Antimicrobial Activity of *Borreria Verticillata* Stem Bark Extracts

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Article history: Received 15 May 2013, Received in revised form 16 June 2013, Accepted 20 June 2013 Published 26 June 2013.

**Abstract:** *Borreria verticillata* plant is a perennial shrubby false-button weed herb which has immense potential as a medicinal source and been used as a traditional herb. Aqueous solutions of stem bark extracts of *Borreria verticillata* were prepared following cold extraction with hexane, ethyl acetate, acetone, chloroform and methanol sequentially in order of polarity. The extracts were tested on some pathogenic organism and were found to inhibit organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*. The minimum inhibitory activity of the stem bark extracts of *Borreria verticillata* against tested microbes ranges from 100 to 200 mg/mL in almost all the extracts and in few instance 50 mg/mL against the tested organisms. The purified fractions were tested for antimicrobial activity and reveals that ethyl acetate fractions (SEAF1-SEAF3), hexane fractions (SHF1-SHF3), and chloroform (SCF1-SCF3) strongly inhibited the growth of *Salmonella typhi*, *Staphylococcus aureus*, *E. coli* *Pseudomonas aeruginosa*, and *Candida albicans*. This justifies the claims by the traditional healers that the *Borreria verticillata* leaves are used to cure some illness.

**Keywords:** *Borreria verticillata*; stem; bark; antimicrobial activity; microorganism.
1. Introduction

Before the advent of orthodox medicine, most societies depended on traditional medicines for their health care needs. The traditional medicine men, in turn, relied on plants that had therapeutic values. Historically, plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made large contributions to human health and well-being (Achan et al., 1980). The primary benefits of using plant-derived medicines in healing are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments (Kudi and Myint, 1999). Today, phytochemists and pharmaceuticals companies depend on these medicinal Plants. A traditional medicine plant is defined as any plant, which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs (Sofowora, 1993). Most people in the rural areas of the world depend largely on herbs for treatment of several ailments because medicinal herbs constitute indispensable components of traditional medicine practice due to low cost, easy access and ancestral experience (Marini-Bettolo, 1980).

Information on the uses of the plant shows that the leaves of Borreria verticillata are used by indigenes of cross river state for curative purposes such as traditional medicine. Benjamin (1979) pointed out that Borreria verticillata have been commonly used effectively to cure eczema-tinea versicolor, ring worm-tinea capitis scabies and other lesions on the skin surface, (infectious dermatitis). Borreria verticillata leaves have also been used for toothache, headache, and dyspepsia (Sofowora, 1982). Borreria verticillata roots extract exhibited a broad antibacterial activity against multiresistant strains of Pseudomonas aeruginosa (Pedro et al., 2002).

The juice obtained from aerial part of the plant is applied topically for the treatment of skin diseases. A lotion is prepared to relieve skin itches (Liogier, 1990). In Gambia a lotion of the plant is used for febrile children. An essential oil extracted from leaves has been shown to inhibit Escherichia coli and Staphylococcus aureus (Burkill, 2000). It is employed in the form of enema for infantile hyperpnexia and treatment of leprosy, furuncles, ulcers, gonorrheal sores, biharzia and paralysis (Sofowora, 1982).

The plant is a forage plant, but not highly favoured by livestock. The Brazilian species has been shown to stimulate the uterus and duodenum of rat. It has no action on BP and respiration of cat and had effect on guinea pig intestine and striated frog muscle. B. verticillata is one of the plants which have been used in traditional medicine for many years. To the best of our knowledge little or no work has been done on B. verticillata in this part of the world. The present work was designed to provide information that may aid further utilization of B. verticillata for the benefit of mankind. The specific objectives of the study were to: (1) extracting and isolating,
the stem bark of *Borreria verticillata*, (2) confirm or disprove the efficacy of the stem bark of *B. verticillata* by evaluating the antifungal and antibacterial activities.

2. Materials and Methods

2.1. Sample Collection and Preparation

Tender *Borreria verticillata* stem bark were collected from their natural habitat of coastal plain sands in Calabar Municipality (04° 15"N; 08° 25"E), Nigeria. The samples were air-dried for about two weeks and then milled into fine powder using a Thomas-Willey milling machine. Aqueous solutions of the milled stem bark were prepared by soaking 100 g of each in 250 mL hexane for four days. The resulting mixture solutions were subjected to gravity filtration and the filtrates were concentrated by evaporation in a water bath, dried and weighed. The procedure was repeated on the residue using the following solvents: ethyl acetate, acetone, chloroform and methanol sequentially in order of polarity. The extracts were stored in desiccators.

