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Screening of Antibacterial and Antifungal Activities of Ten Medicinal Plants from Ghana

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Abstract

The screening of African medicinal plants, utilized in traditional herbal remedies in Ghana West Africa for their antibacterial and antifungals activities, is reported in this paper. Of ten plants tested, extracts of five (*Phyllanthus niruri*, *Ageratum conyzoides*, *Acanthospermum hispidum*, *Cassia alata*, and *Sida acuta*) had activity against the Gram positive microorganism *Staphylococcus aureus* including methicillin-resistant *Staphylococcus aureus* (MRSA). None of these ten plants have previously had their anti-MRSA activity or lack of activity documented. Hexane extracts from three plants (*Portulaca quadrifida*, *Ageratum conyzoides*, and *Newbouldia lavis*) show remarkable anti-fungal activity against *Aspergillus fumigatus*. Four plants (*Phyllanthus niruri*, *Portulaca quadrifida*, *Ageratum conyzoides*, and *Newbouldia laevis*) have strong anti-*Candida* activity. This paper may be the first documentation of *Portulaca quadrifida in vitro* anti-*C. albicans* and anti-*A. fumigatus* activity.

Introduction

This work is a continuation of our laboratory's search for potential bioactive natural products began with examination of plants used by traditional herbalists in central and West African rain forests (Muaza et al., 1994; 1995) conducted in the 1990's. We are now investigating the traditional natural products used in Ghana.

The Republic of Ghana is a tropical country in Western Africa that lies on the Gulf of Guinea where Africa bulges westward into the Atlantic Ocean. It is about the size of the State of Oregon in the USA but with a population five-times greater (about 20 million). Ghanaian farmers take advantage of the climate, growing a wide variety of tropical cash-crops for export: banana, cocoa, coffee, copra, kola nuts, oranges, palm oil kernels, and pineapples. A fairly large portion of the West African tropical rain forest lays in the Southern half of the country and is a great source of plant diversity. Numerous wild plants that grow in Ghanaian forests have been extensively used by traditional healers to prevent or cure several diseases. Because conventional Western medicines are often too expensive and because of the widely held belief that these traditional herbal based medicines work, upwards of 70% of the population of Ghana depend on herbal medicines made from these plants and their traditional practitioners as the primary source of health care (Botwe, 2003). In spite of their widespread use throughout the continent and the curative values ascribed to them by their users, until recently these traditional African herbal remedies have not been systematically analyzed for bioactive components.

In Ghana, there have been many efforts to catalog herbs used in the country (Dokosi, 1998). One recent example is the publication *Herbs in Ghana* commissioned by the Ghanaian Council for Scientific and Industrial Research and compiled by O.B. Dokosi over a 20 year period. Very little information was reported in this reference regarding the scientific basis for the use of these herbs, which is the basis of this paper.

We have examined extracts from ten medicinal plants commonly used in the Northern part of the Volta Region of

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Ghana by traditional practitioners to treat topical bacterial and fungal infections including boils. These extracts were tested for their *in vitro* antibacterial and antifungal activities. Selected plants included *Sida acuta* Burm. f. (Malvaceae), *Portualaca quadrifida* Linn. (Portulacaceae), *Cassia alata* Pers. (Caesalpiniaceae), *Acanthospermum hispidum* DC. (Compositae), *Newbouldia laevis* (Bignoniaceae), *Setaria megaphylla* (Gramineae), *Luffa aegyptiaca* Mill. (Cucurbitaceae), *Ageratum conyzoides* Lin. (Compositae), *Phyllanthus niruri* var.amarus (Schum and Thonn.) Leandri (Euphorbiaceae), and *Boerhavia diffusa* Linn. (Nyctainaceae). We report on the preliminary results from the antibacterial and antifungal tests of 10 crude methanol extracts from these plants and 40 fractions obtained from successive partition of the crude methanol extracts between water and organic solvents (hexane, chloroform, and ethyl acetate).

