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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. Insights from the ethnobotanical survey led to the current research. Hence, the objective of the present investigation was on assessing possible antimicrobial property of ten common medicinal plants within the area of study in tandem with claims made by medicine men within the study area.

Materials and Methods

The study site

The study area 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, Abo-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 interviewees 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 Foresty 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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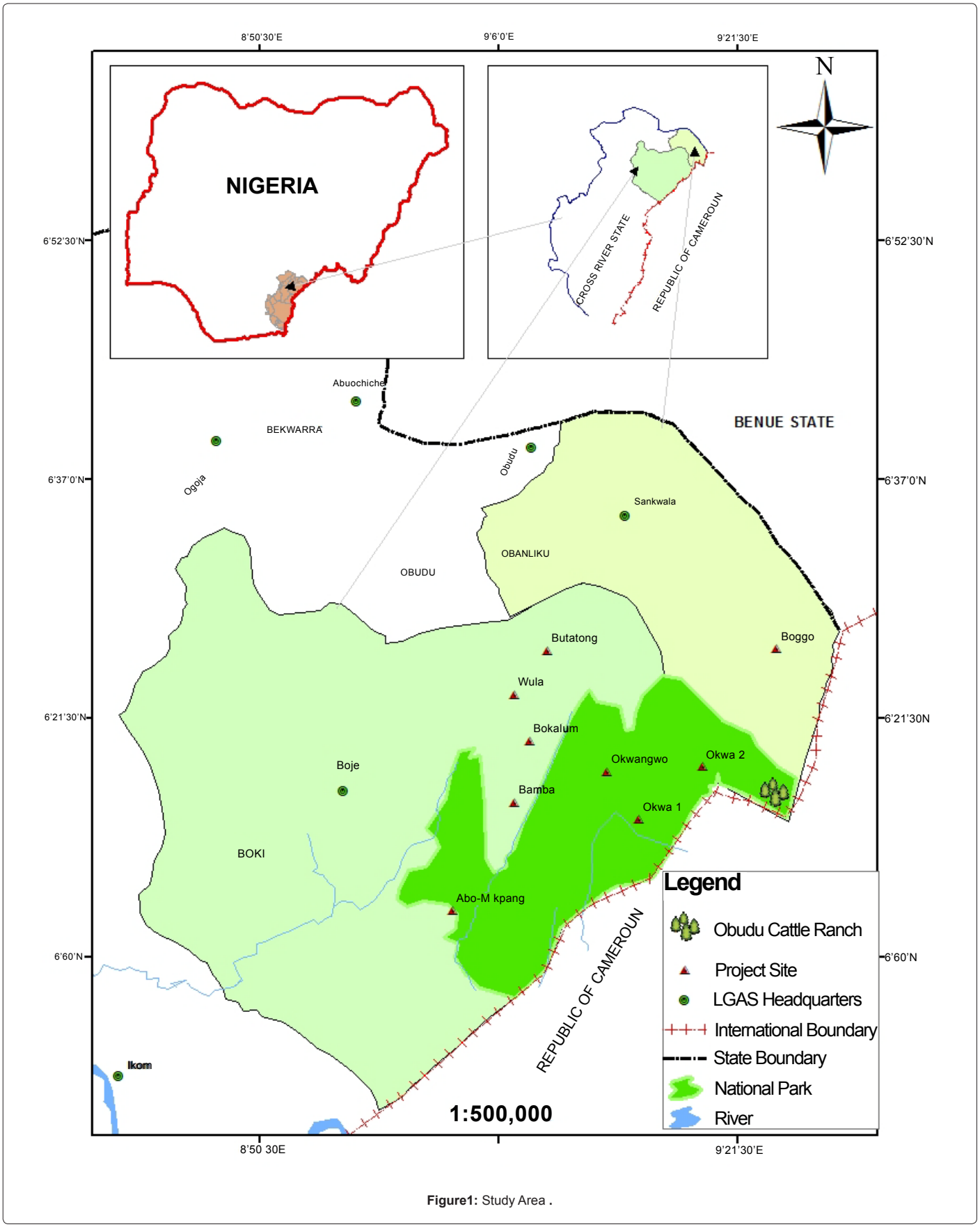

Figure1: Study Area .

