Antimicrobial Potential of Ten Common Medicinal Plants used by the Bokis, Cross River State, Nigeria

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Abstract

Evidence of the continuous use of medicinal plants for primary health care is noted amongst the Bokis living within and on the fringes of the Cross River National Park (Okwangwo division). In consonance with their traditional usage the methano-aqueous extracts of ten common medicinal plants used by traditional medicine men in nine communities of the area were assayed for antimicrobial activity. Four bacteria species and one fungus were used as in-vitro test organisms. Gentamicin was used as standard antimicrobial agent for comparative efficacy. Results of the tests using the diameter of zone of inhibition showed that three i.e. Senna alata and Dillenia indica and Grewia megalocarpa of the total plant assayed at a concentration of 2 mg/ml exhibited some level of activity against Staphylococcus aureus only. The antimicrobial property of these plants is indicative of their usefulness in traditional medicines. Hence, knowledge of medicinal plants practice in this region can be an important source for new drug discoveries. Invariably, such knowledge should act as an important feeder in the development of a robust health care policy and service for the region with recognition and active inputs from traditional medicine practitioners.

Keywords: Zone of inhibition; Methano-aqueous extracts; antibacterial; antifungal; Dillenia indica, Senna alata; Grewia megalocarpa

Introduction

The utilization of plants in traditional healing systems and rites dates back to antiquity and are well documented [1-7]. Importantly, eighty percent of population in most African and Asian countries still rely on traditional medicine for their primary health care, with sales in herbal medicines generating billions of dollars [8]. Traditional medicine practitioners within these societies and elsewhere have used plants for different classes of ailments; examples include the abortifacient effects of Oxalis corniculata [9], and Achyranthes aspera [10]. Others include the use of Senna alata as a purgative [11]; anthelmintic effect of Combretum mucronatum [12] and the widely used neem plant Azadirachta indica as a febrifuge [13]. Utilization involves the usage of different parts, such as the leaves, bark and in some treatments the whole plant [14-16]. Usually plants could be pulverized and applied on affected areas as a poultice or ingested as decoctions and so on. While a majority of plants are used specifically for treating one ailment [17], some on the other hand have multiple uses [18-22].

Communities of the study area are mainly forest enclaves and are far from modern health care facilities. In many instances, villagers have to trek along treacherous terrains for close to five (5) hours to the nearest village for medical care; hence they resort to natural medicines which are cheap and readily available. In view of the importance of plants within the region an extensive ethnobotanical study was conducted involving the nine communities, reported earlier [23], where a total of seventy-three plants used in disease management was recorded.

Materials and Methods

The study site

The study site is within the West African Guinea-Congolian forest, contiguous to Takamanda in the Republic of Cameroon in the Southeast Zone of Nigeria [24]. The nine communities represented are mostly enclaves of the Okwangwo and Okwangwo-Boshi extension forest reserve of the Cross River National Park area, in Cross River State, Nigeria (Figure 1). The Cross River National Park covers a total area of approximately 4000 square kilometres, comprising two divisions the southern axis (Oban division) and the Okwangwo division (about 1000 square kilometres), in the north [25]. Study communities included; Okwangwo, Okwa 1, Okwa 2, Abio-mkpang, Bamba, Bokalum, Butatong, Wula and Boggo.

Plant collections and antimicrobial testing

Semi-structured interviews were conducted with key informants (i.e. traditional medicine men and birth attendants) using a check list drawn from previous interactions with the communities. Subsequently, through a ranking exercise done in conjunction with the interviewee ten plants were selected across the nine communities for antimicrobial testing. Plant specimens were identified in comparison to the specimen preserved in the National Herbarium of the Forestry Research Institute of Nigeria, Ibadan. Voucher specimens have been deposited in the herbarium of the Biological Research Unit, Cross River National Park (Okwangwo division). Specimens for laboratory analysis were air dried for 2-3 days, or until sufficiently dried, after which they were packed in labelled envelopes for further analysis.

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Figure 1: Study Area.
Dillenia indica (7.37%) and Prior to testing the microorganisms were sub-cultured into nutrient potato dextrose agar respectively after a series of sub-culturing at 4°C. The bacteria and fungi were maintained on nutrient agar plates and and Bacillus subtilis Pseudomonas aeruginosa the total plant extracts showed activity against only one of the five micro-

Antimicrobial testing

Agar-dilution method was used for this test [26]. One in twenty dilution of each dried crude extract of plant samples were made in Mueller Hinton agar. Final antimicrobial assay was done using both agar well and filter paper diffusion technique [27,28].

