Anti *Staphylococcus aureus* Activity of the Aqueous Extract and Hexanic Fraction of *Thonningia sanguinea* (Cote ivoire).

*Dinzed Mbengu Rubens*1,2, Okou Obou Constantin3, Akakpo-Akue Moevi1, Guessennd Kouadio Nathalie2, Touré Daouda2, Nguessan Jean David1, Dosso Mireille2, Djaman Allico Joseph1,4

1Biochemical Pharmacodynamie Laboratory, University Felix Houphouet Boigny 22 BP 582 Abidjan 22, Côte d'Ivoire, 2Department of Bacteriology and Virology Institute Pasteur of Côte d'Ivoire, 01 BP 490 Abidjan Côte d'Ivoire
3University Jean Lorougnon Guede (Daloa)
4Department of clinical and fundamental Biochemistry Institute Pasteur of Côte d'Ivoire, 01 BP 490 Abidjan, Côte d'Ivoire

Available Online: 15th March, 2015

**ABSTRACT**

Objective: To evaluate antibacterial activity of methanol, ethanol and aqueous extracts and hexanic fraction of *Thonningia sanguinea*, against clinical isolates of anti *Staphylococcus aureus* (MRSA and MSSA). Methods: The confirmation of the MRSA by disk diffusion method (cefoxitin 30 μg) according to the methods of CA-SFM, 2009. The agar dilution and broth dilution method was used for the determination of the antimicrobial parameters (MIC and MBC) on these sensitive and MRSA strains. Results: The results showed that the inhibition zone diameter of Thos methanolic extracts for *S. aureus* ATCC 25923, MSSA and MRSA were (16.50 ± 0.29) mm, (17.30 ± 0.30) mm and (14.0) mm respectively. While the inhibition zone diameter of the hexanic fraction were (22.33 ± 0.33) mm, (22.0) mm and (19.33 ± 0.33) mm respectively for *S. aureus* ATCC 25923, MSSA and MRSA. The MIC and the MBC were showed value ranged from 0.26 ± 0.07 to 0.45 ± 0.17 mg/ml (MIC) and 1.04 ± 0.26 to 1.30 ± 0.26 mg/ml (MBC). Screening phytochemical showed the presence of Flavonoids, Alkaloid, Steroid, Terpenoids, gallic Tannins and Saponins. Conclusions: The results obtained suggest that methanolic extract of *Thonningia sanguinea* can be used in treating staphylococci infectious diseases.

**Key words:** Phytochemical Screening; Anti staphylococcus activity; MRSA; *Thonningia sanguinea*; MBC, MIC.

**INTRODUCTION**

Antimicrobial resistant bacteria are the causes of numerous clinical problems worldwide1. Control of infections acquired in hospitals and communities caused by multi-drug resistant Gram positive and Gram-negative bacteria has become a major problem not only in developing countries but also in developed countries2. *Staphylococcus aureus* is a gram-positive bacterium responsible for morbidity and mortality. It is one of the leading causes of human infections of the skin, soft tissues, bones and joints, abscesses, and normal heart valves. *Staphylococcus aureus* flourishes in the hospital setting and is associated with bloodstream and surgical wound infections and has become important nosocomial organism3.

Methicillin resistant *Staphylococcus aureus* (MRSA) represents a major public health challenge in many health care institutions worldwide. It is a common cause of outbreaks of cross infection and has become endemic in many regions where it adds to the morbidity, mortality, and cost of care associated with hospital acquired infection1. The importance of methicillin resistant *S. aureus* (MRSA) in comparison with methicillin sensitive strains (MSSA) lies not only in their resistance to all beta-lactams but also in their resistance to various other important antimicrobials1. The worldwide problem of antibiotic resistance impacts negatively on antibiotic therapy thus making successful empiric therapy much more difficult to achieve. The emergence of drug resistance is an evolutionary process that is based on selection for organisms that have an enhanced ability to survive and reproduce in the presence of a drug6. The emergence and spread of resistant bacteria make a serious threat which challenges the validity of the antibiotic arsenal currently available. The lack of new molecules, as well as the diversity of resistance mechanisms require the development of new therapeutic agents in the fight against this scourge6,7. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Their role is two fold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases. Traditional medicine using plant
The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases. In the present study, we have chosen this ivorian medicinal plant *T. sanguinea* to screen its antimicrobial activity (MIC and MBC) against others multidrug resistant namely Methicillin resistant *S. aureus* (MRSA) and methicillin sensitive strains (MSSA) and *E. coli*, *K. pneumoniae* (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* strains by the crude aqueous extract of *T. sanguinea*. In the Ivory Coast, the flowers of *Thos* are used for the treatment of diarrhea which is known to be one of the symptoms of salmonellosis. Previous studies have shown inhibition of the MDR strain *Salmonella enteritidis* lysotype 6, and Extended-Spectrum-β-Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* strains by the crude aqueous extract of *Thos*. In the present study, we have choosen this ivorian medicinal plant *T. sanguinea* to screen its antimicrobial activity (MIC and MBC) against others multi-drug resistant namely Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive strains (MSSA).

