In vitro Antibacterial Properties of Total Alkaloids Extract from Mitragyna Inermis (Willd.)
O. Kuntze, a West African Traditional Medicinal Plant

C. Zongo, 1,2 Etienne-François O. Akomo, 1 A. Savadogo, 1 Louis Clement Obame,
3 Jean Koudou and 1 Alfred S. Traore
1Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles,
UFR/SVT, Université de Ouagadougou, 03 BP 7131, Ouagadougou 03, Burkina Faso
2Institut de Recherche en Ecologie Tropicale/CENAREST Gabon, BP13354, Gabon
3Centre de Recherche en Pharmacopée et Médecine Traditionnelle,
Université de Bangui, BP 1450, Bangui République Centrafricaine

Abstract: The antimicrobial activity of the total alkaloids from the leaves of Mitragyna inermis (Willd.)
O. Kuntze (Rubiaceae) From Burkina Faso was evaluated using disc diffusion essay and broth microdilution
assay. The extract was used against ten (10) reference bacterial strains and three (3) clinical isolates including
Gram(+) and Gram(-) strains. The alkaloids showed moderate activity against microorganisms tested. The highest
Diameter of Inhibition Zone (DIZ) was obtained with S. aureus ATCC9244 (23±1 mm). The lowest Minimum
Inhibitory Concentration (MIC) obtained was 0.625 mg mL⁻¹ recorded with three (3) Gram(+) bacteria (S. aureus
ATCC 25923, S. aureus (clinical isolate) and S. carmorum LMG 13567) and one Gram(-) strain (P. mirabilis
CIP104588). Results showed that Gram(+) bacteria are more sensitive to alkaloids from M. inermis than Gram(-)
bacteria. This study confirmed the use of the plant in traditional medicine against some infectious diseases.

Key words: Bacteria, inhibition, infectious diseases, time-kill assay, Burkina Faso

INTRODUCTION

Indigenous medicinal plants are often the only means for the treatment of several infections in Africa (Fennell et al., 2004; Taylor et al., 2001). According to WHO (2003) 80% of the population use traditional medicine for their primary health care.

Mitragyna inermis (Willd.) O. Kuntze (Rubiaceae) is a medicinal plant widely known and used in folk medicine in West Africa. This plant is a shrub growing on low alluvial plains and swampy savannah of many countries of West Africa (Pillay, 1964; Shellard and Wade, 1969; Kerharo Adam, 1974).

In traditional medicine, M. inermis is used to treat several diseases such as fever, headache, diarrhoea, dysentery, cholera, malaria and other diseases. (Nacoulma/Ouedraogo, 1996; Ouedraogo et al., 2007). According to the traditional knowledge, many scientific studies have been carried out to confirm the activities the plant is assumed to exert in vivo.

The in vitro antiplasmodial activity of the plant has been demonstrated by Traore-Keita et al. (2000), Mustofa et al. (2000), Kohler et al. (2002), Azas et al. (2002) and Fiott et al. (2005). The cardiovascular properties of the aqueous extract have been also demonstrated (Ouedraogo et al., 2004). Toxicity studies including cytotoxicity, genotoxicity, acute and chronic toxicity have been carried out (Azas et al., 2002, Toure et al., 1996; Traore et al., 2000; Monjanel-Mouterde et al., 2006). Several compounds including alkaloids and non-alkaloids have been purified from M. inermis (Shellard and Sarpong, 1969, 1970; Shellard et al., 1971; Cheng et al., 2002; Fiott et al., 2005). The chloroformic extract from the plant showed antibacterial activity against Escherichia coli, Staphylococcus aureus and Streptococcus sp. (Umeh et al., 2005). Some studies showed that the pharmacological and biological properties were mostly due to alkaloids (Traore-Keita et al., 2000). More recently, antimicrobial activity of different extracts from M. inermis against bacteria and fungi has been demonstrated and chemical analysis revealed the presence of alkaloids in the active extracts (Asase et al., 2008).

However, there is a few scientific data on the antibacterial properties of the plant. In the present study, in vitro antibacterial activity of the total alkaloids of M. inermis was evaluated against clinical and reference Gram-positive and Gram-negative bacteria.
MATERIALS AND METHODS

Plant material: The leaves of *Mitragina inermis* were collected in December 2007, at 45 km from Ouagadougou in Burkina Faso. The samples were carefully dried in the laboratory under continuous ventilation, away from sunlight and dust. The leaves were then crushed to fine powder with a mechanical crusher and the powder was kept in plastic bags and stored away from light and moisture until required.

The plant was taxonomically authenticated at the Laboratory of Plant Biology and Ecology of the University of Ouagadougou where a voucher specimen was deposited.

