Phytochemical and antimicrobial studies of *Xylopia aethiopica* stem bark extracts

Babalola SA¹*, Gabriel AF²

**ABSTRACT**

The preliminary screening of phytochemical constituents of extracts of *Xylopia aethiopica* was evaluated. The *n*-hexane extract revealed the presence of terpenoids, alkaloids, and steroids, while the presence of alkaloids, terpenoids, saponins, steroids, and phenolics was indicated in ethyl acetate extract, and the methanol extract contained tannins, flavonoids, phenolics, terpenoids, saponins, alkaloids, steroids. The medicinal property of the extracts was investigated using *in-vitro* antimicrobial assays. The hexane extract indicates potent activity against *Pseudomonas aeruginosa* with MIC of 100mg/ml and ZI of 20 mm. Methanol and ethyl acetate extracts had inhibitory activities against *Candida albicans* with MIC of 100mg/ml while the standard antibiotics have no inhibitory activities against *Candida albicans* suggesting that these extracts contain certain phytochemical that are active against *Candida albicans*. Saponins which are constituents of methanol and ethyl acetate extracts from the phytochemical screening are known to be antifungal, especially against *Candida albicans*. This suggests that the presence of saponins in both ethyl acetate and methanol extracts may be responsible for the inhibitory activities against *Candida albicans*.

Keywords: *Xylopia aethiopica* stem bark, Antimicrobial, Phytochemical, *Pseudomonas aeruginosa*, *Candida albicans*

1. **INTRODUCTION**

The World Health Organization (WHO) recognizes traditional medicine, plant medicine is of particular importance as a possible substitute for the healthcare delivery system for a large portion of the world’s population. Infectious diseases are the number one cause of death worldwide which accounted for almost one-half of all deaths in tropical countries especially sub-Saharan Africans [1]. The rises are attributed to a high occurrence of respiratory tract infections and HIV/AIDS. Another issue is antibiotic resistance in nosocomial and community-acquired infections [1]. These worrying health trends demand prompt infectious disease solutions from the medical and public health communities, as well as updated treatment and prevention approaches like immunization, improved monitoring, and the creation of novel medicines. One of these treatments is the discovery of new antimicrobials. [2]. It’s possible that man has almost eaten or chewed all kinds of herbs in order to relieve or...
cure illness. He identified the usefulness of different plants against a variety of ailments through trials and errors, which were the first crude extracts trials. Various ethnic cultures have vast amounts of information on the medicinal properties of various plants and animals, which can be used to develop new pharmaceuticals [3].

*Xylopia aethiopica* is an evergreen, aromatic tree with a height approximately 20m high, and a width of 100cm, with unbending boles. It is often found in moist fringe forests and low-land rain forests of savanna zones of Africa. Various parts of the plant have been employed traditionally in different therapeutic formulations [4].

The fruits of the *Xylopia aethiopica* tree are used for various therapeutic purposes such as antitussive, sedative, laxative, and analgesic. The fruit is a common ingredient in some parts of Nigeria. Its spice smoke is used in the treatment of asthma in Liberia [5]. The fruit extract is also used in the treatment of analgesia and chronic inflammatory diseases, headache, neuralgia (pains in the nerves), and colic pain in Ghana [6].

It is also employed in inducing placental discharge post-partum due to its abortifacient [8]. The fruit is often subsumed in formulation for endemic and external uses, owing to its analgesic property for any part of the body associated with pain and in the treatment of acute inflammation. The fruit decoction is useful as of diuretic, anti-inflammatory, and also airway inflammation. The infusion of the stem bark extract in palm wine dosage rate of one or two glasses per day is useful in the treatment of airway inflammation, inflammation of the intestine, and chronic inflammatory diseases and as medicine for bulimia (eating disorder) in Congo [7]. The plant is also used in the treatment of cancer and gastrointestinal ulceration conditions via its various traditional therapeutic preparations in Nigeria [8].

