

Evaluation of the Antistaphylococcic Activity of *Terminalia macroptera* Guill et Perr (*Combretaceae*) Stem Bark Extracts

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Abstract: Antibacterial activity of ethanolic_{70%} and aqueous extracts of *Terminalia macroptera* were carried out against five different strains of *Staphylococcus aureus* Meti-R (*S. aureus* Meti-R 1541; *S. aureus* Meti-R 235; *S. aureus* Meti-R 466; *S. aureus* Meti-R 485; *S. aureus* Meti-R 246) and reference strains *S. aureus* ATCC 25923. Two methods have been used: the method of wells in the agar was used to test the sensibility of bacterial strains while the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by the dilution method in liquid medium. Among all the tested strains, ethanolic_{70%} extract has given the inhibition diameters included between 20 and 24 mm whereas the aqueous extract has varied from 12.67 to 19.33 mm. The values obtained with the Minimum Inhibitory Concentration of (MIC) are between 6.25 and 50 mg/ml and those of Minimum Bactericidal Concentration (MBC) are between 6.25 and 50 mg/ml. Moreover the ethanolic_{70%} extract has shown the best bactericidal activity against all the tested).

Keywords: *Terminalia macroptera*, *Staphylococcus aureus* Meti-R, Aqueous Extract, Ethanolic_{70%} Extract

1. Introduction

Staphylococcus aureus is responsible for many infections as respiratory tract diseases (pneumonias, bronchitis), wounded mucous infections, sinusitis, endocarditis, food poisoning and carbuncles. They are also the germs frequently met during surgical wound infections which are often provoked by the use of intravascular catchers or by the spread of bacteria from another source of infection [1].

Presently, the sequencing has allowed counting many species of strains belonging to the *Staphylococcus* family [2]. Most of these species is part of commensal human flora so they live harmony with the host organism. For immunodepressed and generally when the conditions become unfavorable, these species become quickly pathogenic. So for populations at risk especially the drug addicts, the HIV

positive and even the former prisoners the rate of *S. aureus* resistant to methicillin is very high [3, 4, 5]. Also *Staphylococcus aureus* is the most pathogenic among all the species of *Staphylococcus* and it is responsible for almost 25% of septicemias met in hospitals [6], and the Treatment is long then expensive [7].

Many scientific researchers have allowed to bring out the therapeutic properties of some natural substances from plants in the prevention and the treatment of pathologies caused by the microorganisms resistant to antibiotics commonly used [8, 9]. That makes the medicinal plants some potential sources of new molecules to explore. It's in this context, that our team of research was interested to *Terminalia macroptera* (*combretaceae*), a plant species of the savanna that can be also found in the tropical forests of Africa (Many authors have reported to use the leaves, the stem barks and the roots of *T. macroptera* in the treatment of

many diseases caused by infections in African traditional medicine [10, 11].

The present study was undertaken to evaluate the activities of two extracts of *Terminalia macroptera* on the growth in vitro of five strains of *Staphylococcus aureus* Meti-R and a reference strains.

2. Materials and Methods

2.1. Plant Material

It consists of bark *Terminalia macroptera* Guill. et Perr. (*combretaceae*). These barks were collected in April 2012 in Niakara (north of Ivory Cost). Their authentication was performed by professor Ake-Assi of National Center Floristic (NCF), University Felix Houphouet Boigny of Cocody-Abidjan where a sample is retained.

2.2. Bacterial Strains

The bacteria used for the biological tests are *Staphylococcus aureus* resistants to meticillin (*S. aureus* meti-R 1541; *S. aureus* Meti-R 235; *S. aureus* meti-R 466; *S. aureus* meti-R 485; *S. aureus* meti-R 246) provided by pleural liquid, suppuration and probe button and *S. aureus* ATCC 25923 (reference strain). All the bacteria strains were provided by the department of bacteriology and virology, Institute Pasteur of Ivory Cost. (I.P.IC).