Total alkaloids extraction: The dried powder of the leaves was moistened with ammonia (28%) and extracted with chloroform at room temperature for a total period of 24 h. The chloroform extract was then filtered and a first liquid-liquid partition of the alkaloids was made with hydrochloric acid (HCl) (5%). The aqueous layer from the first partition was made alkaline again (pH 9-10) with ammonia and a second partition with chloroform was made. Finally, chloroform was totally evaporated from the organic phase to give a total alkaloids powder.

Microbial strains: The total alkaloids extract of leaves of *M. inermis* was tested against a panel of microorganisms, including reference strains (*Bacillus cereus* LMG 13569, *Enterococcus faecalis* CIP 103907, *Escherichia coli* CIP 105182, *Listeria innocua* LMG 1135668, *Salmonella enterica* CIP 105150, *Shigella dysenteriae* CIP 5451, *Staphylococcus aureus* ATCC 9244, *Proteus mirabilis* CIP 104588 *Staphylococcus aureus* ATCC 25293 and *Staphylococcus cameron* LMG 13567) and Clinical strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*). These clinical strains were isolated at the Laboratoire de Biologie Médicale Saint Camille in Ouagadougou. The identification of these clinical strains was based on their biochemical profiles as recommended by the manual Bactériologie Médicale (LeMinor and Veron, 1984).

Antibiotics and media: Commercially available antibiotics discs, ampicillin 33 μg and Tetraecyclin 30 μg were purchased from Beckton Dickinson and used as references for the test. All media used were from Fluka BioChemica. Chloroform was analytical grade.

Antibacterial assays

Disc diffusion method: The *in vitro* antibacterial activity of total alkaloids extract of *M. inermis* was studied by the paper disc diffusion method (Bauer et al., 1966; Peleczar et al., 1993; Ayandele and Adebeyi, 2007) using Mueller-Hinton agar plates. Briefly, to activate the microorganisms, they were grown on nutrient broth at 37°C for 18 h. The other night cultures were suspended in saline solution (0.9% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards corresponding to 10⁶ cfu mL⁻¹. Each bacterial suspension was used to inoculate 90 mm diameter Petri plates with a sterile non toxic cotton swab. Six millimeter paper discs (Whatman No. 3) soaked with 10 μL of the total alkaloid extract dilution (100 mg mL⁻¹ in dimethylsulphoxide (DMSO) were placed on the agar. The quantity of extract was then 1000 μg per disc. Paper discs soaked in DMSO without extract were used as negative control and DMSO didn’t show inhibition effects to microorganisms growth. The plates were incubated aerobically at 37°C for 18 to 24 h. All tests were performed in duplicate and the antibacterial activity was expressed as the mean of Diameters of Inhibition Zone (DIZ) produced.

Broth microdilution assay: A microdilution broth susceptibility assay was used, as recommended by the National Committee for Clinical Laboratory Standards (2006) for the determination of the Minimum Inhibitory Concentration (MIC) and the minimum bactericidal concentration (MBC). Briefly, the total alkaloids extract was properly prepared, sterilized by filtration through 0.22 μm sterilizing Millipore express filter and transferred in sterile 96 well-plates previously filled with sterile nutrient broth to obtain a twofold serial dilutions ranging from 19,531 to 2500 μg mL⁻¹. Then plates were inoculated with microbial suspensions diluted from the same 0.5 Mac Farland standards to have 5×10⁶ cfu mL⁻¹ in each well. A number of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no extract added) and the DMSO inhibitory effect. The final volumes in wells were 200 μL. After 24 h aerobically incubation at 37°C, bacterial growth was indicated by the presence of turbidity and a pellet on the well bottom. MIC was defined as the lowest extract concentration demonstrating no visible growth in the broth and the MBC was defined as the lowest concentration of the extract at which 99.99% or more of the initial inoculum was killed. To determine MBC values, 100 μL of bacterial suspension from subculture demonstrating no visible growth were removed to spread onto Plate Count Agar (PCA) medium plates. Plates were incubated at 37°C for a total period of 48 h.

Time-kill assay: In order to evaluate the efficiency of the alkaloids as a function of the time, two bacteria (E. coli...
RESULTS AND DISCUSSION

