Antimicrobial activity of the leaves of three species of Nigerian *Pterocarpus* (Jacq.)

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Abstract: This study investigated the antimicrobial activity of the ethanolic extracts of freshly expanded and older leaves of *Pterocarpus soyauxii*, *P. santalinoides* and *P. osun* against some human pathogens (*Escherichia coli*, *Salmonella typhii*, *Shigella flexneri*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Candida albicans*). The in vitro antimicrobial activity was performed using the Agar well diffusion method in nutrient agar, macconkey agar, blood agar and sabouraud dextrose agar. The ethanolic extracts of the samples showed moderate to high activity against all the tested pathogens. The inhibition zone ranged from 3.00 mm to 25 mm. Generally, the freshly expanded leaves (3.00 mm to 18 mm) tend to have higher antimicrobial activity when compared to that of older leaves (4.33 mm to 14.50 mm). *P. osun* leaves showed more antimicrobial activity on the test pathogens, when compared with those of the leaves of the other species. The concentration of the extract also affected the ability of the extract to inhibit the growth of the pathogens. The higher the concentration of the extract, the higher the rate of inhibition of the pathogens. The minimum inhibition concentration (MIC) ranged from 0.25 to 11.00 mg/ml. The result obtained indicates that the leaves of the plants have antimicrobial activity against the human pathogenic microorganism tested and can be exploited as alternative antimicrobial drug use for the treatment of infectious diseases caused by these pathogens.

Keywords: *Pterocarpus* species; Ethanolic extracts; Antimicrobial activity; Inhibition zone; Minimum inhibitory concentration.

Introduction

Plants role in the maintenance of good health cannot be overemphasized. Studies have shown that plants play important role in maintenance of good health (Burkill 1995; Edeoga and Eriata 2001). The bases of many modern pharmaceuticals used today are plants and plant-based products (Kamba and Hassan 2010). Plants have been generally utilized for the treatment of diseases worldwide. The report of WHO (2001) indicates that estimated 80% of world population depend on plant based medicine for their health care. WHO (1996) also observer that the majority of the population in the developing countries still rely on herbal medicine to meet their health needs.

The use of plants and plant based products to meet societal health need stems from the fact that indiscriminate use of commercial antimicrobial drugs commonly utilized in the treatment of infectious diseases has led to the development of multiple drug resistance (Gupta *et al*. 2008), the adverse effect on host, associated with the use of conventional antibiotics (Gupta *et al*. 2008), the safety and cost effectiveness of the use of plants in traditional as well as in modern medicine (Koche *et al*. 2011), and high cost, adulteration and increasing toxic side effects of these synthetic drugs (Shariff 2001). Thus there has been the need to develop alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases; because antimicrobials of plant origin have been found to have enormous therapeutic potential (Werner *et al*. 1999). Furthermore, the studies by Perumalsamy and Ignacimuthu (2000) showed that antimicrobials from plant origin are effective in the treatment infectious diseases and on the other hand simultaneously mitigates many of the side effects that are linked with synthetic antimicrobials.

The usefulness of plant based products in medicine is due to the presence of bioactive substances such as alkaloids, tannins, flavo-
noids, phenolic compounds, steroids, resins and other secondary metabolites which they contain and are capable of producing definite physiological action in the body (Bishnu et al. 2009). The phytochemical screening of the three *Pterocarpus* species has been carried out and they are found to be rich in alkaloid, phenols, saponins, tannins and flavonoids (Osuagwu et al. 2007). The proximate and vitamin content of their leaves have been carried and they are found to have high nutritive value (Osuagwu 2008).

The antimicrobial activities of plants and their products have been well documented (Pirbalouti et al. 2010; Arshad et al. 2010; Kamba and Hassan 2010; Koche et al. 2011). These plants are therefore used in the treatment of many diseases such as rheumatism, diarrhea, malaria, elephantiasis, cold, obesity, desentry, high blood pressure, malnutrition, gonorrhoea and others (Burkill 1995; Edet et al. 2009; Batram 1998; Akudor et al. 2010).

*Pterocarpus* species belong to the family Papilionaceae and they occur throughout the tropics (Keay et al. 1964). The leaves of *P. osun* and *P. santalinoides* have medicinal properties, they are used in the treatment of skin diseases such as eczema, candidiasis and acnes (Gill 1992). The leaves of *P. soyaxuii* and *P. santalinoides* are used as vegetables in food preparation (Osuagwu 2008). *P. santalinoides* leaves are used as fodder for feeding livestock, while the fruits of *P. soyaxuii* are edible (FAO, 1990).

The objective of this research is to determine the antimicrobial activity of the leaves of these *Pterocarpus* species in view of their utilization as alternative source of antimicrobial drugs in the treatment of infectious diseases.

**Materials and methods**

**Plant samples**

The leaves of *Pterocarpous santalinoides* and *P. soyaxuii* were collected from the forest strip of the Forestry Department of the College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, while those of *P. osun* were collected from a homestead bush in Okwuta-Ibeku, Umuahia North LGA, Abia State. The plant samples were identified by the Taxonomic unit of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State.

