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, gonorrhoea or diarrhea. The aim of this study was to evaluate the antimicrobial activities of 7 plants (*Srombosis tetrandra, Terabeilina 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, Tetramerlinia bifoliolata, Dichapetalum barbatum, Gubourcia demeusii, Dacyrodes normandii, Manniophyllum 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 determine 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 × 10⁸ 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 10⁸ CFU/ml). Final inocula were adjusted to 10⁴ 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>
<thead>
<tr>
<th>Plant species</th>
<th>Voucher no.</th>
<th>Family</th>
<th>Part</th>
<th>Traditional uses</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strombosiopsis tetrandra</td>
<td>523 HNG</td>
<td>Olacaceae</td>
<td>L</td>
<td>Kidney and dysentery</td>
<td>0.27</td>
</tr>
<tr>
<td>Tetramerlinia bifoliolata</td>
<td>556 HNG</td>
<td>Fabaceae</td>
<td>S</td>
<td>Unknown</td>
<td>12.6</td>
</tr>
<tr>
<td>Dichapetalum barbatum</td>
<td>566 HNG</td>
<td>Dichetagalaece</td>
<td>L</td>
<td>Unknown</td>
<td>2.03</td>
</tr>
<tr>
<td>Gubourcia demeusii</td>
<td>515 HNG</td>
<td>Caesalpiniaceae</td>
<td>L</td>
<td>Wounds</td>
<td>0.39</td>
</tr>
<tr>
<td>Dacyrodes normandii</td>
<td>524 HNG</td>
<td>Burseraceae</td>
<td>L</td>
<td>Wounds, burns and diareah</td>
<td>30.6</td>
</tr>
<tr>
<td>Manniophyllum fulvum</td>
<td>567 HNG</td>
<td>Euphorbiaceae</td>
<td>L</td>
<td>Wounds, diarrhea and dysentery</td>
<td>1.65</td>
</tr>
<tr>
<td>Paropsia grewoides</td>
<td>590 HNG</td>
<td>Passifloraceae</td>
<td>L</td>
<td>Malaria, fever</td>
<td>3.45</td>
</tr>
</tbody>
</table>

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