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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 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 number of 30 bioactive compounds were identified based on their peak areas. 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 those less than 1 % (< 1 %) were; 2- methylenebornane (0.66 %), 2-methoxy-2-prophenyl (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-trimethylsilypent-1-en-4-yne (0.03 %), bicyclo[13.1.0] hexade can-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 – hyroxy (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.

Introduction

The demand of herbal medicines has increased globally due to the growing recognition with common consideration that plant based medicines are safe, non-toxic, environmental friendly, easily available and affordable (Nikul, 2020; Alagbe *et al*., 2023). The efficacy of herbs can be

linked to the presence of phytochemicals or bioactive compounds which performs 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 being and animals (Singh *et al*., 2022; Oluwafemi *et al*., 2019; Agubosi *et al*., 2022). Phytochemicals are generally regarded as chemicals of plant origin used by plants for growth, defense against competitors, predators and pathogens (Akintayo and Alagbe, 2020; Shittu and 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 plant is *Xylopia aethiopica*.

Xylopia aethiopica (African pepper) belongs to Annonaceae family is an aromatic, evergreen tree native to low land rain forest in the savannah zones of Africa and most parts of Asia (Orwa *et al*., 2009; Burkill, 1985). The tree can grow up to 15 – 30 meters high and about 60 – 70 cm in diameter. The fruits have a 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, flavonoids, anthraquinones, phlobatannins and glycosides making them exhibit a wide range of biological effects (Tapsell *et al*., 2006; Tan *et al*., 2010).

Various parts of *Xylopia aethiopica* 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, tooth ache, sexually transmitted infections, cough, malaria, diabetes, uterine fibroids, hemorrhoids, asthma, rheumatism and female sterility (Feste *et al*., 2016). A decoction of *Xylopia aethiopica* root and stem bark can be used to treat tooth ache due to the presence of minerals (copper, zinc, calcium, phosphorus and potassium) (Obodo *et al*., 2013) 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). *Xylopia aethiopica* 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 performs various biochemical functions in the body (Kiran and Devi, 2007). Drug research makes the use of ethno botany to search for pharmacologically active substance 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 (Nikul, 2020). Therefore, this experiment was designed to examine the bioactive compounds of *Xylopia aethiopica* using gas chromatography technique.

Materials and Methods

Experimental Site

The study was carried out at the Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India located between the coordinate 23º 13'N 72º41'E with a coastline of 1600 Km (Bose Ashish, 1991).

Collection, authentication and processing of *Xylopia aethiopica* leaf extract

Fresh leaves of *Xylopia aethiopica* 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 *Xylopia aethiopica* were grinded into powder form with the aid of an electric blender. 200 grams of *Xylopia aethiopica* powder was imbibed with 1000 mL of 90 % ethanol for 2 days with occasional stirring. Finally, the ethanolic extract of *Xylopia aethiopica* was obtained by sieving the sample using Whatman's No.1 filter paper, stored in a sterile air tight container and stored in a cool dry place before transporting it to the laboratory for further analysis.

Quantitative determination of phytochemical components

Total flavonoids, tannins and phenols were estimated using Aluminium chloride and Folin – Ciocalteau method described by Otles and Yacin (2012). Saponins and alkaloids were quantified using colourimetric and gravimetric technique described by Madhu *et al*. (2016). Glycosides, steroids and phytates were analyzed using anion exchange methods described by Adeniyi *et al*. (2009).

Analysis of bioactive compounds of *Xylopia aethiopica* leaves using GC-MS technique

Analysis of bioactive compounds in ethanolic extract of *Xylopia aethiopica* leaves were analyzed using Skyray GC-MS 6800 (USA). The GC specifications are; inlet temperature (Max. 450 **ºC**), 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 **ºC** ˷ 450 **ºC**), heating rate (up to 120 **ºC**/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 **ºC**), filament emission current (0 - 350 µ A), GC-MS interface temperature (Max. 450 **ºC**), stability (± 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).