The leaves were separated into freshly expanded leaves and older leaves. This is due to the fact that the freshly expanded ones are the type usually used in the preparation of food. The leaves were then sun-dried for 29 days and ground using Thomas Willey Milling Machine. The milled samples were stored in clean sample bottles, corked and stored at room temperature in the laboratory.

**Determination of antimicrobial activity**

**Preparation of plant extract**

The ethanolic extracts of the leaves of *Pterocarpus santalinoides*, *P. sayaxuii* and *P. osun* were prepared using the method of Ijeh et al. (2005).

Fifty grams of the powdered sample were soaked in 200ml of absolute ethanol and allowed to stand for 24 hours. They were filtered using Whatman No1 Filter Paper. The filtrates were evaporated to dryness with rotary evaporator at 40°C to thick residues. The residues were dissolved in deionised water to obtain the desired plant extracts for the antimicrobial tests.

**Preparation of Innocular**

The human pathogens *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Candida albicans* used in the research were obtained from the stock culture of the Microbiology Laboratory, Federal Medical Centre, Umuahia, Abia State, Nigeria. Viability test of each isolate was carried out by resuscitating the organism in buffered peptone broth and thereafter sub-cultured into nutrient agar medium and incubated at 37°C for 24 hours.
Antimicrobial activity test

The sensitivity of the test organism to the ethanolic extracts of the leaves of the Pterocarpus species was carried out using the diffusion method described by Ebi and Ofoefule (1997).

20ml of the molten nutrient agar was seeded with 0.2ml of broth culture of the test organisms in sterile Petri dishes. The Petri dishes were rotated slowly to ensure a uniform distribution of the organisms. They were left to solidify and dish cups of 8.0mm diameter were made in the agar using a sterile Pasteur pipette. The Petri dishes were allowed to stand for about 30 minutes at room temperature to allow for the proper diffusion of the extracts to take place. The plates were then incubated at 37°C for 24 hours. The zones of inhibition in millimetre were measured and recorded.

The test was carried out in the Laboratory of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

Minimum Inhibitory Concentration (MIC) Test

The agar dilution method described by Baron and Finegold (1990) was used to determine the minimum inhibitory concentration.

Six grams of nutrient agar were dissolved in 250ml of distilled water in a conical flask. After sterilization, the nutrient agar was poured into sterilised petri dishes to solidify. The microorganisms were introduced into the wells using swap sticks. Extracts of 5mg/ml, 15mg/ml, 20mg/ml and 25mg/ml were made from the original test samples. The petri dishes were then placed in the incubator at 37°C for 24 hours. The inhibition zones in millimetres were measured and recorded.

Preparation of Antibiotics Stock Solution

500mg of Penicillin was dissolved in 5ml of distilled water for the antimicrobial assay. 12g of nutrient agar was dissolved in 250ml of distilled water in a conical flask. The nutrient agar was poured into sterilized Petri dishes after sterilization. After solidification, wells were made using a sterilized cork borer and microorganisms were introduced. The dissolved antibiotics solution was poured into the wells using a dropping pipette after which the Petri dishes were incubated for 24 hours at 37°C. The inhibitory zones in millimetre were measured and recorded.

Statistical Analysis

The tests were carried out in triplicate, data obtained were analysed using mean and standard deviation.

Results and discussion

The results of the antimicrobial activity of the leaves of the three Nigerian Pterocarpus species are summarized in Tables 1-4.

The ethanolic leaf extracts of the three Nigerian Pterocarpus species significantly inhibited the growth of the test microorganisms (Tables 1 and 2). The antimicrobial activity of other plants has also been reported (Arshad et al. 2010; Kamba and Hassan 2010; Koche et al. 2011). The ability of the extracts to inhibit the growth of the microorganisms might be as a result of the presence of bioactive substances such as alkaloids, saponins, tannins, phenol in their leaves (Osuagwu et al. 2007). Research reports indicate that the antimicrobial property of plants is conferred in them by the presence of secondary metabolites (Edeoga et al. 2005; Ebana et al. 2007; Bishnu et al. 2009). Generally, the ethanolic extracts of freshly expanded leaves tend to have more inhibitory effects on the microorganisms when compared with those of old leaves (Tables 1-2). This might be due to the fact that the freshly expanded leaves in plants seem to contain more metabolites than older leaves (Osuagwu et al. 2007; Brenes-Arguedis et al 2006) hence having more inhibitory ability. P. osun leaves had the highest antimicrobial activity (12.00-18.25mm), while those of the leaves of P. soyauxii had the least antimicrobial activity (3.00-11.75mm). This observed trends might be related to the concentration of these bioactive constituents in them. P. soyauxii had more inhibitory effect on Klebsiella pneumonia and least on Staphylococcus aureus. the other
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hand, *P. santalinoides* had its highest effect on *Shigella flexneri* and least on *Candida albicans*, while the extracts of *P. osun* had the highest effect on *Salmonella typhi*.

