Natural Product Sciences
17(1) : 33-37 (2011)

Evaluation of the Antimicrobial Activity of Seven Gabonese Medicinal Plants against Methicillin-Resistant *Staphylococcus aureus* and *Salmonella*

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Abstract – The plant species reported here are used by traditional healers in Gabon for different ailments such as wounds, malaria, fever, gonorrhea or diarrhea. The aim of this study was to evaluate the antimicrobial activities of 7 plants (*Strombosiopsis tetrandra, Tetraberlinia bifoliolata, Dichapetalum barbatum, Guibourtia demeusii, Dacryodes normandii, Manniophytum fulvum, Paropsia grewoides*) against different strains of both Methicillin-Resistant *Staphylococcus aureus (MRSA)* and *Salmonella*. Disc diffusion was first used to determine the antimicrobial effectiveness of the plants' ethanolic extracts. Then the minimum inhibitory concentrations of the crude extracts of either leaves or stem barks of the 7 plants were determined using broth micro-dilution. The ethanolic plant extracts showed very good activity against both MRSA and *Salmonella* strains where the MICs ranged from 250 μg/ml to 1000 μg/ml. The study shows that many of the tested plants used by Gabonese traditional healers have antimicrobial activities and give support to their traditional use.
Keywords – Medicinal plants, antibacterial activity, traditional medicine, Gabon

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a problem since the 1960s as its infection is associated with high mortality and increased cost in hospitals (Choi *et al*., 2009). On the other hand, *Salmonella enterica*, which are Gram-negative bacteria capable of infecting humans and animals, has caused significant morbidity and mortality worldwide (Fink *et al*., 2007). *S. enterica serovar typhimurium* is a clinically important intracellular bacterial pathogen that causes food poisoning and gastroenteritis in millions of people worldwide each year (Grassl *et al*., 2008). Since bacteria such as MRSA and *Salmonella* have developed different ways to nullify the action of antibiotics (Tenover, 2006, Cloutier, 1995, Cabrera *et al*., 2004), new approaches such as the use of natural products from plants as an alternative to synthetic antibiotics should be strongly considered as plant products are very effective with minimal or no side effects.

The last decade has witnessed the explosion in research for African plants as possible therapeutic agents. Africa has rich flora and for centuries its population has used traditional medicine and natural health products (Mills *et al*., 2005) as primary treatments. While efforts are being to understand the safety and efficacy of plants, it is well known that most of these plants have been used to treat or prevent illness since recorded history. For example, the sacred Vedas dating back between 3500 B.C and 800 B.C gave many references for these medicinal plants (Himal *et al.,* 2008). Plant-derived drugs occupy a central place in Africa and most developed countries (Lamidi *et al*., 2005, Mothana *et al*., 2008) as the premier source of therapies. This popularity of plant-based drug is even taking center stage in developed countries such as the United States as it is recorded that approximately one-third of people surveyed used at least one "unconventional" therapy (Cowan, 1999). Despite the fact that 80% of the world's population relies on traditional medicines for their primary health care need (Himal *et al*., 2008, Lamidi *et al*., 2005), only very few plants are now being used as anti-microbials. In this study, we investigated for the first time the antimicrobial activity of 7 Gabonese medicinal plants against MRSA and *Salmonella*.

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Experimental

Plants material and extraction – Plants (*Strombosiopsis tetrandra, Tetraberlinia bifoliolata, Dichapetalum barbatum, Guibourtia demeusii, Dacryodes normandii, Manniophytum fulvum, Paropsia grewoides*) were collected from different regions of Gabon by the technicians of the Institute of Traditional Pharmacopoeia and Medicine. Botanical determination was performed by taxonomists from the Herbier National du Gabon (HNG) and a voucher specimen for each plant was deposited at the same herbarium.

The leaves and stem barks were washed with water, air dried and powdered in an electric blender. Then 5 g of the powder was suspended in a 50 ml of ethanol for 18 hours in a 37 °C microprocess controlled bench-top (bain-marie). The mixture was filtered using a filter paper (Avantec 2, 110 mm). The ethanol was then removed from the sample using a rotary evaporator (Eyela). The resulting extracts were subsequently weighed to different yielding percentage specific to the plant and the part used.

Bacterial strains – For the *S. aureus* strains used in this study, the 6 clinical isolates (MRSA) were obtained from six different patients at the Wonkwang University Hospital (Iksan, South Korea). The other two strains were *S. aureus* ATCC 33591 (methicillin-resistant strain) and *S. aureus* ATCC 25923 (methicillin-susceptible strain). ATCC 25923 (American Type Culture Collection, Manassas, VA) and ATCC 33591 were purchased. Before use, all bacteria were stored in 30% glycerol and frozen at −70 °C. The bacteria were cultured in Mueller–Hinton broth (MHB) and Mueller–Hinton agar (MHA) (Difco Laboratories, Baltimore, MD) and incubated at 37 °C for 20 h.

Salmonella typhi (ATCC 19943) was used with other local isolates sampled from humans, cattle, pigs, and chicken feces.