2.3. Preparation of Plants Extracts

The stem barks of *T. macroptera* collected were washed cut and has been dried shelter powder by a type IKAMAG-RCT grinder. According to the methods described by [12, 13], 100 g of plant powder have been macerated in 1 L of distilled water then homogenized under magnetic agitation for 24 hours at 25°C with a IKAMAG-RCT type agitator. The homogenate obtained, has been filtered successively two times through hydrophilic cotton (cotton wool) then once through whattman paper n°2. The volume of filtrate obtained is first reduced with a rot vapor Büchi at 60°C. Then, the rest of the filtrate is evaporated with a med center vent cell drying oven at 50°C to give a brown powder which is the aqueous extract. The same process was carried out by using ethanol 70% instead of distilled water to obtain ethanolic extract 70% [14]. The end, all the plant extracts obtained are kept in refrigerator until used for testing antibacterial.

2.4. Study of the Antibacterial Activity of Different Extracts

For each bacterial strain, inoculums was prepared by homogenizing 0.1 ml of a suspension opalescent 3 hours in to 10 ml of Muller-Hinton broth concentrate twice in order to obtain a bacterial load estimated at 5.10^6 CFU/ml and constitute the dilution 10^0 .

2.5. Determining Zones of Inhibition of Growth

The susceptibility test have been carried out on Muller-

Hinton agar (Bio-Rad, France) by using wells [15] so, like in the case of classic anti-biogram realization, each well or hole of 6 mm in diameter has been filled with 80 µl of extract concentration 200 mg/ml. Taking care to separate two holes at least 20 mm. A control well was carried out for each bacterial strain with 80 µl of the solution mixture of DMSO/Sterile distilled water V/V [16, 17]. After a pre-release of 45 minutes at room temperature under the hood, the whole was incubated in an oven at 37° C for 18 to 24 hours. Meanwhile, the oxacillin (5 µg) and cefoxitin (30 µg) were used as positive controls. After incubation; the action of extracts is determined by measuring an area of growth inhibition (lack of colonies) around the well.

2.6. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Dilution method in liquid medium was used to determine these antimicrobials parameters [18]. This, in a series of 8 hemolytic tubes numbered C₁ to C₉ was introduced 1 ml of the bacterial inoculums esteemed to 5.10^6 UFC. Then 1 ml of a plant extract with a known concentration according to the range of prepared concentration has added in the same tubes. This distribution of plant extract is made so that 1 ml of plant extract 200 mg/ml is transferred to the tube C₁, that of 100 mg/ml, to the tube C₂ and so on until to C₇ tube which receive 1 ml of plant extract of 3.125 mg/ml. The C₈ receive, instead of plant extract, 1 ml of DMSO/distilled water (1/9, V/V) was used as control. This distribution of plant extract concentration is well known to each tube containing 1 ml of inoculums already reduced the concentration of the plant extract in the middle half. So the tube C₁ concentration increased from 200 mg/ml to 100 mg/ml, 100 mg/ml to 50 mg/ml for C₂ so on until a concentration of 3.125 mg/ml for T₇. This experiment was performed identically for each extract tested. The eight (8) first tubes (C₁ to C₈) are collected "experimental tubes" and the last tube C₈ is rated "growth control tube or T_C". These loaded tubes are incubated at 37°C for 24 hours. The experience has been reported three times.

The MIC is the concentration of the first tube where it finds no trouble visible to the naked eye. From the MIC lowest concentration that allows at most 0.01% survival of bacteria in the first suspension to survive within 24 hours corresponds to MBC. Therefore, the calculation of the ratio MBC/MIC of the extracts has permitted to determine their antibacterial power.

3. Results and Discussion

The diameters of zone inhibition have varied from 12.67 to 19.33 mm (aqueous extract) and 20.00 to 24.67 mm (ethanolic extract) table 1. Aqueous and ethanolic_{70%} extracts have proved an activity against all the tested microorganisms. On the basis of these inhibition diameters, ethanolic_{70%} extract has been proved as the more active of the two extracts studied with all diameters superior 20 mm for all strains of *S.*

aureus studies. Besides any inhibition zone proved by the oxacillin (5 µg) and the cefoxitin (30 µg) can be observed against *S. aureus* Meti-R (235; 466; 485 and 246). From the analyses of these results, aqueous and ethanolic_{70%} extracts are active on the whole tested bacterial strains because they have induced some inhibition diameters superior to 10 mm

[19]. We can notice that in spite of the inefficacy of the commercialized antibiotic against *S. aureus* Meti-R 235; 466; 485 and 246 (0 mm), they have been more active against the two other strains *S. aureus* Meti-R 1541 and *S. aureus* ATCC 25923.

