Antimicrobial Activities of the Leaves of Four Ethno Varieties of Lasiandra africana
Kola’ K. Ajbinsin1* and Margaret E. Bassey 2

Abstracts: Four ethno varieties of Lasiandra africana are used in Nigeria to treat microbial diseases including dysentery, diarrhea and skin diseases. The ethanol extracts, n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions of the leaves of the four varieties 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 and tube dilution techniques. Among the extracts, the ethanol extract of the leaves of black variety gave the best antimicrobial activity, while the ethanol extract of the forest variety exhibited the least. The fractions derived from the extracts generally elicited better activity. However, no extracts or fractions showed any activity against A. flavus. The most inhibitory effect was elicited by the ethyl acetate fraction of the black variety against E. coli, while the least inhibitory effect was shown by butanol fraction of the forest variety against P. vulgaris and ethyl acetate fraction of the rivet variety against S. aureus. Aqueous fractions of all the varieties showed no antimicrobial effect against any of the organisms tested. The minimum inhibitory concentration of the extracts and fractions ranged between 25 and 250 µg/mL.

Key Words: Antimicrobial activity, Ethanol extracts, Fractions, Ethno variety, Lasiandra africana.

INTRODUCTION

Lasiandra africana is a perennial glabrous shrub of the family Lecadseacae. It is the only species endemic in West Tropical Africa (Hutchinson and Dalziel, 1950), found only in southeast of Nigeria (Akwa Ibom and Cross River States) and Mokoko region of Cameroon. The leaves are oblong-elliptic, caudate-acuminate and cuneate at the base. Flowers are white and occur in head-like clusters. The plant locally referred to as "Editan" in Akwa Ibom and Cross River States, grows wild in the forest and at riverbanks. However, it is now brought under cultivation, and together with the wild sources, four ethno varieties have emerged: Editan akaf (Forest variety), Editan idim (Riverine variety), "Obubuk variety" (Black variety) and "Afiya Editan" (White variety). The black variety is so called due to its characteristic glossy dark green leaves and bitter taste, while the white variety is due to its light green leaves and bland taste. The rest and the riverine varieties are named based on their particular ecological niche.

The four varieties of Lasiandra africana show similarities in the stem characteristics, shorepense position, flower colour, fruit and seed colour and shape. However, distinctions are observed in the leaf shape, apex, leaf length, leaf length-width ratio (Bassey et al., 2004).

Cold infusion of the leaves is administered on a woman to expedite the release of variety was deposited at the herbarium of the Department of Pharmacognosy and Natur Medicine, University of Uyo.

Extraction
1 kg of the fresh leaves of the 4 ethno varieties were separately oven-dried (40 °C), pulverise and macerated in 50 % ethanol for 72 h. The liquid extract was concentrated in vacuo at 4 °C. The dry extract was subjected to physicochemical screening so as to reveal the classes of compounds contained in the plant varieties.

Antimicrobial assay
Microorganisms: The bacteria used in the study were Bacillus subtilis (NCIB 3610), Staphylococcus aureus (NCIB 8588), Pseudomonas aeruginosa (NCIB 950), Proteus vulgaris (67), Escherichia coli (86). However, the fungi used were clinical isolates: Candida albicans and Aspergillus flavus. All the organisms were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The bacteria were maintained on blood agar slants at 40 °C in some prior to use.

Antimicrobial susceptibility testing: The extracts and the fractions were reconstituted in MeOH-H2O (1:1) to obtain a stock solution of 20 mg/mL. 50 µl of this solution was introduced into each of the equidistant well (5 mm) bored on the agar plate surface previously inoculated with each of the test organisms. A control well containing Gentamycin (5 µg/mL) was placed in each row the plates seeded with bacteria, while Nystatin (5 µg/mL) was used as a control for fungal plates. The bacteria were incubated at 37 °C for 24 h, while the fungi were incubated at 2 °C for 7 days (Alade and Irobi, 1993). Antimicrobial activity was expressed as a average diameter of the zones of inhibition calculated as a difference in diameter of the observed zones and those of the wells.

Minimum Inhibitory Concentration: The minimum inhibitory concentration (MIC) was determined by incorporating various concentrations (250 to 6.25 µg/mL) of the solution of extract and fractions into sets of test tubes containing the culture media. 50 µL of the standard test bacterial and fungal broth cultures were added into each of the test tubes. The set of tube containing a mixture of bacteria and fungus samples (extracts and fractions) were incubated at 37 °C for 24 h, while those containing the fungi were incubated at 25 °C for 7 days (Cos et al., 2006).

A positive control tube containing only the growth medium of each of the organisms was also set up. The MIC was regarded as the lowest concentration of the extract or fraction that did not permit any visible growth when compared with that of the control tubes.

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RESULTS
The phytochemical screening revealed the presence of high concentration of alkaloids in the Black variety, while the other varieties showed trace (Table 1). Saponins were trace in White variety but absent in others, while anthraquinones were absent in White varieties, but trace others. Flavonoids and terpenes were present in all the varieties; however, they were in high concentration in the Black variety. Cardiac glycosides were present in equal concentration in all the varieties.

The ethanol extracts of the 4 ethnovarieties of *Lasionthera africana* exhibited differing degrees of antimicrobial effects against the microorganisms tested. The black variety gave the highest activity, while the riverine one gave the least (Table 2). None of the variety was active against *Aspergillus flavus*. The antimicrobial effects of the black and white varieties against *Bacillus subtilis* were comparable with Gentamycin. The mean zones of inhibition obtained for extracts were between 4 and 15 mm.

