Antimicrobial Activities of the Extracts and Fractions of Allanblackia floribunda

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Abstract: Allanblackia floribunda is a tree employed in Nigeria and other countries to treat skin disease and other microbial diseases. The ethanol extract, n-hexane, chloroform, ethyl acetate and butanol fractions of the leaves, stem bark and root bark were evaluated for antimicrobial activities against Staphylococcus aureus NCIB 8588, Bacillus subtilis NCIB 3610, Escherichia coli NCIB 86, Proteus vulgaris NCIB 67, Pseudomonas aeruginosa NCIB 950, Klebsiella pneumoniae NCIB 418, Candida albicans and Aspergillus flavus, using agar diffusion method to validate the ethnomedical uses of the plant. Among the extracts, the ethanol extract of the leaf gave the most significant antibacterial activity. However, no extract showed antifungal activity. Generally, the fractions obtained from the extracts elicited better activity, including antifungal activity against C. albicans. The highest inhibitory effect was exhibited by leaf extract against Ps. aeruginosa NCIB 950, while the ethyl acetate fraction of the stem bark gave the least inhibitory effect against B. subtilis NCIB 3610. The plant extract and fractions produced inhibition zone range between 5 and 35 mm.

Keywords: Antimicrobial activities, ethanol extract, fractions, Allanblackia floribunda

INTRODUCTION

Allanblackia floribunda Oliver ( Clausiaceae), commonly known as Tallow tree reproduces by seeds. Its occurrence is limited to tropical Africa, but centred mostly on the lowland rainforests (Van Rompacy, 2003).

The nuts of the plant produce fine oil taken by the members of local communities in Tanzania to relieve rheumatism (Anonymous, 2004). Also, in Cameroon, the stem bark of the plant mixed with Capsicum frutescens or Solanum anguivi is used for the treatment of cough (Betti, 2004). In Gabon, the bark is pounded and rubbed on the body to relieve painful conditions. There also, a decoction is taken for dysentery and as a mouthwash for toothache and, in Côte D'Ivoire, for stomach pains (Steentoft, 1988). In Ghana, the bark is used for medicinal purposes such as toothache, diarrhoea and as a pain reliever (Abiww, 1990). The bark decoction of the stem and root is also used in Central African Republic and West Africa to treat toothache, dysentery and as an analgesic (Lewis and Elvin-Lewis, 1977).

In Akwa Ibom State of Nigeria, the leaves as well as the bark of the stem and root of the plant are used by the local communities to treat dysentery, diarrhoea, skin diseases and some other microbial diseases.

The fruits and the seed kernels produce a hard white fat (Cunningham, 1993). The use of the fat in soap production has been suggested (Foma and Abdala, 1985). A new prenated xanthone known as Allanxanthone A and some other known xanthones have been identified from the stem bark of A. floribunda and have also been shown to have cytotoxic activity against KB cell line (Nkengfa et al., 2002).

This study aimed at evaluating the leaves, stem bark and root bark of A. floribunda for antimicrobial activities against Staphylococcus aureus NCIB 8588, Bacillus subtilis NCIB 3610, Escherichia coli NCIB 86, Proteus vulgaris NCIB 67, Pseudomonas aeruginosa NCIB 950, Klebsiella pneumoniae NCIB 418, Candida albicans and Aspergillus flavus, in order to validate the ethnomedical use of this plant for microbial diseases.

MATERIALS AND METHODS

Extraction: The fresh leaves (1 kg), stem bark (1 kg) and root bark (1 kg) of Allanblackia floribunda were collected in June, 2006, air-dried for a week and reduced to powder. The powder (300 g) was macerated in 1 L of EtOH-H2O (1:1) for 72 h. The liquid extract obtained was concentrated to dryness in vacuo at 40°C to yield dry ethanol extract (40-50 g).

Phytochemical screening: Applying the methods of Sofowora (1993) and Trease and Evans (2002), the dry

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ethanol extract of each part was subjected to phytochemical screening to reveal the presence of its secondary metabolites.

**Partition chromatography:** The dry crude ethanol extract (30 g) of each plant part was dissolved in 40 mL of distilled water and successively partitioned with n-hexane (50 mL × 3), chloroform (50 mL × 3), ethyl acetate (50 mL × 3) and n-butanol (50 mL × 3) to yield their respective fractions. The fractions were separately concentrated to dryness in vacuo to give dry residues.

