The *in vitro* effect of *Myrianthus arboreus* leaf extract on some pathogenic bacteria of clinical origin

Agwa, O.K., Chuku, W. and Obichi, E.A

Dept. of Microbiology, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria
Regional Center for Bioresource and Biotechnology, South-South zone centre for Excellence, National Biotechnology Development Agency (NABDA), University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria

---

**ABSTRACT**

The effect of aqueous and solvent extracts of *Myrianthus arboreus* leaves were investigated for their antibacterial activities compared to chloramphenicol, amoxicillin and ampicillin studied against four test organisms (*E. coli, Staphylococcus aureus, Klebsiella pneumonia* and *Proteus vulgaris*) using the well-in-agar and disc diffusion methods. The methanol extract showed the highest antibacterial activity on both methods used, followed by ethanol and acetone extracts, but the chloroform extract showed slight activity on only *Proteus vulgaris*. The aqueous hot extract at higher concentration showed antibacterial activity on all the test organisms while the aqueous cold extract was ineffective on the test isolates at all concentration. These results were compared with zones of inhibition produced by commercially available antibiotics. The relatively safer, cheaper and availability of these plants makes them acceptable than their synthetic alternatives for therapeutic purpose because of the undesirable side effects of certain antibiotics.

**Keywords:** Antibiotics, aqueous extracts, disc diffusion, methanol extracts, *Myrianthus arboreus*, well-in-agar diffusion.

---

**INTRODUCTION**

Recently various medicinal plants are used daily to treat diseases all over the world. Most of the world’s population rely on traditional medicine for their primary healthcare needs (1). The relatively cheaper cost of medicinal plants and its availability in Africa have made them attractive as therapeutic alternative when compared to modern medicines because of their antimicrobial properties (2, 3, 4). These infectious and chronic diseases are treated with plants that contain wide range of substances as bioactive compounds which serve as an important
source of natural products for human health (5). Bioactive compounds such as phenolic compounds, tannins, alkaloids and flavonoids have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections (6).

*Myrianthus arboreus* is a common tree in the forest area of West and Central Africa, occurring in rainforest, semi deciduous and swamp forest. A member of the cecropiaceae family, an indigenous wild plant used for food and medicine. The sweet pulp around the seed is edible and the young leaves are eaten as vegetable. Extracts of the leaves are used in Sierreleone and Mt.Cameroun area to treat dysentery, diarrhea and vomiting (7). Within the continents of Africa, such as Nigeria and Congo, the leaves serve as an analgesic given to young children against fever, applied as an enema to treat pain in the back and loins, chopped leaves are eaten raw with salt for heart troubles, pregnancy complications, dysmenorrheal, incipient hernia and a plaster made of beaten leaf applied to boils. Sap from the leaves is applied topically for toothache, to the chest for bronchitis or as throat paint for sore throat. The effect of the leaf extracts of *Myrianthus arboreus* on the liver enzymes of wistar albino rats was studied by (8) and concluded that the leaves are not toxic to the liver, hence can be consumed without restriction.

This study investigates the antibacterial activity of *Myrianthus arboreus* extracts on four selected pathogens namely *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris and Klebsiella pneumonia* isolated from in patients at the University of Port Harcourt Teaching Hospital, traditionally used in the treatment of certain infections and diseases within Africa.

**MATERIALS AND METHODS**

**Collection of plant material, identification and extraction**
The fresh leaves of *Myrianthus arboreus* were collected from Ogbakiri in Emohua Local Govt. Area, Rivers State, Nigeria in November, 2010. The plant was identified at the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria by Mr. Chika Wahua. The leaves were dried for a week, coarsely powdered by using a mechanical grinder and subjected to aqueous (hot/cold), methanol, ethanol, and acetone and chloroform extraction (9). Twenty grams of the fine powder was soaked in each of solvent at room temperature (28±2°C), shakes every 30mins for 6h and allowed to stand for about 48h. Using a sterile muslin cloth, the solution was filtered into a sterile container, left to evaporate to dryness using a rotary evaporator; the extract was preserved in an air tight sterile container until required.