### MATERIAL AND METHOD

**Plant material**

*T. sanguinea* flowers were collected in Adzopé, Côte d’Ivoire (West Africa) and identified by Pr Aké Assi of the Department of Botany, University of Cocody-Abidjan. A voucher specimen (Voucher no. 14162) is deposited in the herbarium of Centre National de Floristique (CNF) of Abidjan.

**Bacterial strains**

The biological assays were carried out on nine (9) hospital isolates and one reference strain (*S. aureus* ATCC 25923) provided by the unit of Surveillance of the Resistance of the Microorganisms to the Anti Infectious (ASSURMI), Department of Bacteriology Virology of the institute Pasteur of Côte d’Ivoire (IPCI). The strains were

Table 1: Zone of inhibition (mm) of the extracts of *Thos* and céfoxitine against *E. coli*, *K. pneumoniae* and *S. aureus*

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Aqueous (mg/ml)</th>
<th>Ethanol (mg/ml)</th>
<th>Methanol (mg/ml)</th>
<th>F1 (mg/ml)</th>
<th>Fox</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td><strong>MSSA</strong></td>
<td>13.80</td>
<td>± 0.11</td>
<td>11.40</td>
<td>± 0.23</td>
<td>14.27</td>
</tr>
<tr>
<td><strong>MRSA</strong></td>
<td>11.83</td>
<td>9.43</td>
<td>± 0.14</td>
<td>12.47</td>
<td>± 0.07</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td>13.93</td>
<td>11.73</td>
<td>± 0.07</td>
<td>14.87</td>
<td>± 0.09</td>
</tr>
<tr>
<td><strong>ATCC</strong></td>
<td>13.93</td>
<td>11.73</td>
<td>± 0.07</td>
<td>14.87</td>
<td>± 0.09</td>
</tr>
</tbody>
</table>

Figure 1: Percentage Yield of *T. catappa* extracts

Aq: Aqueous extract; EtOH: Ethanol extract; MeOH: Methanolic extract; F1: Hexane fraction

extracts continues to provide health coverage for over 80% of the world’s population, especially in the developing world. Higher plants have been shown to be a potential source for the new antimicrobial agents. The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases.

One of the plants used by African populations against skin and systemic infectious diseases is *Thonningia sanguinea* (*Thos*). In Côte d’Ivoire, Togo, and Ghana, *Thos* is traditionally used for the treatment of haemorrhoids and anal lesions, bronchial asthma, skin diseases, dysentery, sore throat and as vermifuge. In Ivory Coast, the flowers of *Thos* are used for the treatment of diarrhea which is known to be one of the symptoms of salmonellosis. Previous studies have shown inhibition of the MDR strain *Salmonella enteritidis* lysotype 6, and Extended-Spectrum-β-Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* strains by the crude aqueous extract of *Thos*. In the present study, we have chosen this ivorian medicinal plant *T. sanguinea* to screen its antimicrobial activity (MIC and MBC) against others multi-drug resistant namely Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive strains (MSSA).
house referenced. According to the activity of known antibiotics, two kinds of strain were distinguished: some strains were meticillino sensitive (079y/09 and 697y/09) and others were meticillino resistant (762c/08; 118c/09; 509y/09; 606y/09; 801/09 and 908c/09).

Extraction procedure
The freshly collected flowers of Thos were air dried at room temperature for 7 days and powdered. From this powder, aqueous, ethanol and methanol extracts were obtained successively following the method described by Touré et al., (2011)14.

Extraction aqueous procedure
Briefly 100g of powder was soaked in 2L distilled water for 24 h with constant stirring. The suspension was further filtered through Whatman (No.1) filter paper. The filtrate was concentrated with a rotary evaporator to obtain the aqueous extract.

Preparation of alcoholic extracts
Preparing ethanolic and methanolic extracts was made using the same method described above using the respective solvents ethanol and methanol. The filtrate was concentrated on a rotary evaporator under reduced pressure at 50°C and then dried in an oven at 40°C. Extracts obtained were stored in a refrigerator until used for bioassays.

To obtain hexane extract of T. Sanguinea, Twenty five grams of extract methanolic (25 g) was extracted with 250 ml of hexane (Merck, Darmstadt, Germany) for 24 h using a Soxhlet extractor. The extract was filtered with Whatman filter paper no.1, and the filtrate was evaporated under vacuum in a rotary evaporator (Buchi) at 55°C.

The different extracts of T. Sanguinea (ETA, EE, EM and EH) obtained were used for the phytochemical screening and in vitro antibacterial tests.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The MIC assay was determined by the twofold serial broth dilutions method in sterile tubes, according to Wilkinson and Gentry revised by N’guessan et al.12 The dried plant extracts were dissolved in sterile distilled water to a final concentration of 50.0 mg/ml, and filtrated...
through a 0.2 μm membrane filter (Whatman). Overnight culture of each test organisms (approximately 10^7 CFU) was seeded into the tubes containing nutrient broth (Mueller-Hinton broth) and the plant extracts were tested at concentration from 50.0 to 0.024 mg/ml. The tubes were incubated for 24 h at 37 °C. MIC was determined as the lowest concentration of the plant extract resulting in the complete inhibition of visible growth. The MBC was determined based on the lowest concentration of the extracts required to kill 99.9% of bacteria from the initial inoculum as determined by plating on agar. Therefore, the calculation of the ratio MBC/MIC of the extracts has permitted to determine their antibacterial power.