2.2. Bioassay

This is the study of antimicrobial activity of the crude or purified extracts against microorganism. It was used as a guide to determine the active components of the stem bark of *Borreria verticillata*. The crude extracts were tested for antibacterial and antifungal activities.

The organisms (pathogen) tested are based on their implication in human diseases such as skin diseases, typhoid, pneumonia, dysentry, urinary tract, respiratory problems and others. The test organisms were collected from Bauchi specialist Hospital. They are as follows; *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *Rhizopus spp.*

The bacterial assay procedures of Water Worth (1978) and perez et al. (1990) were employed with small modification. The methods involved the preparation of the culture medium and inoculation. Aseptic technique was used to avoid contamination.

2.3. Preparation of the Media

Two media were employed for this research: NA (Nutrient agar) for bacteria culture and MEA (Malt extract agar) for fungi culture. The media were prepared by dissolving 28 g of NA (Nutrient agar) in 1 litre of distilled water, while 50 g of MEA (Malt extracts agar) was dissolved in 1 litre of distilled water. They were sterilized at 121 °C for 15 min in an autoclave and subsequently allowed to
cool to about 45 °C (temperature at which the agars remains molten) and pour in plate (petri dishes) allow to gel or solidified.

2.4. Standardization of Innoculums

The seven test organisms were sub-cultured with nutrient broth using a wire loop (done aseptically) and incubated for 24 hours at 35 °C for bacteria and 48 hours at 25 °C for fungi. The growth of the microorganisms in the broth by the turbidity produced was adjusted to match 0.5 McFarland standards (10^8 cfu/mL), which was further adjusted to 10^5 cfu/mL and 10^3 cfu/mL for bacteria and fungi respectively.

2.5. Innoculation of the Plates and Application of the Extracts

The agar plates NA (nutrient agar) and MEA (Malt extract agar) were inoculated by spreading a small volume (0.05 mL to 0.10 mL) of the liquid inoculums (sub-cultured nutrient broth) by means of an L-shaped glass rod (or a “spreader”) in such a way that the surface of the agar in the plates were covered with microbes. One microbe was inoculated to one plate making a total of seven plates for seven microbes. Five wells for hexane, chloroform, ethyl acetate, acetone, and methanol extracts and two for the control (tetracycline, fulcin) were made.

The plant extracts are diluted using dilution method and in each of the appropriately labeled well (hole) diluted plant extract were introduced. Tetracycline and fulcin were also introduced in the other two wells (holes) as control. The inoculated plates were left on the bench for about an hour to allow the extracts diffuse into the agar. The NA (nutrient agar) and MEA (malt extract agar) were aerobically incubated at 37 °C for 23 hours for the bacteria and 48 hours for the fungi. The diameter of zones of inhibition was measured by means of linear instrument in millimeter (venier calliper) and recorded.

3. Results

3.1. Nature and Yield of Crude Extracts from the Stem Bark of Borreria verticillata

The following are the results obtained from the extraction of secondary metabolites from the stem bark of *Borreria verticillata* using hexane, chloroform, ethyl acetate, acetone and methanol in order of polarity gave yields of 4.00 g (4.1%), 3.55 g (3.8%), 1.8 g (1.9%), 3.10 g (3.5%), and 2.76 g (3.2%) as presented in Table 1.
Table 1. Nature and yield of extract from stem bark of *Borreria verticillata*

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Colour of Extract</th>
<th>Texture of Extract</th>
<th>Yield of Extract (g)</th>
<th>Percentage Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hexane</td>
<td>brownish</td>
<td>hard solid</td>
<td>4.00</td>
<td>4.1</td>
</tr>
<tr>
<td>chloroform</td>
<td>brown</td>
<td>hard powder</td>
<td>3.55</td>
<td>3.8</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>dark brown</td>
<td>sticky powder</td>
<td>1.80</td>
<td>1.9</td>
</tr>
<tr>
<td>acetone</td>
<td>light brown</td>
<td>powder</td>
<td>3.10</td>
<td>3.5</td>
</tr>
<tr>
<td>methanol</td>
<td>light brown</td>
<td>powder</td>
<td>2.76</td>
<td>3.2</td>
</tr>
</tbody>
</table>

3.2. Antimicrobial Activity of Stem Bark Extracts

Activity of hexane, chloroform, ethyl acetate, acetone and methanol crude extracts from the stem bark of *Borreria verticillata* was tested on seven clinical isolates. The measured zone of inhibition of the pathogens by the crude extracts are summarized in the Table 2.