The antimicrobial effects of the extracts were increased by purification through partition chromatography, though the increase was marginal in some other extracts such as the ethanol extract of the forestry variety (Table 3). The chloroform and ethyl acetate fractions of the black and white varieties elicited increase in their activity. However, the aqueous fractions of all the varieties were inactive to all the test organisms. Minimum inhibitory concentration values of 50 - 250 μg/mL were recorded for the fractions of the 4 varieties against the bacteria, while a range of 100 - 200 μg/mL was obtained against *Candida albicans*, the only susceptible fungus (Table 4).

DISCUSSION
The result of the study shows that the extracts and fractions of the 4 ethnovarieties gave good inhibitory effects against some of the test microorganisms implicated in the pathogenesis of human infections (Duguid et al., 1978). The result further revealed that the black and white varieties gave better antimicrobial effects than the forestry and riverine varieties. This may be due to the higher concentration of bioactive constituents such as alkaloids, terpenes, flavonoids and tannins detected in the black and white varieties during phytochemical screening.

The fractions obtained from the extracts by partition chromatography showed higher activity against all the test organisms except *flavus*. *Proteus vulgaris* that showed resistance to the extracts of black and white varieties was found to be susceptible to the chloroform and ethyl acetate fractions of black and white varieties, respectively. Similar result has been reported for *Allanblackia floribunda* (Ajibesin et al., 2008). This commutes antimicrobial effect was enhanced purification of bioactive constituents acting in synergy, *Aspergillus flavus* resistant to all the extracts and fractions. Similarly, aqueous fractions of all the varieties were inactive to all the test organisms.

Suggests that almost all the antimicrobial principles have been extracted from aqueous fractions during fractionation. Highest inhibitory effect was exhibited by ethyl acetate fraction of the black variety against *E. coli*, while the least effect was butanol fraction of the forest variety against *vulgatus*.

The highly active fractions such as chloroform and ethyl acetate fractions of black variety showed very low values underpinning their potency. The range was 25 - 250 μg/mL.

The presence of bioactive compounds detected in the plant varieties may account for the observed in vitro antimicrobial effects. Further in vivo studies are necessary to fully evaluate the potential of these ethnovarieties as natural antimicrobial agents.
Table 3. Antimicrobial activity of the fractions of the four ethno varieties of L. africana

<table>
<thead>
<tr>
<th>MO</th>
<th>Black variety</th>
<th>White variety</th>
<th>Forest variety</th>
<th>Riverine variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>C</td>
<td>E</td>
<td>B</td>
</tr>
<tr>
<td>B.s</td>
<td>0</td>
<td>18</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>S.a</td>
<td>0</td>
<td>15</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>P.a</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>K.p</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>P.v</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E.c</td>
<td>0</td>
<td>0</td>
<td>21</td>
<td>7</td>
</tr>
<tr>
<td>C.a</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A.f</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

MO= Microorganism; B.s= Bacillus subtilis; S.a= Staphylococcus aureus; P.a= Pseudomonas aeruginosa; K.p= Klebsiella pneumoniae; P.v= Proteus vulgaris; E.c= Escherichia coli; C.a= Candida albicans; A.f= Aspergillus flavus; B= hexane; C= chloroform; E= ethyl acetate; B= butanol; a= aqueous; *a= values are mean of quadruplicate

Table 4. Minimum inhibitory concentration of the fractions of four ethno variety of L. africana

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Black variety</th>
<th>White variety</th>
<th>Forest variety</th>
<th>Riverine variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>E</td>
<td>B</td>
<td>H</td>
</tr>
<tr>
<td>B.subtilis</td>
<td>50</td>
<td>25</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>S.aureus</td>
<td>100</td>
<td>50</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>100</td>
<td>200</td>
<td>&gt;250</td>
<td>200</td>
</tr>
<tr>
<td>K.pneumonia</td>
<td>100</td>
<td>200</td>
<td>&gt;250</td>
<td>200</td>
</tr>
</tbody>
</table>

H= Hexane; C= chloroform; E= ethyl acetate; B= butanol; a= values are mean of quadruplicate

the antimicrobial effects of the extracts and the enhanced effects of the fractions. The antimicrobial effect of terpenes and phenolics has been reported in some plants 11(Habtemariam et al, 1993; 12Adesina et al, 2000). 13Garou et al, (2006) also reported the antimicrobial effect of alkaloids from Sida acuta.

The extracts and the fractions of the varieties that elicited good zones of inhibition against the test organisms have validated the ethnobotanical claims on this plant as a remedy for treatment of infections caused by these organisms 14(Ajibesin et al, 2008). Similar reports on the validation of claims from ethno botanical survey of other plants used to treat microbial diseases have been made and corroborated by this finding 15(Rajakaruna et al, 2002; 16 Ekpo et al, 2007). From the result, the plant varieties were found to be effective against bacterial and candidal infections. However, the black and white varieties were far more active than the forest and riverine varieties. Thus, the black variety that gave the best activity is recommended for use, followed by the white variety.

Phytochemical investigation should be performed on the active fractions of these varieties so as to identify the active constituents responsible for the enhanced activity observed in this study. The active constituents identified may give a lead to the production of effective antibiotics.

CONCLUSION
The four ethnovarieties of Lagerthia africana gave varying degrees of antimicrobial effects, validating their use in traditional medicine of Nigeria. However, the black variety showed the best activity suggesting its preference over the use of others.

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