2. EXPERIMENTAL METHODS

A fresh sample of *Xylopia aethiopica* stem bark was collected from Iyara town, Ijumu local government, Kogi state. The plant material was authenticated in the herbarium of the University. The stem bark of *Xylopia aethiopica* was then air-dried and pulverized into powdery form in the Chemistry department laboratory, University of Abuja. The powdered stem bark was then weighed.

**Extraction of Xylopia aethiopica stem bark with n-hexane, ethyl acetate, and methanol**

The extraction method followed that of Gabriel et al., 2016 [9]. 69.73g powdered sample was successively extracted with solvents of varying polarities which includes n-hexane, ethyl-acetate, and methanol using soxhlet extractor in the order of their increasing polarities.

**n-Hexane extract**

250 ml of n-hexane was used to extract 69.73 g of the powdered stem bark using soxhlet. The sample was extracted at 65 °C temperature of the heating mantle. The sample was continuously extracted under reflux until the yellow color of a solvent-extract system in the glass thimble turned colorless. A yellow-colored n-hexane extract was obtained and the extract was poured into a 100ml flask. n-Hexane extract of the sample was evaporated on a thermostat water bath to afford gummy yellow extract.

**Ethyl acetate extract**

150 ml of ethyl acetate was used to extract 69.73 g of the powdered stem bark using soxhlet. The sample was extracted at 78 °C temperature of the heating mantle. The sample was continuously extracted under reflux until the green color of a solvent-extract system in the glass thimble turned colorless. A green-colored ethyl acetate extract was obtained and the extract was transferred into a 100ml flask. Ethyl acetate extract of the sample was evaporated on a thermostat water bath to afford a sticky green extract.

**Methanol extract**

150 ml of methanol was used to extract 69.73 g of the powdered stem bark using soxhlet. The sample was extracted at 68 °C temperature of the heating mantle. The sample was continuously extracted under reflux until the brown color of a solvent-extract system in the glass thimble turned colorless. A brown-colored methanol extract was obtained and the extract was transferred into a 100ml flask. Methanol extract of the sample was evaporated on a thermostat water bath to afford slurry brown extract.

**Phytochemical screening of the extracts**

The qualitative phytochemical screening procedure followed those described by Barathidasan et al. [10], Jennifer Adline et al. [11], and P. Brindha et al. [12].
Antimicrobial analysis of the extracts

Bacteriological techniques

The bacteriological techniques followed were those described by Cheesbrough et al. [12], Burdon and Williams et al. [14], Brooks et al. [15].

The standard bacterial strains Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and a fungus Candida albican were obtained from the Microbiology laboratory of the University of Abuja Teaching Hospital, Gwagwalada, Abuja.

The strains were: gram-positive: Staphylococcus aureus, Candida albican. And gram negative: Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa.

The bacteria and fungus strains were activated and cloned three successive times in CLED agar and nutrient agar respectively, and they were stored in nutrient slants at 4 °C, subsequent activation and test were done on nutrient agar medium.

Control

Cotrimoxazole (septrin), Gentamycin, and Tetracycline were used at concentrations ranging from 40mg/ml to 100mg/ml as positive control while the solvents were used as the negative control.

3. RESULTS

Table 1 shows the physical characteristic, masses of extracts of powdered Xylopia aethiopica stem bark obtained, and their percentage yield from 69.73 g powdered sample. The percentage yield was calculated using the formula below.

Phytochemical screening of t n-hexane, ethyl acetate, and methanol extracts of X. aethiopica stem bark was carried out and the result is as shown in Table 2.

Table 3 shows the Zones of Inhibition (mm) and the Minimum Inhibitory Concentration (MIC) of n-hexane, ethyl acetate, and methanol extracts.

\[
\text{% Yield} = \frac{\text{Weight of crude extract}}{\text{Weight of the sample}} \times 100\% 
\]

Figure 1: Percentage yield formula

Table 1: Physical characteristics, masses, and percentage yield crude extract yield and their percentage yield of the extracts.