Table 2. Diameter of zone inhibition of antimicrobial activity of crude extract in mm from the stem bark of *Borreria verticillata*

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>HE</th>
<th>CE</th>
<th>EAE</th>
<th>AE</th>
<th>ME</th>
<th>TCN</th>
<th>FUL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus</em></td>
<td>14</td>
<td>15</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>22</td>
<td>NA</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>8</td>
<td>10</td>
<td>18</td>
<td>17</td>
<td>10</td>
<td>24</td>
<td>NA</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em></td>
<td>-</td>
<td>-</td>
<td>29</td>
<td>13</td>
<td>11</td>
<td>26</td>
<td>NA</td>
</tr>
<tr>
<td><em>Pseudomonas Aeruginosa</em></td>
<td>8</td>
<td>16</td>
<td>23</td>
<td>11</td>
<td>13</td>
<td>24</td>
<td>NA</td>
</tr>
<tr>
<td><em>Candidas albican</em></td>
<td>-</td>
<td>11</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>7</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>6</td>
<td>9</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>9</td>
</tr>
<tr>
<td><em>Rhizopus</em></td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>15</td>
</tr>
</tbody>
</table>

NOTE: HE = Hexane extract, CE = Chloroform extract, EAE = Ethyl acetate, AE = Acetone extract, ME = Methanol extract, FUL = Fulcin, TCN = Tetracycline, - = No Zone of clearance, NA = Not applicable.

3.3. Minimum Inhibitory Concentration of the Crude Extract from *Borreria verticillata* Stem Bark on Clinical Isolates.

Table 3 presents the minimum inhibitory concentration (MIC) of the crude extract from the stem bark of *Borreria verticillata* against the tested microbes.
Table 3. Minimum inhibitory concentration in mg/mL of the crude extract of the stem bark of the *Borreria verticillata*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Ps</th>
<th>Sa</th>
<th>St</th>
<th>Ec</th>
<th>Ca</th>
<th>As</th>
<th>Rh</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CE</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>EAE</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>200</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>AE</td>
<td>50</td>
<td>50</td>
<td>200</td>
<td>-</td>
<td>200</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>ME</td>
<td>-</td>
<td>200</td>
<td>100</td>
<td>-</td>
<td>200</td>
<td>-</td>
<td>200</td>
</tr>
</tbody>
</table>

Note: Ps...*Pseudomonas aeruginosa*, Sa...*Salmonella typhi*, St...*Staphylococcus aureus*, Ec...*Escherichia coli*, Ca...*Candidas albican*, As...*Aspergillus niger*, Rh...*Rhizopus* spp., HE...Hexane, CE...Chloroform, EAE...Ethyl acetate, AE...Acetone, ME...Methanol.

3.4. Antimicrobial Activity of Fractions of Column Chromatography of the Stem bark Hexane Extract of *Borreria verticillata*

Table 4 shows the result of the activity tests carried out on the fractions obtained from the hexane extract of the stem bark of *Borreria Verticillata*. The fractions were tested on the seven pathogens used for the crude extract, and tetracycline and fulcin were used as control.