**Table 1:** List of Common Plants Used for Medicines in Bokiland.

<table>
<thead>
<tr>
<th>S/N</th>
<th>FAMILY</th>
<th>NAME OF PLANT</th>
<th>ETHNOMEDICAL INFORMATION USES</th>
<th>PART USED</th>
<th>MODE OF USE</th>
<th>BOKI NAMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CAESALPINACEAE</td>
<td>Senna alata 507SH</td>
<td>Fungal skin infection (Ok, Ab)</td>
<td>Le</td>
<td>Surface of leaf is rubbed gently on affected skin</td>
<td>Kichokrala (Ok)</td>
</tr>
<tr>
<td>2</td>
<td>COMBRETACEAE</td>
<td>Combretum tarquense 501SH</td>
<td>Fresh wounds (K1)</td>
<td>Le</td>
<td>Leaves are squeezed into fresh wounds</td>
<td>Misheraso (K1)</td>
</tr>
<tr>
<td>3</td>
<td>DILLENIACEAE</td>
<td>Dillenia indica 967SH</td>
<td>STDs (Ok, K1, K2)</td>
<td>Le</td>
<td>Leaves are boiled in water or steeped in palm wine with potash</td>
<td>Kitka cowa (Ok), Jagish (K1, K2)</td>
</tr>
<tr>
<td>4</td>
<td>TILIACEAE</td>
<td>Grewia megalocarpa 635SH</td>
<td>Unspecified eye problem (K1, Wu); infertility (K1)</td>
<td>Le</td>
<td>For eye problem. Leaf sap is squeezed into the eye. For infertility, leaves are boiled with other ingredients</td>
<td>Menolakeni-meshi (K1), Kati-kereu (Wu)</td>
</tr>
<tr>
<td>5</td>
<td>MENISPERMACEAE</td>
<td>Jateorrhiza macrantha 526SH</td>
<td>Dysentery (Ok, K1, K2)</td>
<td>Le</td>
<td>Leaves are boiled in water, sometimes with Tinospora bakis and decoction drunk</td>
<td>Illelo (Ok), Elelo (K1, K2)</td>
</tr>
<tr>
<td>6</td>
<td>RUBIACEA</td>
<td>Morinda lucida 630SH</td>
<td>Ulcerating abscess (K2)</td>
<td>Le &amp; Ba</td>
<td>Exudates from crushed leaves or bark exudates is rubbed on affected area</td>
<td>Gekege (K2)</td>
</tr>
<tr>
<td>7</td>
<td>RUBIACEA</td>
<td>Psychotria brennian 509SH</td>
<td>Cough (Ok)</td>
<td>St</td>
<td>Stem is chewed</td>
<td>Onwo-okwa (Ok)</td>
</tr>
<tr>
<td>8</td>
<td>APOCYNACEAE</td>
<td>Rauvolfia vomitoria 679SH</td>
<td>Curb haemorrhage at child birth (Ok); Malaria (K2)</td>
<td>Ba</td>
<td>Bark is scrapped and steeped in cold water for some minutes</td>
<td>Kato (Ok) Meso-kebei (K2)</td>
</tr>
<tr>
<td>9</td>
<td>SMILACACEA</td>
<td>Smilax kraussiana 506SH</td>
<td>Snake bite (Ok)</td>
<td>Le</td>
<td>Leaves are ground with seven seeds of hot alligator pepper</td>
<td>Ole-oshuo (Ok)</td>
</tr>
<tr>
<td>10</td>
<td>ASTERACEAE</td>
<td>Spathanthus filicula</td>
<td>Mouth sores and boils (Ok, K1, K2)</td>
<td>Le &amp; Fi</td>
<td>Leaves and flowers + seven hot alligator pepper seeds are ground and applied to sores or boils</td>
<td>Ochiche-chibuo (Ok) Manliukwu (K1) Manii-ulkuo (K2)</td>
</tr>
</tbody>
</table>

Entries comprise, plant family, botanical name with herbarium identity number, ethnomedical information (specific ailment treated, part of plant used and mode of usage), followed by the Boki names. Names of Communities: Ab – Abu-mkpang, Bl – Balegete, Ba – Bamba, Bk – Bokalum, Bo – Boggo, Ok – Okwangwo, K1 – Okwa 1, K2 – Okwa 2, Wu – Wuila. Diseases: STDs – Sexually Transmitted Diseases. Part of Plants Used: Le – Leaves, Ba – Bark, Pl – Whole Plant, St – Stem, Fi – Flowers, Se – Seed, Ro – Root. Three of the test organisms were American Type Culture Collection (ATCC). These included Staphylococcus aureus ATCC 13709, Pseudomonas aeruginosa ATCC 7853, Escherichia coli ATCC 9637, while Bacillus subtilis and Candida albicans were clinical isolates. Cultures of the bacteria and fungi were maintained on nutrient agar plates and potato dextrose agar respectively after a series of sub-culturing at 4°C. Prior to testing the microorganisms were sub-cultured into nutrient broth at 37°C for 24 hours and then used for the tests.