Table 1: The antimicrobial activity of the ethanolic extracts of the freshly expanded leaves of the three Nigerian *Pterocarpus* species on *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Staphilococcus aureus*, *Klebsiella pneumonia* and *Candida albicans*.

<table>
<thead>
<tr>
<th>Pathogenic Organisms</th>
<th><em>P. soyauxii</em> Zone of Inhibition</th>
<th><em>P. santalinoides</em> Zone of Inhibition</th>
<th><em>P. osun</em> Zone of Inhibition</th>
<th>Penicillin stock solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>4.33 + 2.62</td>
<td>9.33 + 3.68</td>
<td>17.50 + 2.50</td>
<td>7.25 + 2.17</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>3.75 + 1.48</td>
<td>9.25 + 5.31</td>
<td>18.25 + 2.93</td>
<td>6.75 + 3.27</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>7.25 + 1.92</td>
<td>5.50 + 1.50</td>
<td>17.00 + 2.65</td>
<td>10.25 + 2.95</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>3.00 + 1.73</td>
<td>13.00 + 4.30</td>
<td>14.33 + 3.35</td>
<td>12.25 + 3.96</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>11.00 + 5.10</td>
<td>7.50 + 2.29</td>
<td>11.00 + 1.00</td>
<td>8.75 + 3.90</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>6.50 + 3.35</td>
<td>4.00 + 1.00</td>
<td>15.33 + 4.11</td>
<td>5.25 + 1.79</td>
</tr>
</tbody>
</table>

The concentration of the extract affected the rate of inhibition of growth of the pathogens. Generally, extracts of both freshly expanded leaves and older leaves antimicrobial activity increased with increase in concentration of the three Nigerian *Pterocarpus* species ranged from 0.25mg/ml to 11.00mg/ml (Tables 3-4). Lower concentration of the ethanolic extracts of the leaves of *P. soyauxii* and *P. santalinoides* had no antimicrobial activity on *Candida albicans* and *Klebsiella pneumonia*. However, extracts of *P. osun* at all concentration had antimicrobial effects on all the test pathogens. This observation might suggest that *P. osun* contains more of the bioactive substances.

Table 2: The antimicrobial activity of the ethanolic extracts of the older leaves of the three Nigerian *Pterocarpus* species on *E. coli*, *S. typhi*, *S. flexneri*, *S. aureus*, *K. pneumonia* and *C. albicans*.

<table>
<thead>
<tr>
<th>Pathogenic organisms</th>
<th><em>P. soyauxii</em> Zone of Inhibition</th>
<th><em>P. santalinoides</em> Zone of Inhibition</th>
<th><em>P. osun</em> Zone of Inhibition</th>
<th>Penicillin stock solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>7.00 + 2.00</td>
<td>6.00 + 2.77</td>
<td>14.50 + 3.35</td>
<td>7.25 + 2.17</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>11.00 + 2.16</td>
<td>4.33 + 1.25</td>
<td>13.00 + 2.74</td>
<td>6.75 + 3.27</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>11.75 + 3.19</td>
<td>9.25 + 3.77</td>
<td>12.00 + 3.16</td>
<td>10.25 + 2.95</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>4.66 + 2.16</td>
<td>7.00 + 1.00</td>
<td>14.00 + 2.24</td>
<td>12.25 + 3.96</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>11.66 + 1.29</td>
<td>7.33 + 2.49</td>
<td>14.33 + 4.78</td>
<td>8.75 + 3.96</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>8.75 + 2.59</td>
<td>11.66 + 4.19</td>
<td>14.00 + 1.00</td>
<td>5.25 + 1.79</td>
</tr>
</tbody>
</table>

Table 3: The minimum inhibitory concentration (mg/ml) of the ethanolic extracts of freshly expanded leaves of the three Nigerian *Pterocarpus* species on *E. coli*, *S. typhi*, *S. flexneri*, *S. aureus*, *K. pneumonia* and *C. albicans*.

<table>
<thead>
<tr>
<th>Pathogen Organism</th>
<th><em>P. soyauxii</em> Conc. (mg/ml)</th>
<th><em>P. santalinoides</em> Conc. (mg/ml)</th>
<th><em>P. osun</em> Conc. (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>11.00</td>
<td>4.00</td>
<td>4.50</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>0.62</td>
<td>0.25</td>
<td>1.75</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>2.00</td>
<td>2.75</td>
<td>5.00</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.50</td>
<td>2.25</td>
<td>4.25</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>0.00</td>
<td>0.75</td>
<td>2.75</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>0.00</td>
<td>2.50</td>
<td>4.75</td>
</tr>
</tbody>
</table>
The results obtained from this investigation revealed that the leaves of the three Nigerian Pterocarpus studied have antimicrobial activity on the human test pathogen used in the research and that they could be utilized in the formation of alternative antimicrobial drugs.

References


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