Determination of antibacterial activity using the disc diffusion method – The paper disc diffusion method was used to determine antibacterial activity (Joung *et al*., 2010). Bacterial strains grown on MHA at 37 °C for 18 h were suspended in MHB and adjusted to a turbidity of 0.5 McFarland standard scale (approximately 1.5×10^8 CFU/ml). The MHA was poured into petri dishes and inoculated with 100 µl of the suspension. Sterile paper discs (diameter 6 mm) were punched in the agar and filled with 100 and 200 µg of plant extracts per disc. The dissolution of the organic extracts was facilitated with the addition of 50% (v/v) DMSO (50% DMSO was not active against any strains). Ampicillin was used as positive controls, and the discs treated with DMSO were used as the negative control. The plates were placed in a plant growth chamber at 37 °C for 24 h. The inhibition zone diameter around each of the discs was measured and recorded at the end of the incubation period.

Determination of the minimum inhibitory concentration (MICs) – The minimum inhibitory concentration (MIC) was determined using the broth micro-dilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2000) guidelines. Briefly, a preparation of the microorganisms' inocula was done on 24-h broth cultures and the suspensions were adjusted to a 0.5 McFarland standard turbidity (approximately 10^8 CFU/ml). Final inocula were adjusted to 10^4 CFU/ml. The MHB was supplemented with serial ampicillin or the ethanol plant extracts. The MIC was defined as the lowest concentration in which there is no visible growth after 24 h of incubation at 37 °C.

Results and Discussion

This article describes the antimicrobial activities of a number of plants used in Gabonese traditional medicine. A total of 7 extracts belonging to 7 different plants were investigated. Table 1 shows the scientific name, plant family, part used, traditional uses, and voucher specimen

Table 1. Ethnobotanical data of the investigated medicinal plants

Plant species	Voucher no.	Family[a]	Part	Traditional uses [a]	Yield (%)[b]
Strombosiopsis tetrandra	523 HNG	Olacaceae	L	Kidney and dysentery	0.27
Tetraberlinia bifoliolata	556 HNG	Fabaceae	S	Unknown	12.6
Dichapetalum barbatum	566 HNG	Dichapetalaceae	L	Unknown	2.03
Guibourtia demeusii	515 HNG	Caesalpiniaceae	L	Wounds	0.39
Dacryodes normandii	524 HNG	Burseraceae	L	Wounds, burns and diareah	30.6
Manniophytum fulvum	567 HNG	Euphorbiaceae	L	Wounds, diarhrea and dysentery	1.65
Paropsia grewoides	590 HNG	Passifloraceae	L	Malaria, fever	3.45

L: Leaves, S: Stem barks
[a] Information provided by the Institute of Traditional Pharmacopoeia and Medicine in Gabon
[b] Yield (%): (Quantity of extract obtained/ original plant extract quantity) × 100

Table 2. Antimicrobial activities of ethanolic extracts of the investigated plants

		Diameter of Zone of Inhibition (mm)[a]			
Plant species	Part	MRSA	MSSA	*S. typhi*	*S. paratyphi*
Strombosiopsis tetrandra	Leaves	[a]ND	ND	ND	ND
Tetraberlinia bifoliolata	Stem barks	16	12	ND	ND
Dichapetalum barbatum	Leaves	8	ND	ND	ND
Guibourtia demeusii	Leaves	16	14	ND	ND
Dacryodes normandii	Leaves	25	12	ND	ND
Manniophytum fulvum	Leaves	20	8	ND	ND
Paropsia grewoide	Leaves	10	ND	ND	ND
Ampicillin[a]		14	43	14	20

[a:] 200 µg per disc was used for the plants and 50 µg per disc for ampicillin
[b]ND: Not detected

Table 3. Antimicrobial activities of the ethanolic plant extracts

		Minimum Inhibitory concentrations (MIC) (µg/ml)[a]			
Plant species	Part	MRSA	MSSA	*S. typhi*	*S. paratyphi*
Strombosiopsis tetrandra	Leaves	>1000	**1000**	>1000	>1000
Tetraberlinia bifoliolata	Stem barks	>1000	>1000	>1000	>1000
Dichapetalum barbatum	Leaves	>1000	>1000	>1000	>1000
Guibourtia demeusii	Leaves	**500**	**250**	>1000	>1000
Dacryodes normandii	Leaves	**500**	**250**	**1000**	**250**
Manniophytum fulvum	Leaves	**1000**	**1000**	**1000**	**500**
Paropsia grewoide	Leaves	**1000**	**500**	>1000	>1000
Ampicillin		500	0.06	0.97	1.95

L: Leaves, S: Stem barks
[a] Values in bold are considered very active (1000 µg/ml or lower)

of the medicinal plants. Some plants have no clear medicinal characterizations as they are classified based solely on the report of traditional doctors.