Statistical analysis
Three different samples of Thos were assayed. All the experiments were conducted in triplicate unless stated otherwise and data were analyzed by one-way ANOVA followed by Dennett’s t-test using Instat® (Graph Pad software, U.S.A). At 95% confidence interval p<0.05 was considered statistically significant.

RESULTS
Extract efficiency
The total mass of plant material extracted by the different solvents (water, ethanol, methanol and hexane) from 100 g of plant material is presented in the Figure 1. Yields for the various extracts showed values between 13.23 ± 0.23 % and 21.90 ± 0.06 %. Among the three solvents used, methanol gave the highest yields.
Phytochemical analysis
Phytochemical constituents present in the extracts and hexanic fraction of Thos included tannins, saponins, flavonoids, steroids and terpenoids, Alkaloids, coumarins, and phenolic (Figure 2). The presence of these constituents has been reported to account for the exertion of antimicrobial activity by plant.
Antibacterial activity
Results of the bioactivities measured in terms of zone of inhibition of the plant extracts are shown in Table 1. The result shows that all extracts (aqueous, ethanolic and methanolic) and fraction of Thos showed activity against the tested S. aureus strains. The highest activity (diameter of zone of inhibition 22.33 ± 0.33 mm) was demonstrated by the hexanic fraction against S. aureus ATCC while the lowest activity (diameter of zone of inhibition 11.83 ± 0.17 mm) was demonstrated by the water extract against MRSA. Cefoxitin (30 μg) were used as positive control and had a diameter of inhibition zone of 25.67 ± 0.88 ± 0.88 to 30.67 ± 0.67 mm. (Table 1). During the antibacterial susceptibility test it was observed (Table 1) that the control drug Cefoxitin showed the largest and significance (P<0.05) zone of inhibition compared to all other extracts on all S. aureus strains investigated in this study.
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): Results of MIC and MBC are shown in Table 2. The result showed that MRSA had the highest MIC (3.12 mg/ml) and MBC (12.50 mg/ml) with the aqueous extract, while the lowest MIC (0.024 mg/ml) and MBC (0.07 ± 0.03 mg/ml) was shown by MSSA and S. aureus ATCC with the hexanic fraction of Thos. We notice that the values of the MIC and MBC agree with that of the diameters of the inhibition zone growth because the extracts that have induced the largest diameter of inhibition have presented the smallest values of MIC on the corresponding strains.

DISCUSSION
Medicinal plants are commonly available resources, have less if no side effects, economic and have antimicrobial properties. The majority of these medicinal plants used in this study are applied in traditional medicine in many African regions to cure different disorders. Medicinal plants are considered one of the most valuable resources for antibiotic development. Pathogenic strains of antibiotic resistant bacteria have emerged due to the misuse of antibiotics. As a result, bacteria become resistant to antibiotics, which are in turn less effective after extended periods of use. Pharmaceutical companies whose efforts are focused on the production and manufacture of antibiotics strive to manufacture new generations of antibiotics capable of treating such antibiotic resistant bacterial strains. In recent years, publications from several countries have reported the use of active compounds extracted from medicinal plants, which may benefit antibiotic development[6,17]. Methanol, ethanol, water extracts and hexanic fraction of Thos were used in this study to evaluate the, antibacterial activities against S. aureus. In this study, we was observed that methanol and ethanol were good solvents for extraction with yield of 21.90 ± 0.06 % and 17.23 ± 0.28 % respectively while water was the least 15.47 ± 0.24 %. The methanolic extract has given better yield. Generally the methanol extract was more active than other extracts against the selected bacterial isolates, because most of the antimicrobial agents in plants are soluble in methanol. The results correspond to those of Masoko et al (2008)[19] which have respectively shown agreat concentration of the active principals with the methanol. That justifies the fact that the methanolic extract of Thos presented a better activity than most of the tested strains. (P<0.0001)
In the present investigation, we have evaluated the antimicrobial activity of aqueous, ethanol, methanol extracts and hexanic fraction of T. sanguinea against strains of MRSA, MSSA and S. aureus ATCC. From the analysis of these results of disk diffusion we noticed that all extracts (methanolic ethanolic and aqueous) and hexanic fraction of T. sanguinea are active on the whole tested bacterial strains because they have induced some inhibition diameters superior to 9 mm[18]. Our results clearly indicated the inhibitory effects of all extracts of T. sanguinea on these bacteria. Ohiri and Uzodimma[19] had already shown the inhibitory activity of T. sanguinea on sensitive strains of S. aureus. For an extract considered or fraction, comparatively to the control inoculum there was a progressive decrease in the thickness of the layer of colonies on the streak but also a