**Sterilization of materials**
All glassware used in this research were washed with detergent, rinsed with distilled water, air dried and sterilized on a hot air oven at 121°C for 2h.Each of the material was wrapped with aluminium foil before sterilization. Distilled water and all prepared media were sterilized in the autoclave at 121°C for 15mins. Cork borers and glass rods were sterilized by dipping into 70% alcohol prior to flaming in a Bunsen burner. The working bench was swabbed with 70% alcohol before and after each experiment.
Proximate analysis
The dried leaves were subjected to proximate analysis using the recommended methods (10). The following parameters were determined: carbohydrate determination, crude fat, ash content, moisture content, crude protein and crude fiber.

Phytochemical screening of plant extracts
A qualitative phytochemical test to detect the presence of tannins, phytic acids, cyanogenic glycosides, alkaloids, oxalates and flavonoids was carried (10).

Testorganisms
Pure cultures of *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris* and *Klebsiella pneumonia* were obtained from the Medical Laboratory of the Microbiology and Parasitology unit of the University of Port Harcourt Teaching Hospital and used as test organisms. A 24 h fresh culture was prepared in Nutrient Broth and was used for the antibacterial activity.

Antibacterial Activity
The extracts were obtained from the leaves of the plant with four solvents (methanol, ethanol, chloroform and acetone) and aqueous (hot/cold) extracts were studied for antibacterial activity using the disc and well-in-agar diffusion methods.

The well-in-agar diffusion method described by (9) was adopted for the study. Broth cultures of the test isolates (0.1ml) containing $1 \times 10^5$ cells/ml of organism was introduced into a sterile Petri dish, added 15ml of molten nutrient agar, thoroughly mixed and allowed to solidify. Six holes were made in the Petri dishes (about 5.00mm in diameter) using a sterile cork borer. Equal volumes of the plant extract were transferred into the holes using a Pasteur pipette. Three Petri dishes containing a particular organism was used for each Concentration of plant extract. The Petri dishes were allowed to stand for 1h for pre-diffusion of the extract to occur and were incubated at 37°C for 24 h.

At the end of the incubation period, the Petri dishes were collected and zones of inhibition that develop were measured in millimeters. The average zone of inhibition was calculated and the minimum inhibitory concentration (MIC) was obtained by plotting the logarithm of the concentration of extracts against the square of the zones of inhibition. The antilogarithm of the intercept on the concentration axis gave the MIC values.

The Disc diffusion method was adopted (11). Filter paper discs of 7mm were soaked with 0.1ml of extract. The Petri dishes were sterilized in an oven at 35°C for 2 h. The sterilized Petri dishes were properly labeled with the names of the test isolates, extracts and the inner content was divided into six parts. One millimeter of the inoculums was aseptically transferred from the test tubes into the Petri dish containing the nutrient agar, allowed to solidify at room temperature. The filter paper discs soaked with different extracts were placed in the Petri dishes at their labeled positions, incubated and zones of inhibition that developed were measured. Subsequently, the MIC was determined with the method stated in the well-in-agar method.
Another set of Petri dishes were prepared in which three different commercially available antibiotics: chloramphenicol, amoxicillin and ampicillin were placed on the medium, used as positive control.

RESULTS AND DISCUSSION

Table 1 illustrates the result of the nutritive composition of *Myrianthus arboreus* leaves. The moisture content was high but all other parameters were low including the lipid content. The presence of bioactive compounds such as tannins, cyanogenic glycosides, phytic acid, flavonoids and alkaloids were found, but oxalates were absent (Table 2).