<table>
<thead>
<tr>
<th>Powdered Sample</th>
<th>Extraction Solvent</th>
<th>Physical Characteristic</th>
<th>Mass of Crude Extract (g)</th>
<th>% yield of Crude extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem bark</td>
<td>n-Hexane</td>
<td>Yellow color</td>
<td>2.14</td>
<td>3.07</td>
</tr>
<tr>
<td>of Xylopia</td>
<td>Ethyl acetate</td>
<td>Green color</td>
<td>0.94</td>
<td>1.35</td>
</tr>
<tr>
<td>aethiopica</td>
<td>Methanol</td>
<td>Brown color</td>
<td>4.29</td>
<td>6.15</td>
</tr>
</tbody>
</table>
**Table 2:** Phytochemical screening results of stem bark extracts of *X. aethiopica*.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>n-Hexane extract</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenolics</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Keywords: +ve = Present, -ve = Absent

**Table 3:** Result for antimicrobial tests on n-hexane, ethyl acetate, and methanol extracts.

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Zones of inhibition (ZI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONC.(mg/ml)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td>Candida albican</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
</tbody>
</table>
**ETHYL ACETATE EXTRACT**

**Pseudomonas**

- *Pseudomonas aeruginosa*: 6 mm
- *Escherichia coli*: 4 mm
- *Candida albican*: 2 mm
- *Klebsiella pneumonia*: ----
- *Staphylococcus aureus*: ----

**n-HEXANE**

**Pseudomonas**

- *Pseudomonas aeruginosa*: 31 mm
- *Escherichia coli*: 18 mm
- *Candida albican*: ----
- *Klebsiella pneumonia*: 4 mm
- *Staphylococcus aureus*: ----

**Table 4: Positive Control (known antibiotics)**

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Tetracycline</th>
<th>Gentamycin</th>
<th>Cotrimoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>30 mm</td>
<td>27 mm</td>
<td>15 mm</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>30 mm</td>
<td>25 mm</td>
<td>18 mm</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>
4. DISCUSSION

Phytochemical Screening
The result of preliminary screening of phytochemical constituents of n-hexane extract indicates the presence of alkaloids, terpenoids, and steroids in the extract. Ethyl acetate extract showed the presence of saponins, alkaloids, terpenoids, steroids, and phenolics while in methanol extract the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, steroids, and phenolics were all observed according to the result in Table 2 above. Preliminary studies showed that *X. aethiopica* stem bark contains pharmaceutical constituents such as saponins which are known to be antifungal, especially against *Candida albican*, it is also known to lower cholesterol levels in the body. Saponins have been found to increase the effectiveness of vaccines [16].

Alkaloids and terpenoids which are bioactive have been known to be bactericidal, pesticidal, and fungicidal [17]. Alkaloids are also used in medicine because of their quick action on specific areas of the central nervous system, the effectiveness of alkaloids on humans has led to the development of powerful painkiller vaccines. They are the active components of many relaxants, tranquilizers, sedatives, stimulants, and anesthetics [18]. Some terpenoids are useful flavoring agents. Steroids are mostly secondary metabolites that are capable of producing definite physiological actions on the body [17].

Tannins in plants are known to be astringents, which help in wound healing and the treatment of hemorrhoids, tonsillitis, pharyngitis, and skin eruptions [19]. They are used in treating intestinal disorders such as diarrhea and dysentery and are known to show curative activity against several pathogens [20]. Tannins are also used as antidotes for metallic, alkaloidal, and glycosidic poisons [18].

Antimicrobial Analysis
Table 3 showed the antimicrobial activity of n-hexane, ethyl acetate, and methanol extracts of *X. aethiopica* stem bark at different concentrations.