Agar disc diffusion was used to qualitatively determine which extracts contained antimicrobial activities. As shown in Table 2, all extracts were not effective against *Salmonella* but there was considerable activity against MRSA and MSSA. *Dacryodes normandii* extract had the greatest zone of inhibition with diameters of 25 mm and 12 mm for MRSA and MSSA, respectively. While there is no activity found for all plants against *Salmonella* through disc diffusion, 2 of the seven plants (*Dacryodes normandii, Manniophytum fulvum*) have MICs in the range of 250 to 1000 µg/ml against *Salmonella* (Table 3). This shows that the absence of diffusion was not necessary equal to the absence of antimicrobial activity. Also, when tested against more strains (Table 4) of MRSA and *Salmonella*, the two plants further demonstrated their antimicrobial activity against *Salmonella* strains with MICs ranging as low as 250 to 1000 µg/ml. Further, 6 of the 7 plants screened show very good activity against MRSA strains.

In search for new antimicrobial products from plants, we screened 7 plants collected in Gabon. This is the first report mentioning their antimicrobial activities on MRSA and *Salmonella*. Evaluation of the antimicrobial activities of these plant extracts showed good potency, especially against the MRSA strains.

As shown in Table 1, the selected plants have a wide variety of therapeutic uses including kidney dysfunctions, malaria, fever wounds, gonorrhea or dysentery. Even though there is controversy surrounding the primary care delivery by traditional healers in Africa regarding the use of natural products as medicine (Mills *et al*., 2005), collaboration with them remains the starting point for the advancement of these medicines. The knowledge of traditional healers has led to effective anti-malaria (Adjuik M *et al*., 2004) anti-cancer (Mills *et al*., 2005), anti-oxidative (Farombi, 2003) or antimicrobial activities (Amoo *et al*., 2009) which compels the WHO to promote the

Table 4. Minimum inhibitory concentrations of screened plants and ampicillin (AC) against 6 strains of *Staphylococcus aureus* and *Salmonell*a

		MICs (μg/ml)[b]							
S.aureus strains	Class	523H[a]	556H[a]	566H[a]	515H[a]	524H[a]	567H[a]	590H[a]	AC
ATCC 33591	MRSA	>1000	>1000	>1000	500	500	500		500
ATCC 25923	MRSA	>1000	>1000	>1000	250	250	250		0.06
Clinical isolates									
DPS-1	MRSA	>1000	>1000	>1000	>1000	>1000	>1000	>1000	250
DPS-2	MRSA	>1000	500	1000	250	500	1000	250	62.5
DPS-3	MRSA	>1000	500	1000	250	500	1000	250	250
DPS-4	MRSA	>1000	500	1000	250	250	1000	250	62.5
Salmonella									
JOL 380	S.typhi ATCC 19943	>1000	>1000	>1000	>1000	1000	1000	>1000	0.97
JOL 381	S.paratyphi A	>1000	>1000	>1000	>1000	250	500	>1000	1.95
JOL 386	S.enteritidis	>1000	>1000	>1000	>1000	>1000	1000	>1000	1.95
JOL 387	S.typhimurium	>1000	>1000	>1000	>1000	500	1000	>1000	1.95
JOL 388	S.typhimurium	>1000	>1000	>1000	>1000	500	1000	1000	0.97
JOL 389	S.typhimurium	1000	>1000	1000	1000	250	500	500	2000

[a]**523H**; *Strombosiopsis tetrandra* **556H**; *Tetraberlinia bifoliolata* **566H**; *Dichapetalum barbatum* **515H**; *Guibourtia demeusii* **524H**; *Dacryodes normandii* **567H**; *Manniophytum fulvum* **590H**; *Paropsia grewoide*

development of traditional medicine (Elujoba *et al*., 2005, Akinyemi *et al.*, 2005).

Screening of the ethanol extracts from the 7 plants showed that most plants are very active mainly against MRSA strains.

As for the remaining plants, the low potency values observed could be due to the fact that the extracts were still in an impure form and thus there could be some compounds with potent microbial effects (Amoo *et al*., 2009) but were at low concentrations in the extract (Rabe *et al*., 1997). On the other hand, it is reported that multidrug resistance pumps (MDRs) of gram-positive bacteria do not present a serious problem for the penetration of most clinically antibiotics (Lewis *et al*., 2006).In contrary, gram-negative bacteria have a permeability barrier that does not allow the penetration of drugs. In this study, plant extracts used against *Salmonella* that showed higher MICs were likely restricted penetration through the resistance-nodulation-cell division (RND) MDRs efflux amphipathic substance across this barrier. This mechanism is likely the origin of less available broad-spectrum antibiotics capable of killing both gram-positive and gram-negative bacteria (Lewis *et al*., 2006).

The results from our study are encouraging since many of the plant extracts are active at 250 μg/ml, which can be considered to be potent (Chea *et al*., 2007). The study showed that many of the plants tested used by Gabonese traditional healers have antimicrobial activities. And some of these plants extracts may be more potent in vivo due to metabolic transformation into highly active intermediates as well as potential interaction with the immune system (Garcia *et al*., 2003, Amoo *et al*., 2009,). More studies are therefore needed to fraction the ethanol extracts and determine which sub-fraction and ultimately which isolated compounds are responsible for the antibacterial activities of those plants.

Acknowledggements

Dr Lamidi Maroufath of the Institute of Traditional Pharmacopoeia and Medicine in Gabon is thanked for collecting medicinal plants and providing traditional use of those plants.

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Received November 11, 2010
Revised February 18, 2011
Accepted March 5, 2011