopherol (vitamin E), which perform various biochemical functions in the body ([Ravi Kiran & Sita Devi, 2007](#)).

Drug research makes the use of ethno botany to search for pharmacologically active substances in nature, and has, in this way, discovered hundreds of useful compounds ([Sushila, 2017](#)). Phyto-medicinal reports for each of the medicinal plants, including information on physiological effects, efficacy, and references, needed to be developed. Therefore, this experiment was designed to examine the bioactive compounds of *Xylophia aethiopia* using the gas chromatography technique.

2. Materials and methods

2.1. Experimental site

The study was carried out at the Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India, located between the coordinate 23° 13'N 72° 41'E with a coastline of 1600 Km ([Bose et al., 1991](#)).

2.2. Collection, authentication, and processing of *Xylophia aethiopia* leaf extract

Fresh leaves of *Xylophia aethiopia* were collected within the premises of Sumitra Research Institute, Gujarat, India, and authenticated by a certified taxonomist. It was washed with distilled water and shade-dried for 14 days. Dried leaves of *Xylophia aethiopia* were ground into powder form with the aid of an electric blender. 200 grams of *Xylophia aethiopia* powder was imbibed with 1000 mL of 90 % ethanol for 2 days with occasional stirring. Finally, the ethanolic extract of *Xylophia aethiopia* was obtained by sieving the sample using Whatman's No.1 filter paper, stored in a sterile airtight container, and stored in a cool, dry place before transporting it to the laboratory for further analysis.

2.3. Quantitative determination of phytochemical components

Total flavonoids, tannins, and phenols were estimated using Aluminium chloride and the Folin-Ciocalteu method described by [Otlés and Yalcin \(2012\)](#). Saponins and alkaloids were quantified using the colourimetric and gravimetric techniques described by [Madhu et al. \(2016\)](#). Glycosides, steroids, and phytates were analyzed using anion exchange methods described by [Adeniyi et al. \(2009\)](#).

2.4. Analysis of bioactive compounds of *Xylophia aethiopia* leaves using the GC-MS technique

Analysis of bioactive compounds in the ethanolic extract of *Xylophia aethiopia* leaves was performed using Skyray GC-MS 6800 (USA). The GC specifications are; inlet temperature (Max. 450 °C), pressure range (0 - 100 psi), pressure control mode (electronic pressure control), split mode (split/splitless, max. split ratio: 1000:1), column oven working temperature (+4 °C - 450 °C), heating rate (up to 120 °C/min), temperature programming (7 stages/8 platforms) and auto sampler (optional) and MS specifications: EI source ionization energy (5eV - 250 eV), mass range (1.5 - 1000 amu), resolution (unit resolution), ion source temperature (100 - 350 °C), filament emission current (0 - 350 μ A), GC-MS interface temperature (Max. 450 °C), stability (\pm 0.10 amu/48 hours), sensitivity (full scan. 1 pg OFN at m/z 272 with S/N > 30:1), scan rate (up to 1000 amu/s), vacuum (Turbo molecular pumps: 67 L/s) and detector (high energy dynode electron multiplier).

3. Results and discussion

3.1. Phyto-constituents of *Xylophia aethiopia* leaf extract

Phyto-chemical constituents of *Xylophia aethiopia* leaf extract are presented in Table 1. The values of flavonoids, tannins, phenols, alkaloids, glycosides, oxalates, steroids, and saponins were 951.82 mg/g, 282.70 mg/g, 603.25 mg/g, 188.47 mg/g, 190.32 mg/g, 23.74 mg/g, 91.20 mg/g, and 11.47 mg/g, respectively. Flavonoids had the