Aqueous extract of the plants tested recorded significant antibacterial activity with the hot extract only while cold extract did not inhibit the growth of the test isolates. The well-in-agar antibacterial activity exerted high zone of inhibition of 12mm with *E.coli* at 500mg/ml, while the least zone of inhibition of 2mm was exerted by *Staphylococcus aureus* at the same concentration, but the 375mg/ml, 250mg/ml and 125mg/ml did not exhibit any zone of inhibition. But with the disc diffusion method, the same high activity of 10mm was recorded by the same isolate at the same concentration and *Staph aureus* did not show any activity.

The activities of four solvent extracts (methanol, ethanol, acetone and chloroform) of *Myrianthus arboreus* leaves were used studied on the test organisms using the well-in-agar diffusion method. The methanol extract showed highly significant antibacterial activity followed by ethanol, acetone extract showed least the antibacterial activity and chloroform exerted activity on only *Proteus vulgaris* with a diameter of 4mm at 500mg/ml and 1mm at 375mg/ml respectively (Fig 1 and 2). A comparison of the zone of inhibition revealed that the solvent extraction activity was higher than that of the aqueous extraction between the two methods used.

Among the three antibiotics tested (Fig 3), chloramphenicol and amoxicillin were very effective against the test organisms but ampicillin was least effective against all the test isolates especially on *Staph aureus*.

Comparative efficacy of the test organisms with the different antibacterial methods revealed that the well-in-agar method gave better, higher and clear inhibition zone. The methanol extract were at the same range of inhibition with the antibiotics, but the other extracts showed lesser susceptibility.

**TABLE 1: Proximate analysis of *Myrianthus arboreus***

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULTS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>1.60</td>
</tr>
<tr>
<td>Moisture</td>
<td>77.61</td>
</tr>
<tr>
<td>Crude protein</td>
<td>2.63</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>3.08</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>15.63</td>
</tr>
<tr>
<td>Fat</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Available online at www.scholarsresearchlibrary.com
TABLE 2: Phytochemical constituents of *Myrianthus arboreus*

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin</td>
<td>Present</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>Present</td>
</tr>
<tr>
<td>Phytic acid</td>
<td>Present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>Oxalate</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Fig 1: Zones of inhibition (mm) of the different extracts using well in agar diffusion method

The result of the MIC of the extracts on the test organisms were shown in Table 3. The lowest MIC was obtained with *E.coli* which had 63.1mg/ml using the well-in-agar method but with the disc diffusion method on the same organism was 79.4mg/ml.

Available online at www.scholarsresearchlibrary.com
Table 3: minimum inhibitory concentration of different extracts of Myrianthus arboreus on test organisms

<table>
<thead>
<tr>
<th>Extracts</th>
<th>E. coli (WIA)</th>
<th>E. coli (DDM)</th>
<th>Proteus sp (WIA)</th>
<th>Proteus sp (DDM)</th>
<th>Klebsiella sp (WIA)</th>
<th>Klebsiella sp (DDM)</th>
<th>S. aureus (WIA)</th>
<th>S. aureus (DDM)</th>
<th>Staphylococcus sp (WIA)</th>
<th>Staphylococcus sp (DDM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>63.1</td>
<td>79.4</td>
<td>79.4</td>
<td>125.9</td>
<td>125.9</td>
<td>79.4</td>
<td>251.2</td>
<td>251.2</td>
<td>251.2</td>
<td>251.2</td>
</tr>
<tr>
<td>Ethanol</td>
<td>63.1</td>
<td>79.4</td>
<td>125.9</td>
<td>79.4</td>
<td>125.9</td>
<td>125.9</td>
<td>251.2</td>
<td>251.2</td>
<td>398.1</td>
<td>398.1</td>
</tr>
<tr>
<td>Acetone</td>
<td>158.5</td>
<td>125.9</td>
<td>125.9</td>
<td>251.2</td>
<td>125.9</td>
<td>251.2</td>
<td>251.2</td>
<td>251.2</td>
<td>398.1</td>
<td>398.1</td>
</tr>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>-</td>
<td>251.2</td>
<td>251.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous hot</td>
<td>125.9</td>
<td>125.9</td>
<td>251.2</td>
<td>398.1</td>
<td>251.2</td>
<td>251.2</td>
<td>251.2</td>
<td>251.2</td>
<td>501.2</td>
<td>501.2</td>
</tr>
</tbody>
</table>