Results and Discussion

Phyto-constituents of *Xylopia aethiopica* leaf extract

Phytochemical constituents of *Xylopia aethiopica* leaf extract is 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 group of compounds with antioxidant activities against free radicals, cellular signaling, inflammation allergies and platelet aggregation (Akintayo and Alagbe, 2000; Agubosi *et al*., 2021). Alkaloids have a wide range of pharmacological activities including; antimalarial, antiarrhythmic and analgesics (Okwu, 2004). Plants rich in alkaloids have bitter taste thus preventing consumption from insects and chordates (Sexena *et al*., 2013; Stary, 1998). Tannins are complex mixtures of organic compounds used as astrigents as they precipitate tissue protein (Saxena *et al*., 2013). They can also be used for the treatment of diarrhea (Sczkowski *et al*., 1988). The presence of phenols in *Xylopia aethiopica* leaf extracts supports its use as anti-inflammatory and antioxidant thus preventing the incidence of coronary disease (Poumarad *et al*., 2006). Plants containing glycosides and steroids can be used as flavouring agents and cardiac drugs (Saker and Nahar, 2007). In addition, steroids possess medicinal properties such as; anti-carcinogenic, antispasmodic and fertility boosting activity (Feste *et al*., 2016). Overload of oxalate in a body can cause kidney stones and heart diseases (Kuete, 2014). The results on phyto-constituents of *Xylopia aethiopica* leaf extract is in agreement with the findings of Aguoru *et al*. (2016).

Table 1: Phyto-constituents of *Xylopia aethiopica* 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

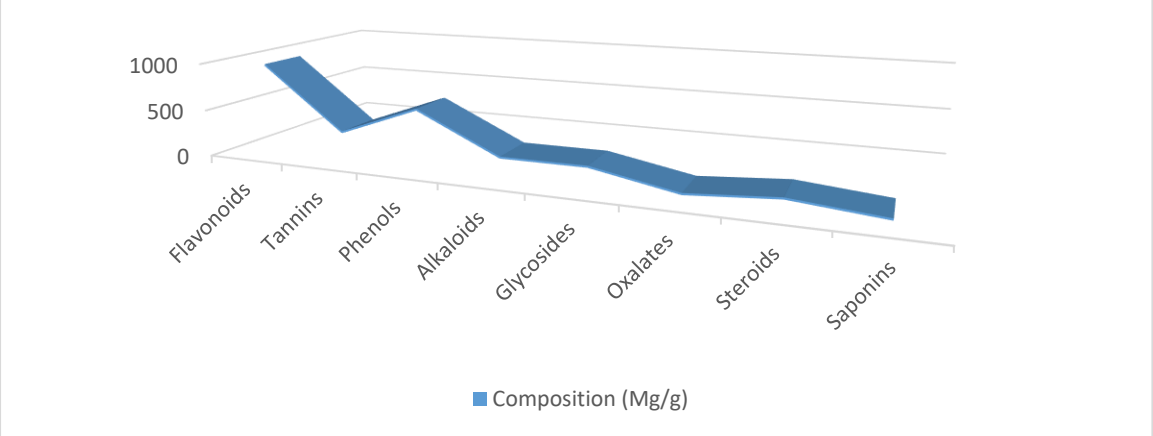

Figure 1: Phyto-constituents of *Xylopia aethiopica* leaf extract

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-prophenyl (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-trimethylsilypent-1-en-4-yne (0.03 %), bicyclo[13.1.0] hexade can-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 – hyroxy (0.09 %). Ethyl oleate, γ-elemene and β-ocimene was reported to be found in *Luffa aegyptiaca l*eaves (Alagbe *et al*., 2023) and *Strychnos innocua* root bark. Hexadecanoic acid was found in *Delonix regia* root and leaves (Alagbe *et al*., 2020). Hexade can-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, gastro-intestinal disease and skin infections (Paula *et al*., 2008; Singh *et al*., 2010; Adams *et al*., 2020). Caryophyllene, copaene and methyl stearate have been detected in *Prosopis africana* oil, *Baccharis spp*, *Strychnos spinosa*, *Zollingeriana indigofera* stem bark (Agubosi *et al*., 2021; Hoet *et al*., 2007). They have a wide range of therapeutic properties including; anti-inflammatory, anti-carcinogenic, cytotoxic and antioxidant (Hongxiang *et al*., 2005).

Conclusion

It was concluded that *Xylopia aethiopica* leaf extract have several phyto-constituents which have a wide range of pharmacological or therapeutic functions making them useful in the treatment of gastro-intestinal 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-vinylphenol	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-prophenyl	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-dimethylphenyl heptyl ether	204	1.26	8.22
1,1,5 –Trimethyl -1,2-dihydronaphthalene	270	0.01	9.08
Propane, 1,1 –Oxybis -3- chloro	292	0.08	9.27
1-Trimethylsilypent-1-en-4-yne	280	0.03	9.63
Ethyl Oleate	310	1.07	10.04
Bicyclo[13.1.0] hexade can-2-one	236	0.02	10.32
Methyl octadeca-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 – hyroxy	98	0.09	28.93

M.W: Molecular weight

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