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Research Article

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

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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 worldwide[1]. 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 countries[2].

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 organism[3].

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 infection[4]. 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 antimicrobials[5].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 drug[6].

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 scourge[6,7].

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

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

Bacterial strain	Aqueous (mg/ml)		Ethanol (mg/ml)		Methanol (mg/ml)		F_1 (mg/ml)		Fox
	100	50	100	50	100	50	50	25	
MSSA	13,80 ± 0,11	11,40 ± 0,23	14,27 ± 0,27	11,83 ± 0,17	17,30 ± 0,30	13,20 ± 0,11	22	18.23 ± 0,23	30.67± 0.67
MRSA	11,83 ± 0,17	9,43 ± 0,14	12,47 ± 0,07	10,27 ± 0,18	14	11,47 ± 0,24	19,33 ± 0,33	16,47 ± 0,12	25.67 ± 0.88
S. aureus ATCC	13,93 ± 0,06	11,73 ± 0,07	14,87 ± 0,09	12,17 ± 0,12	16,50 ± 0,29	13 ± 0,06	22,33 ± 0,33	18,73 ± 0,14	30.17 ± 0.44

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Thos*

Bacterial strain	Aqueous				Ethanol			
	CMI	CMB	CMB/CMI	Antibacterial activity	CMI	CMB	CMB/CMI	Antibacterial activity
MSSA	1,56	3,12	2	Bactericidal	0,78 ± 0,13	3,12	4	Bactericidal
MRSA	3,12	12,50	4	Bactericidal	2,08 ± 0,52	5,21 ± 1,04	2,50	Bactericidal
S.aureus ATCC	1,56	3,12	2	Bactericidal	0,65 ± 0,13	2,60 ± 0,52	4	Bactericidal
	Methanol				F1			
Bacterial strain	CMI	CMB	CMB/CMI	Antibacterial activity	CMI	CMB	CMB/CMI	Antibacterial activity
MSSA	0,26 ± 0,07	1,04 ± 0,26	4	Bactericidal	0,024	0,07 ± 0,03	2,92	Bactericidal
MRSA	0,45 ± 0,17	1,30 ± 0,26	2,88	Bactericidal	0,195	0,195	1	Bactericidal
S.aureus ATCC	0,26 ± 0,07	0,78	3	Bactericidal	0,024	0,07 ± 0,03	2,92	Bactericidal

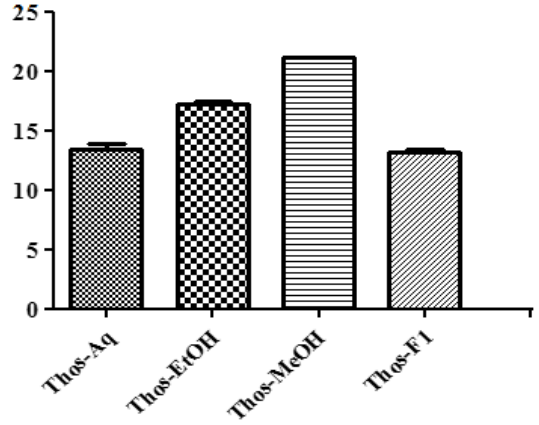

Figure 1: Percentage Yield of T. catappa extracts
Aq: Aqueous extract; EtOH: Ethanolic extract; MeOH: Methanolic extract; F1: Hexane fraction

extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world[8]. Higher plants have been shown to be a potential source for the new antimicrobial agents[9,10].

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[11]. 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[12,13].