Table 1. Inhibition diameters (mm) of plant extracts and antibiotic against *Staphylococcus* strains.

Bacterial strains	Plant extracts		Antibiotics	
	Eaq	Eeth70	OX	FOX
<i>S. aureus</i> Meti-R 1541	19.33 ± 1.53	20.33 ± 2.89	18.63 ± 1.25	21.00 ± 1.73
<i>S. aureus</i> Meti-R 235	19.00 ± 1.00	21.00 ± 1.73	0.00	0.00
<i>S. aureus</i> Meti-R 466	16.67 ± 1.53	21.67 ± 1.53	0.00	0.00
<i>S. aureus</i> Meti-R 485	18.00 ± 1.73	20.00 ± 1.00	0.00	0.00
<i>S. aureus</i> Meti-R 246	12.67 ± 1.25	24.33 ± 1.00	0.00	0.00
<i>S. aureus</i> ATCC25923	18.00 ± 2.00	24.67 ± 1.53	20.33 ± 1.73	19.67 ± 1.00

Results are given by (m±sd); E_{aq}(aqueous extract); E_{eth70}(ethanolic extract70%); OX (oxacillin 5µg); FOX (céfoxitin 30µg)

The antimicrobial parameters of the aqueous extract on the tested germs are presented in table 2. MBC has advanced 6.25 to 50mg/ml. *S. aureus* meti-R; *S. aureus* 1541 and *S. aureus*-R 235 recorded a MBC equal to 6.25 mg/ml. As for the other strains, they presented a MBC equal to 50 mg/ml. The determination of antibacterial effect of some active substances depends on the ratio MBC/MIC [20]. According those authors, when this ratio is inferior or equal to 3, the substance is considered bactericidal and bacteriostatic if the ratio is superior t or equal to 3. Thus, aqueous extract was bactericidal against *S. aureus* meti-R 1541; 235 and ATCC 25923 strain for ratio of MBC/MIC equal to 1. Besides, the ratio was equal to 4 for the over bacteria studied, showing bacteriostatic activity on these strains. These results corroborate with those of Toty [21]. Indeed, these authors determined a ratio CMB/MIC equal to 1, showing that aqueous extract of bark *Harungana madagascariensis* had a bactericidal activity on *S. aureus* ATCC25923 and *S. aureus* meti-R 926. These results can be compared to the results [22]. These authors have found MBC equal to 1600mg/ml of aqueous extract of plant against *S. aureus* meti-R. Seen that value, we can say our results are better.

Table 2. Antibacterial activity of aqueous extract of *Terminalia macroptera*.

Bacterial strains	Antimicrobial parameters (mg/ml)			
	MIC	MBC	MBC/MIC	Interpretation
<i>S. aureus</i> Meti-R 1541	6.25	6.25	1	Bactericidal
<i>S. aureus</i> Meti-R 235	6.25	6.25	1	Bactericidal
<i>S. aureus</i> Meti-R 466	12.50	50	4	Bacteriostatic
<i>S. aureus</i> Meti-R 485	12.50	50	4	Bacteriostatic
<i>S. aureus</i> Meti-R 246	12.50	50	4	Bacteriostatic
<i>S. aureus</i> ATCC25923	50	50	1	Bactericidal

MIC: Minimum Inhibition Concentration; MBC: Minimum Bactericidal Concentration

MBC of the ethanol extract were ranged enter 6.25 and 25 mg/ml (table 3). Strains of *S. aureus* meti-R1541; 235; 246 and *S. aureus* ATCC 25923 showed the smallest MBC