S/N	FAMILY	NAME OF PLANT	ETHNOMEDICAL INFORMATION			BOKI NAMES
			USES	PART USED	MODE OF USE	
1	CAESALPINIACEAE	*Senna alata* 507SH	Fungal skin infection (Ok, Ab)	Le	Surface of leaf is rubbed gently on affected skin	Kichokrala (Ok)
2	COMBRETACEAE	*Combretum tarquense* 501SH	Fresh wounds (K1)	Le	Leaves are squeezed into fresh wounds	Misheraso (K1)
3	DILLENIACEAE	*Dillenia indica* 967SH	STDs (Ok, K1, K2)	Le	Leaves are boiled in water or steeped in palm wine with potash	Kitka cokwa (Ok), Jagish (K1, K2)
4	TILIACEAE	*Grewia megalocarpa* 635SH	Unspecified eye problem (K1, Wu); Infertility (K1)	Le	For eye problem. Leaf sap is squeezed into the eye. For infertility, leaves are boiled with other ingredients	Menolakeni-meshi (K1), Katie-keru (Wu)
5	MENISPERMACEAE	*Jateorhiza macrantha* 526SH	Dysentery (Ok, K1, K2)	Le	Leaves are boiled in water, sometimes with *Tinospora bakis* and decoction drunk	Illelo (Ok), Elelo (K1, K2)
6	RUBIACEA	*Morinda lucida* 630SH	Ulcerating abscess (K2)	Le & Ba	Exudates from crushed leaves or bark exudates is rubbed on affected area	Gekegbe (K2)
7	RUBIACEA	*Psychotria brenani* 509SH	Cough (Ok)	St	Stem is chewed	Onuo-okwa (Ok)
8	APOCYNACEAE	*Rauvolfia vomitoria* 679SH	Curb haemorr-hage at child birth (Ok); Malaria (K2)	Ba	Bark is scrapped and steeped in cold water for some minutes	Kato (Ok) Meno-kebeli (K2)
9	SMILACACEA	*Smilax kraussiana* 506SH	Snake bite (Ok)	Le	Leaves are ground with seven seeds of hot alligator pepper	Ole-oshuo (Ok)
10	ASTERACEAE	*Spilanthes filicaulis*	Mouth sores and boils (Ok, K1, K2)	Le & Fl	Leaves and flowers + seven hot alligator pepper seeds are ground and applied to sores or boils	Ochiche-chibuo (Ok) Manliukwo (K1) Manii-ukwo (K2)

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 – Wula. Diseases: STDs – Sexually Transmitted Diseases. Part of Plants Used: Le – Leaves, Ba – Bark, Pl – Whole Plant, St – Stem, Fl – Flowers, Se – Seed, Ro – Root

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

Test organisms

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 micro organisms 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.

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].

Results

Table 1 shows the ten common plants, specific disease treated and mode of usage within the nine communities. Thirty percent (30%) of the total plant extracts showed activity against only one of the five micro-organisms at a test concentration of 2 mg/ml (Table 2). The three plants that showed activity at this concentration were; *Senna alata* (leaves), *Dillenia indica* (leaves) and *Grewia megalocarpa* (leaves). Table 3 shows comparative activity level of the three plants and the standard antibiotic (Gentamicin) against the susceptible organism (*Staphylococcus aureus*). All three plants showed activity at all concentrations examined except at 0.06 mg/ml. Also, activity decreased as the concentration of extracts decreased, with the standard antibacterial agent showing the largest zone of inhibition at the 2 mg/ml test concentration. *Dillenia indica* showed the greatest activity amongst the three plant extracts used, with a zone of inhibition of 7 mm at 2 mg/ml. The activities are expressed as less active if the zone of inhibition was 1-3 mm, moderate (4-7 mm), high (8-12 mm) and very active (>12 mm). In order of decreasing activity amongst the three plant samples, *Grewia megalocarpa* showed very low activity of 2 mm at 2 mg/ml.

Discussion

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* [27], the crude methanolic extract however showed no inhibition against the same *Candida albicans* and *Saccharomyces* [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

Sample No	Name of Plant	Part Used	Micro-organisms				
			Pa	Bs	Ec	Sa	Ca
1	*Senna alata*	Le	-	-	-	+++	-
2	*Combretum tarquense*	Le	-	-	-	-	-
3	*Dillenia indica*	Le	-	-	-	+++	-
4	*Grewia megalocarpa*	Le	-	-	-	+++	-
5	*Jateorhiza macrantha*	Le	-	-	-	-	-
6	*Morinda lucida*	Le	-	-	-	-	-
7	*Psychotria brenani*	Wp	-	-	-	-	-
8a	*Rauvolfia vomitoria*	B	-	-	-	-	-
8b	*Rauvolfia vomitoria*	Le	-	-	-	-	-
9	*Smilax kraussiana*	Wp	-	-	-	-	-
10	*Spilanthes filicaulis*	Wp	-	-	-	-	-

All plant samples were subjected to same treatment and activity was examined at 2mg/ml. Plates were examined after 24 hours and re-examined after 48 hours. Part of plant: Le – Leaves, Wp – Whole plant, B – Bark. Organisms: Ps – *Pseudomonas aeruginosa*, Bs – *Bacillus subtilis*, Ec – *Escherichia coli*, Sa – *Staphylococcus aureus*, Ca – *Candida albicans*. Activities are reported 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.

Plant extract/antibiotic	Zone of Inhibition (mm) at 6 different concentration					
	0.06 mg/ml	0.125 mg/ml	0.25 mg/ml	0.50 mg/ml	1.00 mg/ml	2.00 mg/ml
Senna alata	0.0	1	2	3.25	3.5	3.5
Dillenia indica	0.0	2	2	2.5	3.5	7
Grewia megalocarpa	0.0	0.5	0.5	0.5	1	2
Gentamicin	NT	NT	NT	NT	NT	11.75

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 activity of 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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