**Test organisms:** The bacteria used in this study were typed cultures obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, while the fungi were clinical isolates collected from the same source. The bacteria: Staphylococcus aureus NCIB 8588, Bacillus subtilis NCIB 3610, Escherichia coli NCIB 86, Proteus vulgaris NCIB 67, Pseudomonas aeruginosa NCIB 950 and Klebsiella pneumoniae NCIB 418 were sustained on nutrient agar (Oxoid) slant at 4°C prior to use. However, the fungi Candida albicans and Aspergillus flavus were sustained on Sabouraud's Dextrose Agar (Oxoid) slants at 4°C before use.

**Antimicrobial test:** The dry ethanol extract and the dry fractions were evaluated against the test microorganisms using agar-gel diffusion method described by Alade and Irobi (1993). The ethanol extract and the fractions were redissolved in Dimethyl sulfoxide (DMSO). The ethanol extract and the fractions were tested at concentration levels of 40 and 80 mg mL⁻¹. Fixed volumes (150 µL) of the ethanol extract, fractions and DMSO were separately introduced into equidistant wells bored on the surface of the agar and Sabouraud's plates, which had been previously inoculated with one of the test organisms. A well containing a standard drug, chloramphenicol was made in the bacteria plates, while the fungal plates had a hole containing Nystatin as standard drug.

The bacteria were incubated at 37°C for 24 h, while the fungi were incubated at 25°C for seven days. The presence of zones of inhibition surrounding the wells was taken as an evidence of antimicrobial activity.

**RESULTS**

The extracts of the leaves, stem bark and root bark showed various classes of compounds (Table 1) inherent in the plant. The extracts of the three parts contained tannins and cardiac glycosides in high concentration, while flavonoids and terpenes occurred in moderate concentration. Anthraquinones, phlobatannins and alkaloids were absent in all the parts, while saponins were abundant in the leaves and absent in others.

The extracts of the leaves, stem bark and root bark exhibited varying degrees of antimicrobial effects, with leaves showing the highest antibacterial activity and the root bark, the least (Table 2). It is noteworthy that none of the extracts was effective against the test fungi. Also, E. coli was resistant to all the extracts. The inhibitory effect of the leaf extract against P. aeruginosa was much better than that of Chloramphenicol. The purification of the extracts by partition chromatography showed some improvement on the antimicrobial activity of all the extracts. For instance, the ethyl acetate fraction of the leaf extract elicited an improvement on the activity of the extract, though fungal activity was still lacking. With the exception of aqueous fraction, all the other fractions generally showed improved activity than the stem bark extract. This improved activity included fungal effect against C. albicans. However, n-hexane fraction gave the highest activity. This pattern is similar for root bark extract.

**DISCUSSION**

The result of this study showed that the extracts and fractions of the leaves, stem bark and root bark of Allamblaica floribunda gave good inhibitory effects against all the test microorganisms except A. flavus.

The result also revealed that the leaf extract gave more significant inhibitory effects than those of stem bark and root bark. However, all the extracts exhibited only antibacterial effects.

The fractions obtained from the extracts through partition chromatography gave improved activity against
test organisms, including activity against *C. albicans* which the extracts failed to inhibit. A similar result has been observed for the leaves of *Heinsia crinita* (Ajibesin et al., 2003). This suggests that antimicrobial activity was increased by purification of active constituents of the plant. Generally, all the extracts and fractions failed to inhibit *A. flavus*. However, aqueous fraction did not show significant inhibitory effect against all the test organisms except *B. subtilis*. This may be due to the fact that virtually all the antimicrobial principles had been extracted from the aqueous phase during fractionation. The best inhibitory effect was elicited by the leaf extract against *P. aeruginosa*, while the least was by ethyl acetate fraction of the stem bark against *B. subtilis*. Furthermore, the antimicrobial activities of the extracts and the fractions were dose-dependent.