Prior to laboratory work, a literature search was conducted to determine the extent of published antimicrobial activity for each of the plants. Many of the plants collected for this study are used medicinally in tropical regions worldwide. *Ageratum conyzoides* is distributed throughout the tropics, among many traditional uses, it is used as a bactericide in India, South America, and Africa (Ming, 1999). *Portulaca quadrifida*, found growing in the most inhospitable rock pile in the center of a remote Volta Region village, is used topically in India to treat erysipelas, and used to treat fever in Java. It is eaten as a vegetable in the Congo, Malaysia, and Sri Lanka (Dokosi, 1998). A decoction of *Phyllanthus niruri* is used to treat thrush in Togo (Dokosi, 1998) and in India and other places it is attracting attention for activity against hepatitis B (Subramoniam et al., 1999). *Boerhavia diffusa* is considered one of the 12 most commonly used plants in Indian herbal formulations (Subramoniam *and Pushpngadan*, 1999). The roots of *Newbouldia laevis* (the leaves are investigated here) are utilized by indigenous people of South Africa and has shown remarkable antimicrobial properties including anti-*C albicans* and *E. coli* activity (Gafner et al., 1996).

Materials and methods

Plant materials

Plant samples were collected with the aid of herbalists in rural areas in the north of Ghana's Volta Region. They were identified by botanists at the Department of Botany's Herbarium at the University of Ghana Legon branch located in Accra Ghana. Voucher specimens are kept at this herbarium. The following plant parts were collected: roots, leaves, and stem of entire plant (*Phyllanthus niruri*, *Portualaca quadrifida*), leaves and stems (*Sida acuta*, *Boerhavia diffusa*, *Acanthospermum hispidum*, *Setaria megaphylla*), leaves, stems and flowers (*Luffa aegyptiaca*, *Ageratum conyzoides*) and leaves only (*Cassia alata*, *Newbouldia laevis*).

Microorganisms

Microorganisms were provided from the clinical microbiology laboratory at the University of Texas M.D. Anderson Cancer Center. Those with American type culture collection numbers were *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 20096). Clinical isolates included *Aspergillus fumigatus* (293), and methicillin-resistant *Staphylococcus aureus* (2-220).

Extraction

Standard solvent extraction methods were performed as outlined by Muanza et al. (1994). Plant material (32 g) was ground and exhaustively extracted with 1000 ml of methanol using a Soxhlet extractor for 24 h. The solvent was evaporated at low temperature under reduced pressure to yield a thick syrup which was suspended in a methanol-water mixture (1 : 9) and then successively extracted with hexane, chloroform, and ethyl acetate. Afforded organic solutions were similarly evaporated to give organic extracts. Samples from methanol, hexane, chloroform, and ethyl acetate extracts, as well as lyophilized aqueous fractions, were further used to test for the antibacterial and antifungal properties.

Antimicrobial tests

The microorganisms were grown in appropriately fortified agar poured in 25 cm × 25 cm bioassay plates. Mueller-Hinton agar (DIFCO, Detroit, MI) was prepared for the bacteria. RPMI 1640 standard cell culture (Sigma Chemicals, St. Louis, MO) buffered with a 0.165 M MOPS buffer and fortified with 20 g of glucose/liter of 1.5% agar was utilized for the fungi (Hoffman and Pfaller, 2001). All extracts (50 in number) were simultaneously tested on each bioassay plate. Each extract was poured in individual 6.2 mm diameter wells made in the agar (Barry, 1986). Additional wells were made so that standard commercial antibiotics and all the pure solvents could be placed as quality controls. Activity of a given extract was determined by measuring the zone of inhibition (ZOI). Because zones of inhibition were often asymmetrical, ZOI measurements were made three times for each well at different orientations and the average recorded. The standard antibiotics were applied in concentrations of 1 mg/ml, and the amount of extracts and QCs placed in each well was 100 µl. The standard antibiotics were: ciprofloxacin for *E. coli* and *P. aeruginosa*, linezolid for *S. aureus* and MRSA, amphotericin B for *A. fumigatus*, and fluconazole for *C. albicans*. Bacteria were added directly to the cooling agar (at about 55 °C) and spores for the fungus and yeast were applied as a lawn just prior to coring and filling the wells. Plates were stored for both bacteria and fungi screening at 48 h in an incubator at 37 °C after which measurements of zones of inhibition were done.