The aim of this research was to study the antibacterial activity of total alkaloids of *M. inermis*. Present results exhibited moderate to significant antibacterial activity against bacterial strains used for this test. The diameters of inhibition zone (DIZ) indicated the susceptibility of all tested bacteria to alkaloids except to *P. aeruginosa*. An antibacterial activity is recorded when a DIZ more than 9 mm is observed around the paper disc (Kitzberger et al., 2006). The largest DIZ (23 ± 1 mm) were obtained with *S. aureus ATCC92449*. It has frequently been reported that Gram-positive bacteria are more sensitive to plant extract and their components than Gram-negative bacteria (Kehmanon et al., 2000; Masika and Afolayan, 2002; Sahin et al., 2002; Karaman et al., 2003; Karou et al., 2006; Masoodi et al., 2008). The results of this study confirmed these observations. Diameter of inhibition values obtained with gram-positive bacteria are larger than those obtained with gram-negative bacteria (Table 1). Earlier study with alkaloids of *Sida acuta* (Karou et al., 2006) gave DIZ values greater than those obtained in this study. However, it is difficult to make a comparison because alkaloids from *M. inermis* are different from those from *S. acuta*. It is also indicated that the DIZ value is determined by the initial population density of the microorganisms, their growth rate and the rate of diffusion of the antimicrobial agent (Hugo and Russell, 1998).

Table 1: Diameters of inhibition zone by disc diffusion assay

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>ALK (mm)</th>
<th>AMP (mm)</th>
<th>TET (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram negative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> CIP105182</td>
<td>9 ± 0</td>
<td>44 ± 0</td>
<td>23 ± 0</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> CIP104588</td>
<td>9 ± 1</td>
<td>20 ± 1</td>
<td>19 ± 1</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em> CIP5451</td>
<td>10 ± 1</td>
<td>22 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td><em>Salmonella enterica</em> CIP105150</td>
<td>11 ± 0</td>
<td>34 ± 0</td>
<td>33 ± 0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (clinical isolate)</td>
<td>6 ± 0</td>
<td>36 ± 0</td>
<td>22 ± 0</td>
</tr>
<tr>
<td><strong>Gram positive</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25293</td>
<td>15 ± 1</td>
<td>55 ± 1</td>
<td>30 ± 1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC9244</td>
<td>23 ± 1</td>
<td>42 ± 1</td>
<td>33 ± 1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (clinical isolate)</td>
<td>14 ± 1</td>
<td>43 ± 1</td>
<td>28 ± 1</td>
</tr>
<tr>
<td><em>Staphylococcus carmorum</em> LMG113567</td>
<td>12 ± 0</td>
<td>54 ± 0</td>
<td>22 ± 0</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> LMG13569</td>
<td>13 ± 1</td>
<td>32 ± 1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td><em>Listeria innocua</em> LMG13568</td>
<td>12 ± 1</td>
<td>46 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> (clinical isolate)</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
<td>22 ± 1</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> 103907 CIP</td>
<td>13 ± 1</td>
<td>36 ± 1</td>
<td>22 ± 1</td>
</tr>
</tbody>
</table>

**ALK**: All alkaloids of *M. inermis*; **AMP**: Ampicillin; **TET**: Tetracycline

Table 2: MIC and MBC values of total alkaloids of *M. inermis* in the microdilution assay

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>MIC (mg mL⁻¹)</th>
<th>MBC (mg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram negative</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> CIP105182</td>
<td>1.25</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em> CIP104588</td>
<td>0.625</td>
<td>1.25</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em> CIP5451</td>
<td>2.5</td>
<td>&gt;2.5</td>
</tr>
<tr>
<td><em>Salmonella enterica</em> CIP105150</td>
<td>1.25</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (clinical isolate)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td><strong>Gram positive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25293</td>
<td>0.625</td>
<td>1.25</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC9244</td>
<td>1.25</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Staphylococcus carmorum</em> LMG113567</td>
<td>0.625</td>
<td>1.25</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> LMG13569</td>
<td>1.25</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Listeria innocua</em> LMG13568</td>
<td>1.25</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> (clinical isolate)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> 103907 CIP</td>
<td>1.25</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**MIC**: Minimum Inhibitory Concentration; **MBC**: Minimum Bactericidal Concentration; **nd**: Not determined

S. carmorum LMG113567 and *S. aureus* ATCC 25293. The antibacterial activity was considered bactericidal when the ratio MBC/MIC is 1 or 2 and bacteriostatic when this ratio is 3 or more. In this case, alkaloids of *M. inermis* can be considerate bactericide to tested bacteria (Table 2). The mode of action of the alkaloids of *M. inermis* which include indole and oxindole alkaloids (Shellard and Sharp, 1969) is not known in detail. They should have a particular mechanism which can justified their actions against Gram negative and Gram positive bacteria because the outer membrane of Gram-negative bacteria is known to present a barrier to the penetration of numerous antibiotic molecules.
REFERENCES


ACKNOWLEDGMENTS

This study was partially supported by CORUS 034. We gratefully think Dr. Imaas H.N. Bassole for his helpful comments on the manuscript and technical help and Mr. Dieudonne Kinda for his help during plant material collection. We also thank Laboratoire de Biologie Medicale Saint Camille for providing clinical bacterial strains.