WIA: Well in agar diffusion method  
DDM: Disc diffusion method

Therapeutic treatment using plant extracts and phytochemicals with known antimicrobial properties can be of great significance because chemically synthesized drugs have undesirable side effects. Antimicrobial compounds from plants inhibit bacterial growth by different mechanisms which may have a significant clinical effect in treatment of resistant microbial strains (12).

The proximate analysis results revealed that the leaves of Myrianthus arboreus are rich in fiber and moisture content, low in fats, protein and carbohydrates. These values fall within the range of some edible Nigerian vegetables (13). The high fiber content is a good health benefit which prevents hemorrhoids, constipation, assists during pregnancy, lows the risk of developing diabetes, colon cancer, kidney and heart diseases by lowering the sugar and fat content as well as...
cuts calories (14, 5, 8). Many plants owe their potency to the presence of certain substances which vary in their solubility; polarity and volatility were used for the extraction of the active agents to identify the principal bioactive compound responsible for antimicrobial activity. The presence of these bioactive compounds from plants inhibit the growth and existence of microorganisms (15,7,13).Flavonoid are good antioxidant substances, tannins are reported to have antimicrobial property with anti-diarrheal, antisecretolytic, anti parasitic and anti irritant effects and are used to treat injuries of the different sense organs of the body (16,17).The test organisms were selected because they are responsible for a number of infections relating to food, gastrointestinal tract, urinary tract and a times can cause certain systemic infections. Six extracts of methanol, ethanol, acetone, and chloroform, aqueous hot and aqueous cold were used for determining the antibacterial activity of *Myrianthus arboreus*.

![Graph showing zones of inhibition (mm) of the antibiotics on the test organisms](image)

The solvent extracts (methanol, ethanol and acetone) and aqueous hot extracts inhibited the growth of the test organisms to varying degree, chloroform extract inhibited only *Proteus vulgaris* but the aqueous cold extracts did not have any effect on the test organisms, but this is not enough to conclude that the leaf does not contain substances that can exert antibacterial activity. Thus they posses active compounds that can inhibit the growth of some microorganisms and the potency of the leaf depends on the method used to obtain the extract (18). This finding is in agreement with the findings of (9, 19, 20). The high antibacterial effect of some of these solvents may be due to the interactions of these solvents with their phytochemical components.
with inhibitory potential and the synergistic activity of the bioactive compounds. In the study it was observed that the methanol extract had a significantly higher antibacterial activity than all other extracts at all concentration on *E. coli*, *Klebsiella pneumonia*, *Proteus vulgaris* and less significant on *Staphylococcus aureus*. Other researchers, while working with other medicinal plants showed that methanol extract had significant high antibacterial activity against all tested microorganisms (21,22,23). Similarly, (19) indicated that methanol extracts were more sensitive than all other extracts used in the study.

*E. coli* was found most sensitive as compared to *Klebsiella pneumonia*, *Proteus vulgaris* and *Staphylococcus aureus*. *E. coli*, *Klebsiella pneumonia* and *Proteus vulgaris* were more susceptible to the three commercial antibiotics and *Staphylococcus aureus* was less susceptible. This proves that these commercial antibiotics were more active than the plant extracts (18). The side effects of these commercial antibiotics, unavailability, adulteration, resistance pattern to microorganisms and high cost make drug users more comfortable with plants with antimicrobial activity (24).

Thus, the result suggests that the leaves of *Myrianthus arboreus* has good antibacterial activity, and could be used in traditional medicine as therapeutic agent for controlling the pathogenic bacteria.

**REFERENCES**