The presence of terpenes observed in the phytochemical screening may be responsible for the enhanced effect of n-hexane fraction, since terpenes are non-polar compounds located in non-polar fractions. In a similar fashion, tannins, flavonoids and saponins may account for the improved activity of the ethyl acetate and butanol fractions. The antimicrobial activity of phenolics (tannins, flavonoids) and saponins has been established in some plants (Buraphadaj and Bunchoo, 1995; Adesina et al., 2000; Birutu and Cordell, 2000;)

Table 2: Antimicrobial activity of the extracts and fractions of the plant parts on the test organisms

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Concentration (mg mL⁻¹)</th>
<th>Zone of inhibition of organisms (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> (NCIB 86)</td>
<td>15±2.45 6±2.45 20±3.45 12±2.45 11±1.41 16±2.45 11±2.45</td>
<td>L1 L2 L3 L4 L5 L6 B1</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> (NCIB 950)</td>
<td>11±1.41 14±2.45 6±2.45 23±1.41 5±1.41</td>
<td>12±1.41</td>
</tr>
<tr>
<td><em>S. aureus</em> (NCIB 8588)</td>
<td>11±1.41 18±1.4 11±1.4 20±2.45 16±1.41 13±2.45 11±0.00</td>
<td>32±2.45 15±1.4</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Microorganisms</th>
<th>Concentration (mg mL⁻¹)</th>
<th>Zone of inhibition of organisms (mm)</th>
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<tbody>
<tr>
<td><em>E. coli</em> (NCIB 86)</td>
<td>26±2.45 11±3.74 16±3.74 7±0.90 11±1.41 15±2.45</td>
<td>L2 L3 L4 L5 B6 B1</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> (NCIB 950)</td>
<td>5±1.41 11±2.45 11±3.74 6±2.45</td>
<td>12±2.45</td>
</tr>
<tr>
<td><em>S. aureus</em> (NCIB 8588)</td>
<td>19±2.45 16±3.74 28±2.45 13±1.41 10±2.45</td>
<td>27±1.41 7±2.45</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Bacteriophages</th>
<th>Concentration (mg mL⁻¹)</th>
<th>Zone of inhibition of organisms (mm)</th>
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<tbody>
<tr>
<td><em>E. coli</em> (NCIB 86)</td>
<td>17±0.00 6±0.00 14±2.45 7±0.00 19±2.45 8±2.45</td>
<td>B2 B3 B4 B5 B6 B1</td>
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<tr>
<td>Zones of inhibition of organisms (mm)</td>
<td>Concentration (mg ml⁻¹)</td>
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<td>R2</td>
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<td></td>
<td>A</td>
<td>B</td>
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<tr>
<td>E. coli (NCIB 86)</td>
<td>21±1.41</td>
<td>11±2.82</td>
</tr>
<tr>
<td>P. vulgaris (NCIB 67)</td>
<td>18±0.00</td>
<td>14±2.45</td>
</tr>
<tr>
<td>P. aeruginosa (NCIB 950)</td>
<td>22±1.41</td>
<td>16±3.74</td>
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<tr>
<td>Staph. aureus (NCIB 8588)</td>
<td>-</td>
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<td>Sptions aureus (NCIB 3619)</td>
<td>-</td>
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<tr>
<td>L. pneumophila (NCIB 418)</td>
<td>18±2.82</td>
<td>-</td>
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**Fungi**

<table>
<thead>
<tr>
<th>Cordyceps sinensis</th>
<th>15±3.74</th>
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<th>-</th>
<th>15±2.82</th>
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<th>14±2.45</th>
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<tbody>
<tr>
<td>Applicability factor</td>
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L1 = Leaf ethanol extract
L2 = Leaf n-hexane fraction
L3 = Leaf chloroform fraction
L4 = Leaf ethyl acetate fraction
L5 = Leaf butanol fraction
L6 = Leaf aqueous fraction
A = 80 mg ml⁻¹
B = 40 mg ml⁻¹
C = Chlorogenic acid (2 μg ml⁻¹)
N = Nyctin (2 μg ml⁻¹)
NA = Not applicable
DM = DMSO

Pistelli et al., 2002; Mandal et al., 2005). The extracts of the plant and their fractions gave significant zones of inhibition against the test organisms, thereby validating the ethnobotanical claims on this plant as a remedy for the treatment of infections and diseases caused by these organisms. This result corroborates the reports on the validation of claims from ethnobotanical surveys on other plants used to treat microbial diseases (Rajakaruna et al., 2002; Khan and Omoloso, 2002; Idowu et al., 2005; Rene et al., 2006; Elko et al., 2007). From the result, the plant was found to be effective against bacterial infections, but weak against fungal diseases. Further, the leaves whose extract and fractions exhibited the best activity are recommended for use in preference to other parts of the plant. Since activity improved with purification, this encourages further investigation to be carried out on the active fractions of the plant in order to isolate and characterize the active constituents responsible for the improved activity recorded in this study. The active constituents if isolated may show much better activity, leading to the production of effective antibiotics.

REFERENCES