Methanol extract has inhibitory activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albican*, and *Klebsiella pneumonia* at all concentrations (100 mg/ml – 600 mg/ml). The extract had no inhibitory effect against *Staphylococcus aureus* at all concentrations. The highest zone of inhibition was observed with *Candida albican*.

Ethyl acetate extract has inhibitory activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albican*. The ethyl acetate extract has no inhibitory effect against *Klebsiella pneumonia* and *Staphylococcus aureus* at all concentrations (100 mg/ml – 600 mg/ml) and only indicated activity against *Pseudomonas aeruginosa* at concentration 300 mg/ml to 600 mg/ml. The highest zone of inhibition was observed against *Klebsiella pneumonia* at 600 mg/ml.
The n-Hexane extract had an inhibitory effect against *Pseudomonas aeruginosa* at all concentrations (100 mg/ml – 600 mg/ml) and only had an inhibitory effect against *Klebsiella pneumonia* at 200 mg/ml to 600 mg/ml. The extract had no inhibitory effect against *Escherichia coli, Candida albicans*, and *Staphylococcus aureus* at all concentrations (100 mg/ml – 600 mg/ml). The n-Hexane extract has very high zones of inhibition against *Pseudomonas aeruginosa* at all concentrations making it the most potent extract of all the three extracts against *Pseudomonas aeruginosa*. The three extracts had no observable inhibitory effect against *Staphylococcus aureus*.

The Minimum Inhibitory Concentration (MIC) of the extracts observed for *Pseudomonas aeruginosa* was 100 mg/ml of methanol extract, 300 mg/ml for ethyl acetate extract, and 100 mg/ml of n-hexane extract. MIC of methanol and ethyl acetate extracts was 100 mg/ml for *Escherichia coli* and *Candida albicans*, and the MIC for *Klebsiella pneumonia* was 100 mg/ml of methanol and n-hexane extracts.

Methanol and ethyl acetate extracts have an inhibitory effect against *Candida albicans* while the standard antibiotics had no inhibitory effect against *Candida albicans* suggesting that these extracts contain certain phytochemicals that are active against *Candida albicans*. Saponins which are constituents of methanol and ethyl acetate extracts from the phytochemical screening result in Table 2 above are known to be antifungal, especially against *Candida albicans*. This suggests that the inhibitory effect of methanol and ethyl acetate extracts may be due to the presence of saponins in the two extracts.

The antimicrobial results of the crude extracts indicate that these extracts are sources of useful potential medicines that will help in the treatments of diseases related to the inhibited pathogens. Generally, an increase in activity with an increase in concentration was observed from the results.

**Control**
The antibiotics used as the positive control showed an inhibitory effect against all the microbes except for *Candida albicans* which showed resistance to all the antibiotics. The highest zone of inhibition was observed against *Klebsiella pneumonia*. Cotrimoxazole (septrin) had no effect against *Staphylococcus aureus* suggesting that septrin has no effect against gram-positive strains (*Staphylococcus aureus* and *Candida albicans*). This indicates that septrin has a selective spectrum of action.

Methanol, ethyl acetate, and n-hexane solvents were used as the negative control. The three solvents did not show any observable inhibition against the micro-organisms.

5. **CONCLUSION**
Phytochemical are essential regimens for infectious diseases caused by bacteria and fungi. More attentions are currently toward phytochemical for their effectiveness in the treatment of infectious diseases and simultaneous alleviation of many of the adverse events caused by conventional antimicrobials drugs. This research demonstrated that *Xylopia aethiopica* stem bark contains bioactive compounds that can be used in the treatment of diseases that are related to inhibited microbes.

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**Ethical approval**
The ethical guidelines for plants & plant materials are followed in the study for experimentation. The ethical guidelines for microbial studies are followed in the study.

**Conflict of Interest:**
The authors declare that there are no conflicts of interests.

**Data and materials availability:**
All data associated with this study are present in the paper.
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