Table 4. Diameter (mm) of zone of inhibition of antimicrobial activity of fractions of column chromatography stem bark hexane extract of *Borreria verticillata*

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Ps</th>
<th>Sa</th>
<th>St</th>
<th>Ec</th>
<th>Ca</th>
<th>As</th>
<th>Rh</th>
<th>TCN</th>
<th>FUL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHF1</td>
<td>5</td>
<td>8</td>
<td>12</td>
<td>16</td>
<td>5</td>
<td>7</td>
<td>12</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>SHF2</td>
<td>0</td>
<td>11</td>
<td>5</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>24</td>
<td>15</td>
</tr>
</tbody>
</table>


3.5. Antimicrobial Activity of Fractions of Column Chromatography of the Stem bark Chloroform Extract of *Borreria verticillata*

Table 5 shows the result of the activity tests carried out on the fractions obtained from the chloroform extract of the stem bark of *Borreria verticillata*. The fractions were tested on the seven pathogens used for the crude extract, and tetracycline and fulcin were used as control.
Table 5. Diameter (mm) of zone of inhibition of antimicrobial activity of fractions of column chromatography stem bark chloroform extract of *Borreria verticillata*

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Ps</th>
<th>Sa</th>
<th>St</th>
<th>Ec</th>
<th>Ca</th>
<th>As</th>
<th>Rh</th>
<th>TCN</th>
<th>FUL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF1</td>
<td>10</td>
<td>7</td>
<td>17</td>
<td>14</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>SCF2</td>
<td>16</td>
<td>7</td>
<td>13</td>
<td>11</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>SCF3</td>
<td>11</td>
<td>4</td>
<td>15</td>
<td>8</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>24</td>
<td>15</td>
</tr>
</tbody>
</table>


3.6. Antimicrobial Activity of Fractions of Column Chromatography of the Stem bark Ethyl Acetate Extract of *Borreria verticillata*

Table 6 shows the result of the activity tests carried out on the fractions obtained from the ethyl acetate extract of the stem bark of *Borreria verticillata*. The fractions were tested on the seven pathogens used for the crude extracts. Tetracycline and fulcin were used as control.

Table 6. Diameter of zone of inhibition of antimicrobial activity of Fractions of Column Chromatography stem bark ethyl acetate extract of *Borreria verticillata* (mm)

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Ps</th>
<th>Sa</th>
<th>St</th>
<th>Ec</th>
<th>Ca</th>
<th>As</th>
<th>Rh</th>
<th>TCN</th>
<th>FUL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEAF1</td>
<td>18</td>
<td>21</td>
<td>20</td>
<td>15</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>SEAF2</td>
<td>19</td>
<td>18</td>
<td>16</td>
<td>14</td>
<td>6</td>
<td>6</td>
<td>9</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>SEAF3</td>
<td>7</td>
<td>13</td>
<td>17</td>
<td>12</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>24</td>
<td>15</td>
</tr>
</tbody>
</table>


4. Discussion

The antimicrobial activity of *Borreria verticillata* stem bark against the growth of organisms (microbes) was observed visually and measured. Sofowora Abayomi (1982) pointed out that *Borreria verticillata* possesses antimicrobial action at different concentration depending on the bacteria species. For some species, for example, *Staphylococcus aureus* and for some other bacterial infections, the
minimum inhibitory concentration is the same as commercial antibiotic. Monica (1984) pointed out that active antimicrobial compound diffuses from the disc into the medium and the organism sensitive to the active antimicrobial compounds are inhibited at a distance from the disc. The in vitro antimicrobial activities results obtained from the stem bark extracts reveals the following. All the crude stem extracts inhibited *Escherichia coli* and *Pseudomonas aeruginosa* significantly. *Rhizopus* spp was negatively inhibited by all the stem extracts except ethyl acetate extract. Hexane, chloroform and ethyl acetate extracts exhibited reasonable antibacterial activities on *Staphylococcus aeru* while ethyl acetate, acetone and methanol extracts inhibited *Salmonella typhi* significantly.

The minimum inhibitory activity of the stem bark extracts of *Borreria verticillata* against tested microbes ranges from 100 to 200 mg/mL in almost all the extracts and in few instance 50 mg/mL against the tested organisms. The purified fractions from column chromatography were tested for antimicrobial activity and reveals that ethyl acetate fractions (SEAF1-SEAF3), hexane fractions (SHF1-SHF3), and chloroform (SCF1-SCF3) strongly inhibited the growth of *Salmonella typhi*, *Staphylococcus aerus*, *E. coli*, *Pseudomonas aeroginosa*, and *Candida albican*.

5. Conclusions

This justifies the claims by the traditional healers that the *Borreria verticillata* stem bark are used to cure some illness and has confirmed that *B. verticillata* stem bark are of medicinal value due to their antipathogenic activity and could be exploited for use in pharmaceutical.

References


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