Literature search

An extensive compilation of published scientific research was obtained for each plant from a NAPRALERT[SM] and internet search. NAPRALERT[SM] is an acronym for the NAtural PRoducts ALERT relational database, containing information from over 150,000 research articles. Over 1.5 million records document the biological activity of more than 151,000 plant, marine, microbial and animal species.

Results and discussion

Tables 1 and 2 display the results of the antimicrobial testing for those plants which had active extracts. Measured zone of inhibitions (ZOIs) for bacterial testing are displayed in Table 1. It can be seen that five plants had activity against the Gram positive *Staphylococcus aureus* (both MRSA and non-MRSA). One plant's methanol extract, *Phyllanthus niruri*, had a MRSA ZOI (24.3 mm) comparable to that of the linezolid antibiotic's ZOI (27.9 mm). Results for the Gram negatives, *Escherichia coli* and *Pseudomonas aeruginosa*, were disappointing. Only *Sida acuta*'s 1 : 9 extract had a measurable zone of inhibition of 10.3 mm against *E coli* compared to a ZOI of 38.2 mm for the QC drug ciprofloxacin.

Table 2 reveals that three plants, *Portulaca quadrifida*, *Ageratum conyzoides*, and *Newbouldia laevis*, had extracts which exhibited very strong anti-fungal activity against both *A. fumigatus* and *C. albicans*. *Phyllanthus niruri* showed activity against *Candida albicans* as well. *Portulaca quadrifida*, *Ageratum conyzoides*, and *Newbouldia laevis* all had hexane extract ZOIs greater than that of fluconazole for *Candida albicans*. These same plants' hexane extracts had ZOIs more than 1.5-times greater than the QC amphotericin B for *Aspergillus fumigatus*. In particular, the hexane fraction of *Newbouldia laevis* had a ZOI (71.0 mm) over 2.5-times that of amphotericin B (26.7 mm). Work with the fungal bioassay plates had to be duplicated with single well Petri dishes since the plate zone of inhibitions surrounding the wells containing these plants' active extracts overlapped and could not be distinguished.

Sida acuta showed modest activity in three of its extracts against *S. aureus*. Not recorded in Table 2 were very impressive partially cleared zones for all *Sida acuta*'s extracts tested against *A. fumigatus*. In these areas, fungal growth was clearly impeded, but some scattered growth still occurred. Most of these zones were slightly larger than the inhibitory QC zones.

Table 3 records that no activity was observed for any extract of *Setaria megaphylla*, *Luffa aegyptiaca*, or *Boerhavia diffusa*. As mentioned in the introduction, *Boerhavia diffusa* finds extensive use in Indian herbal remedies; however, specimens from Ghana showed no antimicrobial activity.

The NAPRALERT[SM] and internet literature search revealed that none of the ten plants had prior published testing against methicillin resistant *Staphylococcus aureus*.

Table 1. Antibacterial activity[a].

Extract	Bacterial Zone of Inhibition (mm)	
	S. aureus	MRSA
QC		
Linezolid	33.2	27.9
All Pure Solvents	–	–
Phyllanthus niruri		
MeOH	24.5	24.3
Hexane	17.8	18.8
CHCl3	10.1	8.8
EtOAc	14.6	12.6
1 : 9	20.1	15.2
Ageratum conyzoides		
MeOH	11.4	–
Hexane	10.5	–
CHCl3	10.4	–
EtOAc	11.1	–
1 : 9	–	9.1
Acanthospermum hispidum		
MeOH	–	–
Hexane	–	–
CHCl3	14.5	15.1
EtOAc	–	–
1 : 9	–	–
Sida acuta		
MeOH	–	–
Hexane	–	–
CHCl3	10.2	–
EtOAc	10.5	10.3
1 : 9	13.3	10.7
Cassia alata		
MeOH	16.2	9.8
Hexane	–	–
CHCl3	21.4	21.5
EtOAc	13.1	15.0
1 : 9	14.3	12.2

[a] Gram negative activity was observed only with *Cassia alata*'s 1 : 9 extract with a ZOI of 10.3 mm against *E. coli* compared to the QC ciprofloxacin with ZOI 38.2 mm.