Preparation of extracts

Ten grams of each plant sample were extracted with 50 ml of 70% methanol for 24 hours. Each individual solution of plant extract after 24 hours was filtered using a Whatman No. 4 filter paper. The filtrates were concentrated to a small volume in-vacuo at 80°C. The lyophilized samples were weighed to obtain yield of extraction. The crude extract yields for the three most active plants were Senna alata (3.64%), Dillenia indica (7.37%) and Grewia megalocarpa (1.90%). Samples were then packed in to clean sample bottles, labelled and kept in desiccators until time of analysis.

Discussions

Different inhibitory patterns of extracts from Senna alata has been established by several workers using different solvents. For instance, crude ethanolic extracts of the plant was positive against common dermatophytes such as the genera Trichophyton and Microsporum [29]. While, methanolic fractionates of the plant was positive against Candida albicans [30]. But, crude petroleum ether and ethanol extracts were fungicidal against C. albicans [11]. Importantly, similar to the current investigation methanol extracts of S.alata inhibited growth of Staphylococcus aureus but, at 0.125 g/ml test concentration [11]. Hence, from the current study and other scholarship the type of solvent used in extraction of active plant constituent, coupled with the test concentration does play a fundamental role in establishing the antimicrobial functions of medicinal plants [31]. Interestingly, the Bokis within the study area use.
is important to note that the antimicrobial tests conducted in this study, the activity of these plants and their efficacy in traditional medicine, it good potency or else higher concentrations are needed for increase.  

Aureus zone of inhibition (7 mm) of S alata  

yield,  

studies are needed in establishing the range of this plant specificity of antibacterial effect on in the treatment of fungal skin diseases (Table 1). But, with its S alata  

Table 3: Activity of the three active plants, with a standard antibiotic tested at different concentrations. Activity of the three plant is represented in descending order as (-) No inhibition in microbial growth, (+++) Inhibition in microbial growth.  

**Table 2:** Result on antimicrobial testing of test plants at highest concentration of 2 mg/ml.  

<table>
<thead>
<tr>
<th>Plant extract/antibiotic</th>
<th>Zone of Inhibition (mm) at 6 different concentration</th>
<th>0.06 mg/ml</th>
<th>0.125 mg/ml</th>
<th>0.25 mg/ml</th>
<th>0.50 mg/ml</th>
<th>1.00 mg/ml</th>
<th>2.00 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senna alata</td>
<td></td>
<td>0.0</td>
<td>1</td>
<td>2</td>
<td>3.25</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Dillenia indica</td>
<td></td>
<td>0.0</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Grewia megalocarpa</td>
<td></td>
<td>0.0</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>11.75</td>
</tr>
</tbody>
</table>

The activity of the three active plants, with a standard antibiotic tested at different concentrations against Staphylococcus aureus. Result shown is average of four readings. NT: Not Tested at this concentration. Diameter of well used for experiment was 6mm. Activity of the three plant is represented in descending order as; Di>Sa>Gm, each generic name is represented by the first letter in capital, while the species name is represented by the second letter.  

**Table 3:** Result showing active plant extracts and standard antibiotic against Staphylococcus aureus using the Agar well technique.  

S alata in the treatment of fungal skin diseases (Table 1). But, with its antibacterial effect on Staphylococcus aureus, it appears that the leaves of Senna alata could be considered broad spectrum. However, more studies are needed in establishing the range of this plant specificity against several microorganisms.  

While lots of studies have been done on Senna alata, in comparison little information is available on the microbiology of Dillenia indica and Grewia megalocarpa. However, recent investigations on methanol extracts and other organic fractions of the bark and leaves of Dillenia indica was shown to have moderate effect on Bacillus subtilis, Staphylococcus aureus and other organisms [31,32], establishing its antimicrobial property. In addition also, results of this study show that both plants (D. indica and G. megalocarpa) have the potential of being potent antimicrobial agents. For instance, despite its low percentage yield, Grewia megalocarpa shows activity. Also, is the diameter of the zone of inhibition (7 mm) of Dillenia indica against Staphylococcus aureus at 2 mg/ml concentration, which is quite close in activity to that of the standard antibacterial agent used. Both plants suggest either good potency or else higher concentrations are needed for increase activity against different micro-organisms. In drawing a conclusion on the activity of these plants and their efficacy in traditional medicine, it is important to note that the antimicrobial tests conducted in this study, utilized crude extracts of the plants.

**Conclusion**

This study has shown that antimicrobial activity exists in plants used by the Bokis for their health care. Leads for new drug discovery can be taken from any of the listed plants investigated in this research. But, further studies are necessary to isolate active compounds of these plants. Also, this study will contribute to the ongoing national medicinal plants database [33], invariably knowledge obtained should act as an important feeder to the development of a robust health care policy and service for the region with recognition and active inputs from traditional medicine practitioners.  

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