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 %), caryophyline (8.15 %), β-oicimene (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- methaneboronane (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-dihydrinaphthalene (0.01 %), propane, 1,1 – oxybis -3-chloro (0.08 %), 1-trimethylsilylpent-1-en-4-yne (0.03 %), bicyclo[13.1.0] hexadec-2-one (0.02 %), 1-methyl octadeca-9-yn-11-trans-enolate (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 – hyroxyl (0.09 %). However, all the compounds have a wide range of pharmacological activities including antimicrobial, antioxidant, anti-malarial, antifungal, anti-arrrhythmic, 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, environmentally 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, antimicrobial, heat-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, and defense against competitors, predators and pathogens (Akintayo and Alagbe; 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 plants is Xylopia aethiopica. Xylopia aethiopica (African pepper) belongs to
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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 ethnobotany to search for a pharmacologically active substance in nature and has in this way discovered hundreds of useful compounds (Sushila, 2017). Phyto-medical 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 the 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 coordinates 23° 13'N 72°41'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 °C), pressure range (0 – 100 psi), pressure control (electronic 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: El 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 (± 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 a 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 a bitter taste thus preventing consumption from insects and chordates (Saxena et al., 2013; Stary, 1998). Tannins are complex mixtures of organic compounds used as astringents as they precipitate tissue protein (Saxena 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; anticancerogenic, 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

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Composition (Mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>951.82</td>
</tr>
<tr>
<td>Tannins</td>
<td>282.70</td>
</tr>
<tr>
<td>Phenols</td>
<td>603.25</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>188.47</td>
</tr>
<tr>
<td>Glycosides</td>
<td>190.32</td>
</tr>
<tr>
<td>Oxalates</td>
<td>23.74</td>
</tr>
<tr>
<td>Steroids</td>
<td>91.20</td>
</tr>
<tr>
<td>Saponins</td>
<td>11.47</td>
</tr>
</tbody>
</table>

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-docosanamide (21.09 %), terpinol (10.07 %), 1,6-cycloptadien (9.37 %), copaene (2.88 %), caryophyllene (8.15 %), β-octimene (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- methylenborne (0.66 %), 2-methoxy-2-prophenyl (0.72 %), hexadec-7-enal (0.23 %), hexadecanoic acid (0.02 %), didodecanol 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-dihydroxynaphthalene (0.01 %), propane, 1,1 – oxybis - 3-chloro (0.08 %), 1-trimethylsilylpen-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-linal oxide (0.22 %), tetradecanoic acid, 10,13 – dimethyl ester (0.18 %), didodecyl benzene 1,2 – dicarboxylate (0.47 %) and 2-cyclopentene -1-one, 2 – hyrox (0.09 %). Ethyl oleate, γ-elemene and β-octimene was reported to be found in Luffa aegyptiaca leaves (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-dihydroxynaphthalene 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
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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 has several phytoconstituents 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>
<thead>
<tr>
<th>Bioactive compounds</th>
<th>M.W(g/mol)</th>
<th>Peak area (%)</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2- Methylenebornane</td>
<td>150</td>
<td>0.66</td>
<td>2.71</td>
</tr>
<tr>
<td>2 – Methoxy-4-vinyl phenol</td>
<td>136</td>
<td>1.72</td>
<td>3.92</td>
</tr>
<tr>
<td>13-Docosanamide</td>
<td>121</td>
<td>21.09</td>
<td>4.02</td>
</tr>
<tr>
<td>Terpineol</td>
<td>173</td>
<td>10.07</td>
<td>4.18</td>
</tr>
<tr>
<td>Copaene</td>
<td>156</td>
<td>2.88</td>
<td>4.26</td>
</tr>
<tr>
<td>1,6-Cyclooctadiene</td>
<td>112</td>
<td>9.37</td>
<td>4.44</td>
</tr>
<tr>
<td>Humulene</td>
<td>160</td>
<td>2.38</td>
<td>4.63</td>
</tr>
<tr>
<td>β-Mycene</td>
<td>188</td>
<td>5.09</td>
<td>4.86</td>
</tr>
<tr>
<td>β-Ocimene</td>
<td>167</td>
<td>6.05</td>
<td>5.11</td>
</tr>
<tr>
<td>Caryophyllene</td>
<td>152</td>
<td>8.15</td>
<td>5.23</td>
</tr>
<tr>
<td>2-methoxy-2-propionyl</td>
<td>216</td>
<td>0.72</td>
<td>5.47</td>
</tr>
<tr>
<td>γ-Elemene</td>
<td>204</td>
<td>1.27</td>
<td>6.39</td>
</tr>
<tr>
<td>β-Elemeneone</td>
<td>218</td>
<td>1.31</td>
<td>6.75</td>
</tr>
<tr>
<td>Hexadec-7-enal</td>
<td>238</td>
<td>0.23</td>
<td>6.97</td>
</tr>
<tr>
<td>Hexadecanoic acid</td>
<td>284</td>
<td>0.02</td>
<td>7.06</td>
</tr>
<tr>
<td>Didodecyl benzene 1,2 dicarboxylate</td>
<td>504</td>
<td>0.09</td>
<td>7.19</td>
</tr>
<tr>
<td>Methyl stearate</td>
<td>298</td>
<td>0.16</td>
<td>7.42</td>
</tr>
<tr>
<td>9,12-Octadecadienoic acid</td>
<td>280</td>
<td>0.08</td>
<td>7.86</td>
</tr>
<tr>
<td>Hexadeca-7,10 – dienal</td>
<td>236</td>
<td>0.47</td>
<td>7.94</td>
</tr>
<tr>
<td>3,4-dimethyl phenyl heptyl ether</td>
<td>204</td>
<td>1.26</td>
<td>8.22</td>
</tr>
<tr>
<td>1,1,5 –Trimethyl -1,2-dihydro naphthalene</td>
<td>270</td>
<td>0.01</td>
<td>9.08</td>
</tr>
<tr>
<td>Propane, 1,1 –Oxybis -3-chloro</td>
<td>292</td>
<td>0.08</td>
<td>9.27</td>
</tr>
<tr>
<td>1-Trimethylsilypent-1-en-4-one</td>
<td>280</td>
<td>0.03</td>
<td>9.63</td>
</tr>
<tr>
<td>Ethyl Oleate</td>
<td>310</td>
<td>1.07</td>
<td>10.04</td>
</tr>
<tr>
<td>Bicyclo[13.1.0] hexade can-2-one</td>
<td>236</td>
<td>0.02</td>
<td>10.32</td>
</tr>
<tr>
<td>Methyl octadeca-9-yn-11-trans-enoate</td>
<td>292</td>
<td>0.51</td>
<td>10.78</td>
</tr>
<tr>
<td>cis-Linaloxide</td>
<td>131</td>
<td>0.22</td>
<td>18.02</td>
</tr>
<tr>
<td>Tetradecanoic acid, 10,13 –dimethyl ester</td>
<td>270</td>
<td>0.18</td>
<td>18.46</td>
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<tr>
<td>Didodecyl benzene 1,2 –dicarboxylate</td>
<td>504</td>
<td>0.47</td>
<td>21.75</td>
</tr>
<tr>
<td>2-Cyclopentene -1-one, 2 – hyroxy</td>
<td>98</td>
<td>0.09</td>
<td>28.93</td>
</tr>
</tbody>
</table>

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