Many lacked previous documented antifungal activity. In particular, no antimicrobial data has been referenced by NAPRALERT[SM] for *Portulaca quadrifida* or *Setaria megaphylla*. NAPRALERT[SM] indicates that *Phyllanthus niruri* has had its crude methanol extract tested for activity against *C. albicans* and *Aspergillus niger*. Both of these extracts were inactive. Strong antifungal activity of *Newbouldia laevis* root extracts has been documented by Gafner et al. (1996), but only for *C. albicans*. Extracts of leaves tested here confirmed this activity and have shown strong activity against *A. fumigatus*. *Ageratum conyzoides* has been screened for antibac-

Table 2. Antifungal activity.

Extract	Fungal Zone of Inhibition (mm)	
	C. albicans	*A. fumigatus*
QC		
Amphotericin	22.1	26.7
Fluconazole	32.9	0
All Pure Solvents	–	–
Phyllanthus niruri		
MeOH	–	–
Hexane	21.1	–
CHCl3	11.2	–
EtOAc	29.0	–
1 : 9	–	–
Ageratum conyzoides		
MeOH	–	–
Hexane	25.4	42.1
CHCl3	17.2	16.2
EtOAc	–	–
1 : 9	–	–
Portulaca quadrifida		
MeOH	–	–
Hexane	45.7	45.2
CHCl3	16.1	18.2
EtOAc	–	16.6
1 : 9	–	–
Newbouldia laevis		
MeOH	–	–
Hexane	36.5	71.0
CHCl3	18.8	31.1
EtOAc	–	–
1 : 9	–	–

Table 3. Plants with no observed extract activity.

Plant
Setaria megaphylla
Boerhavia diffusa
Luffa aegyptiaca

terial properties and has had anti-insect properties verified elsewhere (Ming, 1999).

Conclusion

Seven of the ten plants selected for antimicrobial screening showed activity against the two *Staphylococcus aureus* and/or the two fungal microorganisms. For the first time, five plants *Phyllanthus niruri*, *Ageratum conyzoides*, *Acanthospermum hispidum*, *Sida acuta*, and *Cassia alata* have documented MRSA activity. *Phyllanthus niruri* had the strongest anti-MRSA activity.

Newbouldia laevis, *Portulaca quadrifida*, and *Ageratum conyzoides* show activity against both *Candida albicans* and *Aspergillus fumigatus*. Leaf hexane extracts of *Newbouldia laevis* have particularly strong activity against both these organisms. Absence of reports in NAPRALERT[SM] for *Portulaca quadrifida* may indicate that this paper is the first documentation of anti-*C. albicans* and anti-*A. fumigatus* activity. *Ageratum conyzoides* shows strong antifungal activity. Our work indicates the hexane and EtOAc extracts of *Phyllanthus niruri* show activity against *C. albicans*.

No activity was recorded for any extract of *Setaria megaphylla*, *Luffa aegyptiaca*, or *Boerhavia diffusa*.

Efforts are now underway to begin the work of identifying the chemical compounds responsible for observed activity. Thin-layer chromatography (TLC) plates have been run verifying that every solvent extract produced in the laboratory has a number of constituents.

Preliminary isolation with column chromatography and further anti-fungal testing has confirmed the presence of single active compounds in each of the hexane extracts of *Portulaca quadrifida* and *Newbouldia laevis*. Currently, work is being conducted to further isolate and identify these active constituents.

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