highest concentration (951.82 mg/g) while saponins had the lowest concentration (11.47 mg/g). Plants are complex matrices producing a range of secondary metabolites with different functional groups and polarities ([Oluwafemi et al., 2020](#)). Flavonoids are a group of compounds with antioxidant activities against free radicals, cellular signaling, inflammation, allergies, and platelet aggregation ([Agubosi et al., 2022](#); [Omolere & Alagbe](#)). Alkaloids have a wide range of pharmacological activities, including antimalarial, antiarrhythmic, and analgesic ([Okwu & Josiah, 2006](#)). Plants rich in alkaloids have a bitter taste, thus preventing consumption by insects and chordates ([Saxena et al., 2013](#); [Selvendran, 1984](#)). Tannins are complex mixtures of organic compounds used as astringents as they precipitate tissue protein ([Saxena et al., 2013](#)). They can also be used for the treatment of diarrhea ([Pączkowski et al., 1998](#)). The presence of phenols in *Xylopiya aethiopic*a leaf extracts supports its use as an anti-inflammatory and antioxidant, thus preventing the incidence of coronary disease ([Pourmorad et al., 2006](#)). Plants containing glycosides and steroids can be used as flavouring agents and cardiac drugs ([Nahar & Sarker, 2019](#)). In addition, steroids possess medicinal properties such as anti-carcinogenic, antispasmodic, and fertility-boosting activity. An overload of oxalate in the body can cause kidney stones and heart disease. The results on phyto-constituents of *Xylopiya aethiopic*a leaf extract agree with the findings of ([Alagbe et al., 2023](#)).

Table 1: Phyto-constituents of *Xylopiya aethiopic*a leaf extract

Constituents	Composition (Mg/g)
Flavonoids	951.82
Tannins	282.70
Phenols	603.25
Alkaloids	188.47
Glycosides	190.32
Oxalates	23.74
Steroids	91.20
Saponins	11.47