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

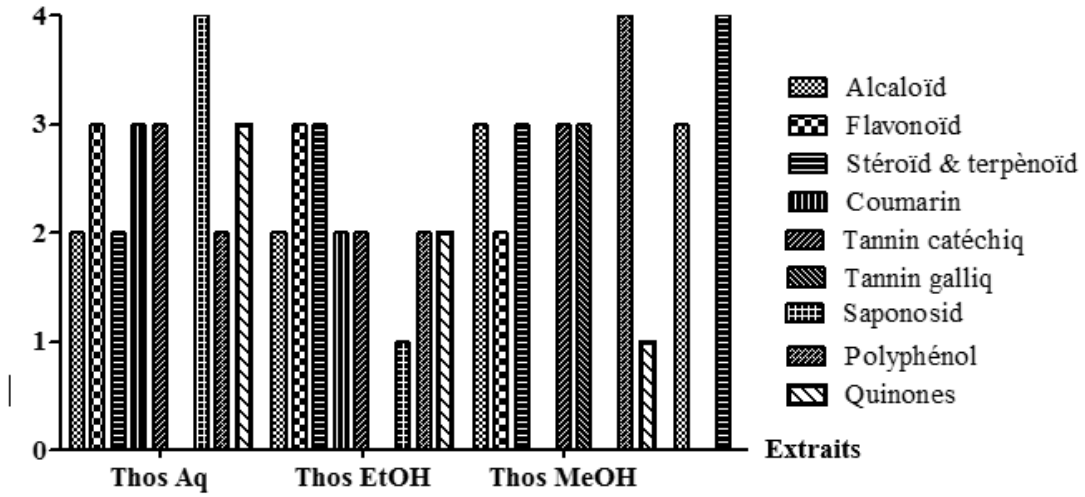

Figure 2 : Screening phytochemical of the extracts of Thos

house refecenced. 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 (N°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 is 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 méthanolic (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 solvent extraction strength

To determine the extraction efficiency of different solvents, 100 g of fresh plant material was macerated in 300 ml of each extracting solvent, and extraction was done as described previously. The mass of each solvent extract was measured and presented in a bar chart, as mass of extract against solvent[15].

Confirmatory test for MRSA

Susceptibility to methicillin was determined by using Kirby-Bauer disk diffusion method according to the Committee of Antibiogramme of the Microbiology Society French (CA - SFM 2012) guidelines. The antibiotic tested was cefoxitin (30 µg).

Quality control was performed with the reference strain of *S. aureus* ATCC 25925.

Screening phytochemical of vegetal extracts

The freshly prepared extracts were subjected to standard phytochemical analyses to test for the presence of the phytoconstituents Alkaloids, Saponins, Tannins, terpènoïdes, flavonoids, coumarins and quinons[7,16].

Determination of antibacterial activity

Paper disc agar diffusion method

The anti MRSA and MSSA activity of the aqueous and organic extracts of *T. Sanguinea* sample was initially determined using the disc diffusion assay[7].

A standardized inoculum (1–2 × 10^7 cfu/ml 0.5 McFarland standards) was introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum.

A sterile paper disc previously soaked in a known concentration of extract (100; 50 and 25 mg/ml per disc) was carefully placed at the centre of the triplicate labeled seeded plate. The plates were later incubated at 37°C for 24 h after which they were observed for zones of inhibition. As positive controls, discs (oxoid) containing cefoxitin (30 µg) 1 µg were used. Water or ethanol and methanol saturated disc (air-dried) were used as negative controls. Antibacterial activity was determined by measurement of zone of inhibition around each paper disc. For each extract three replicate trials were conducted against each organism and the mean of the diameter of the inhibition zones was calculated.

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 terpnoids, 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[3,6,17].

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)[15] 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 Uzodinma [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

decrease in the number of colonies from low concentrations to high concentrations; thus reflecting a dose-dependent sensitivity.

Taken together, the results of disk diffusion, MIC and MBC assays show that all extracts and hexanic fraction of Thos exhibited bactericidal activity against some S. aureus strains. Previously observation indicated that the relationship between inhibition zone diameters, the MIC and the MBC values were correlated. The results showed significant inhibition with promising antibacterial parameters (MIC values between 0.024 and 3.125 mg/ml; MBC values between 0.07 ± 0.03 and 12.50 mg/ml). These results have showed also that all extracts and hexanic fraction were bactericidal for all the tested strains (MBC/MIC ≤ 4)[20]. It appears from the analysis of results, that the most bactericidal extract was the hexanic fraction with a MBC of 0.07 ± 0.03 mg/ml while the least bactericidal extract was aqueous extract because of its higher value of MBC (12.50 mg/ml). Moreover, some settled report established on the basis of the minimal bactericidal concentration confirm that the hexanic fraction are better in activity than the methanolic extract, on the other hand the methanolic extract is more active than the ethanolic extract and itself more active than the aqueous extract. Similar results were also shown by Ohiri and Uzodinma[19]. The extracts of *T. sanguinea* studied were found to contain more of the following phytochemical compounds tannins, flavonoids, steroids and terpnoids, Alkaloids, coumarins, saponins and phenolic with variable concentration according to the extract. The antibacterial activity of this medicinal plant may therefore be due to the presence of flavonoids, tannis, saponins, steroid and terpènoïdes[12,19] and hence, the use of this plant in the anti-infectious and anti-diarrhea treatment.

CONCLUSION

It can be concluded that all extract and hexanic fraction of *T. sanguinea* were revealed significant antibacterial activity. These activities were bactericidal effect upon and were dose dependent all the tested strains. The hexanic fraction of *T. sanguinea* can be used as complementary medicine in treating diseases caused by multidrug resistant strains of *S. aureus*. However, further investigation is needed to determine the bioavailability of the active compounds and to determine the dose and toxicity before it can be used as therapeutic agents.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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