(6.25 mg / ml). Also the largest MBC (25 mg/ml) was observed on *S. aureus* meti-R 466 and *S. aureus* meti-R 485. Unlike the aqueous extract, ethanolic_{70%} extract was bactericidal against all strains studied. Indeed, the ratio MBC / MIC was between 1 and 2. Alone, strains of *S. aureus* meti-R 246 and *S. aureus* ATCC25923 have shown MBC which are the MIC double. For other strains, MBC were equal to 1. Others studies showed bactericidal activity on *S. aureus* ATCC 25923 and *S. aureus* meti-R like the case of [23]. Indeed, these authors studying activity of ethanolic_{70%} extract of *Vitex donania* against *S. aureus* ATCC25923 and *S. aureus* meti-R found a ratio MBC/MIC equal to 2, showed that ethanolic_{70%} extract has a bactericidal power against these strains.

Table 3. Antibacterial activity of ethanolic_{70%} extracts t of *T. macroptera*.

Bacterial strains	Antimicrobial parameters (mg/mL)			
	MIC	MBC	MBC/MIC	Interpretation
<i>S. aureus</i> Meti-R 1541	6.25	6.25	1	Bactericidal
<i>S. aureus</i> Meti-R 235	6.25	6.25	1	Bactericidal
<i>S. aureus</i> Meti-R 466	25	25	1	Bactericidal
<i>S. aureus</i> Meti-R 485	25	25	1	Bactericidal
<i>S. aureus</i> Meti-R 246	3.12	6.25	2	Bactericidal
<i>S. aureus</i> ATCC25923	3.12	6.25	2	Bactericidal

MIC: Minimum Inhibition Concentration; MBC: Minimum Bactericidal Concentration.

In comparison, aqueous and ethanolic extracts showed the same MBC equal to 6.25 mg/ml for *S. aureus* meti-R 1541 and *S. aureus* méti-R 235. But, for *S. aureus* meti-R 466 and *S. aureus* 485, MBC of ethanolic extract was the half of that aqueous extract ($MBC_{Aq}/MBC_{Eth70\%}=2$). Also we can observe that ethanolic extract amelior eight times the effect of aqueous extract for *S. aureus* meti-R 246 and *S. aureus* ATCC 25923 (figure 1). In other studies, similar results have been obtained with some aqueous and ethanolic extract of *Mycroglossa pyrifolia* by [14], (2003). In fact, these authors have proved that ethanolic extract ameliorate 100 times the

effect of aqueous extract showing a ratio ($MBC_{Aq} / MBC_{Eth70\%} = 100$).

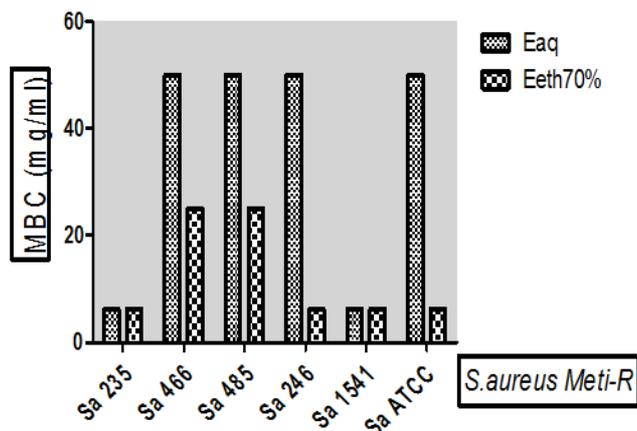


Fig. 1. Compared effect of aqueous and ethanolic 70% extracts of *T. macroptera*.

4. Conclusion

This study has allowed to demonstrate that *Terminalia macroptera* stem barks have some bactericidal activities against the strains of *S. aureus* Meti-R.

Ethanolic 70 % extract has shown the best activity on the whole tested strains. The concentration to which this extract remains active allows us to say that plant could be used against *Staphylococcus* infections. This work is a contribution for a better knowledge of some medicinal plants in Ivory Cost, which can become a new source of antibacterial agent, then it's can be explored by determination the potential secondary metabolites susceptible the origin of antibacterial activity. Also it would be interesting to undertake studies of toxicity of the extracts which are fund to be active and to consider the development of improved traditional medicine (I T M) after purification.

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