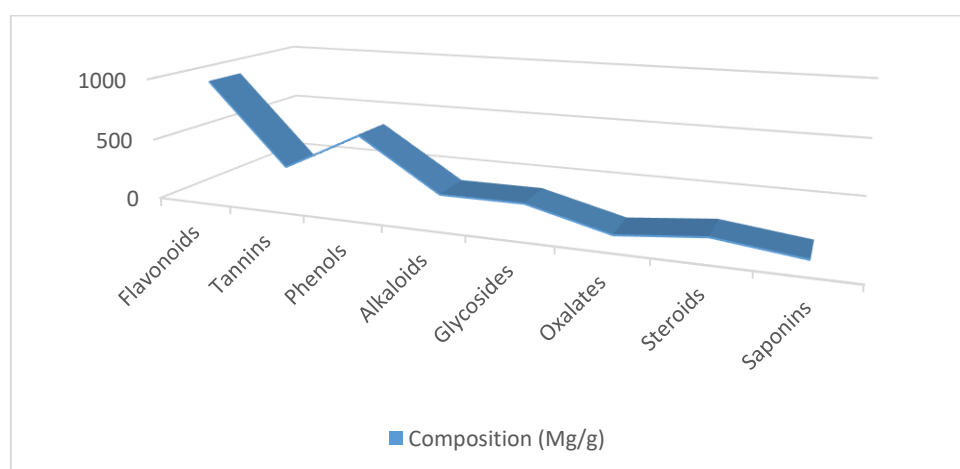


Figure 1: Phyto-constituents of *Xylopiya aethiopic*a leaf extract

4. Bioactive compounds of *Xylopia aethiopica* leaf extracts by Gas chromatography and mass spectrometry technique

The bioactive compounds of *Xylopia aethiopica* leaf extracts by GC-MS is presented in Table 2. Thirty compounds were identified based on their peak areas and retention time. The major compounds greater than 1 % were; 13-docosenamide (21.09 %), terpineol (10.07 %), 1,6-cyclodecadiene (9.37 %), copaene (2.88 %), caryophyllene (8.15 %), β -ocimene (6.05 %), β -myrcene (5.09 %), copaene (2.38 %), 2 - methoxy-4-vinylphenol (1.72 %), β -elemenone (1.31 %), 3,4-dimethylphenyl heptyl ether (1.26 %), ethyl oleate (1.07 %) and γ -elemene (1.27 %) while the minor compounds less than 1 % (< 1 %) were; 2- methylenebornane (0.66 %), 2-methoxy-2-propionyl (0.72 %), hexadec-7-enal (0.23 %), hexadecanoic acid (0.02 %), didodecyl benzene 1,2 dicarboxylate (0.09 %), methyl stearate (0.16 %), 9,12-octadecadienoic acid (0.08 %), hexadeca-7,10 - dienal (0.47 %), 1,1,5 - trimethyl -1,2-dihydronaphthalene (0.01 %), propane, 1,1 - oxybis -3- chloro (0.08 %), 1-trimethylsilylpent-1-en-4-yne (0.03 %), bicyclo[13.1.0] hexadecan-2-one (0.02 %), methyl octadeca-9-yn-11-trans-enoate (0.51 %), cis-linaloxide (0.22 %), tetradecanoic acid, 10,13 -dimethyl ester (0.18 %), didodecyl benzene 1,2 -dicarboxylate (0.47 %) and 2-cyclopentene -1-one, 2 - hydroxy (0.09 %). Ethyl oleate, γ -elemene, and β -ocimene were reported to be found in *Luffa aegyptiaca* leaves ([Alagbe, 2022](#)) and *Strychnos innocua* root bark. Hexadecanoic acid was found in *Delonix regia* root and leaves ([Alagbe et al., 2020](#)). Hexadecan-2-one, 1,1, 5 -trimethyl -1,2-dihydronaphthalene, and methyl octadeca-9-yn-11-trans-enoate have been reported to effectively treat female infertility, gastrointestinal disease, and skin infections ([Adams et al., 2020](#); [Debek et al., 2018](#); [Singh et al., 2010](#)). Caryophyllene, copaene, and methyl stearate have been detected in *Prosopis africana* oil, *Baccharis* spp, *Strychnos spinosa*, and *Zollingeriana indigofera* stem bark ([Agubosi et al., 2022](#); [Hoet et al., 2007](#)). They have a wide range of therapeutic properties, including anti-inflammatory, anti-carcinogenic, cytotoxic, and antioxidant ([Sun HongXiang et al., 2005](#)).

5. Conclusion

It was concluded that *Xylopia aethiopica* leaf extract has several phyto-constituents which have a wide range of pharmacological or therapeutic functions, making them useful in the treatment of gastrointestinal disease, skin infection, cough, malaria, sexually transmitted infections, hemorrhoids, infertility, diabetes, and uterine fibroids, among others.

Table 2: Bioactive compounds of *Xylopia aethiopica* leaf extract

Bioactive compounds	M.W(g/mol)	Peak area (%)	Retention time (min)
2- Methylenebornane	150	0.66	2.71
2 - Methoxy-4-vinyl phenol	136	1.72	3.92
13-Docosenamide	121	21.09	4.02
Terpineol	173	10.07	4.18
Copaene	156	2.88	4.26
1,6-Cyclodecadiene	112	9.37	4.44
Humulene	160	2.38	4.63
β -Myrcene	188	5.09	4.86
β -Ocimene	167	6.05	5.11
Caryophyllene	152	8.15	5.23
2-methoxy-2-propionyl	216	0.72	5.47
γ -Elemene	204	1.27	6.39
β -Elemenone	218	1.31	6.75
Hexadec-7-enal	238	0.23	6.97
Hexadecanoic acid	284	0.02	7.06
Didodecyl benzene 1,2 dicarboxylate	504	0.09	7.19



Methyl stearate	298	0.16	7.42
9,12-Octadecadienoic acid	280	0.08	7.86
Hexadeca-7,10 – dienal	236	0.47	7.94
3,4-dimethyl phenyl heptyl ether	204	1.26	8.22
1,1,5 –Trimethyl -1,2-dihydro naphthalene	270	0.01	9.08
Propane, 1,1 –Oxybis -3- chloro	292	0.08	9.27
1-Trimethylsilylpent-1-en-4-yne	280	0.03	9.63
Ethyl Oleate	310	1.07	10.04
Bicyclo[13.1.0] hexadecan-2-one	236	0.02	10.32
Methyl octadec-9-yn-11-trans-enoate	292	0.51	10.78
Cis-Linaloxide	131	0.22	18.02
Tetradecanoic acid, 10,13 –dimethyl ester	270	0.18	18.46
Didodecyl benzene 1,2 –dicarboxylate	504	0.47	21.75
2-Cyclopentene -1-one, 2 – hydroxy	98	0.09	28.93

M.W: Molecular weight

CRedit authorship contribution statement

Conceptualization: Alagbe J.O; Methodology: Kadiri Mercy; Investigation and Data Collection and Formal Analysis: Oluwafemi R.A; Writing – Original Draft: Agubosi O.C.P; Writing – Review & Editing: Anorue D.N the final manuscript.

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Declaration of competing interest

The authors declared no conflict of interest.

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