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Botanical Study and *in vitro* Antibacterial Activity of *Bersama abyssinica Fresen*. (Melianthaceae) on Multi-Resistant *Staphylococcus aureus* Strains

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Abstract : ***Objective: The objective of this study was to find new natural substances (bioactive)having antibacterial activity on multi-resistant strains of Staphylococcus aureus. Methods and Results: The methods of dissemination swab on Muller-Hinton agar and double dilution were used to evaluate the antibacterial activity of aqueous total extract of the stembark of Bersama abyssinica. All multi-resistant strains of Staphylococcus aureus and the reference strain (ATCC 25923) were sensitive to aqueous total extract (ATE)of the stem bark of B. abyssinica. Conclusion and Application:ATE had a bactericidal effect on different strains tested. MBC ranged from 1.563±0.0 to 0.098±0.0 mg/ml. This result justifies the traditional use of the stem bark of Bersamain treating skin diseases. We have hope on the development of this new substances that can fight the multi-resitantes strains of Staphylococcus aureus at least with the crude extract of the plant.***

Keywords: *Bersama abyssinica*, multi-resistant strains, *Staphylococcus aureus,* aqueous total extract

1. Introduction

Antibiotics widely used for the treatment of infectious diseases are under constant threat due to the emergence of antibiotic resistant pathogens such as meticiline resistant*Staphylococcus aureus*(MRSA) [1] [2]. This antibiotic resistance in pathogenic microorganisms has caused a lot of premature deaths and has become a public health problem worldwide [3]. Many cases of multidrug resistance have been reported inCôte-d'Ivoire and in other sub-Saharan countries[4]. *Staphylococcus aureus* is responsible for endocarditis, and wound infections. It's responsible for bacterial infections and one of the three major causes of nosocomial infections [5]. These strains also cause skin infections: boils, folliculitis, paronychia, breast abscess in women and nursing animals. Mucosal infections are common and can affect the eyes (conjunctivitis), ears (otitis), the genital area (endometritis, pelvic inflammatory disease) or respiratory organ (pneumonia, pleurisy). Among these *Staphylococci*, the strains marked R (Resistant) are those of which there is a high probability of treatment failure, regardless of the type and dose of antibiotic treatment used[6].

In view of the increasingly growing of multiple resistant disease causing bacteria, and the wide spectrum of infections caused by *Staphylococcus aureus*, the search for new antimicrobial substances from medicinal plants, capable of destroying multi-resistant strains becomes necessary. Indeed, antimicrobial compounds from plants extracts are capable of inhibiting bacterial growth by acting on cellular targets different from those covered by the currently used antibiotics such as penicillins,macrolidesortetracyclines. They could also have a significant clinical value in the treatment of infections caused by microbial resistant strains[7]. Thus, an ethnobotanical study was conducted in the Region of Transua, District of Zanzan (Côte d'Ivoire), we found*Bersama abyssinica* Fresen. (Melianthaceae), a plant widely used in the treatment of skin diseases.

Previous work has shown that *Bersama abyssinica* is used in the treatment of various diseases: cancer, spasms, infections, male infertility, diabetes [8], diarrhea, cholera, intestinal worms, amebiasis dysentery, syphilis, gonorrhea [9], malaria and general fatigue [10] [11].

The botanical study conducted aimed at evaluating the antibacterial activity of aqueous total extract of the stem bark of *Bersama abyssinica* on the *in vitro* growth of some multi-resistant strains of *Staphylococcus aureus*.

2. Material

2.1 Plant Material

Going by our ethnobotanical investigation in the Region of Transua (District of Zanzan,Côte d'Ivoire), it appears that *Bersama abyssinica* plant is widely used in the treatment of microbial diseases. The stem bark was harvested, cut, washed with water and dried under the shade. These dried plant parts,using a grinder were then reduced to a fine powder.

Botanical Study of Bersama abyssinica
Synonym: *Bersama engleriana* Gürke (1892).

Phytogeography
Bersama abyssinica belongs to the family Melianthaceae; the genus *Bersama* made up of eight species, all found in Africa. There is a wide variability in *Bersama*, this distinction has enabled the identification of many species, subspecies and varieties. The Ivorian species is the paullinioides variety.

Bersama abyssinica is a very common plant in Africa. It is found in West Africa (except in Republic of Benin), the Horn of Africa and southern part of the continent (Angola, Zambia, Zimbabwe and Mozambique).

Our sample of *B. abyssinica* was compared to that of the National Centre of Floristic : Region of Bondoukou, April 7, 1966, No. 8704 Ake-Assi.

Bersama abyssinica is a shrub up to 6 m in height, often with twisted trunk. The leaves,imparipinnately compound, measuring up to 60 cm long, contain from six to nine pairs (or more) of glabrescent leaflets; the spine is winged. The inflorescences, solitary racemes or few, are axillary near the ends of branches. Flowers, white or yellowish, about 2 cm in length. Fruits, reddish, up to 2 cm in length, are dehiscent at maturity. The seeds are surrounded by an orange-red aril[12].

2.2 Bacterial Material

Made up of a reference strain (ATCC 25923) and five multi-resistant strains of *Staphylococcus aureus* obtained from biological products (**Table 1**). They are Gram-positive bacteria (Gram +), spherical, with a diameter of 1 µm, in diplococcior small cluster (cluster of grapes) motionless,spore, not encapsulated. This bacterium is an aero-anaerobic germ respiratory and fermentative metabolism, catalase positive [13].They are provided by the Antibiotics Unit, Natural Substances and Monitoring of Microorganisms for Anti-Infective (ASSURMI) and the Department of Bacteriology and Virology of the Pasteur Institute of Cote d'Ivoire (IPCI).

Table 1: List of strains studied

Strain	*Codes*	*Profile*	*Biological products*
Staphylococcus aureus	ATCC 25923	Sensitive to methicillin	-
	039C/11	Methicillin resistant	Pus
	237YO/14	Methicillin resistant	Pus
	446UB/15	Methicillin resistant	Pus
	524YO/14	Methicillin resistant	Blood
	833UB/14	Methicillin resistant	Blood

3. Methods

In this part, the tests are done in triplicate.

3.1 Preparation of the aqueous total extract (ATE)

One hundred grams (100 g) of stem bark powder were homogenized in 1 liter of distilled water in a blender (mixer) for three times three minutes at room temperature. The homogenate was first squeezed in a square of clean white cloth (the mac was placed again in the blender to repeat the operation, it's an extraction by exhaustion) and then filtered through cotton wool, and finally on Whatman paper. Using an oven set at 50°C, the extraction solvent was removed. The evaporate was recovered in the form of dry powder and constituted the aqueous total extract (ATE) [14] [15].

3.2 Yield Calculation

The yield is the amount of extract obtained from the plant powder. It is expressed as a percentage or without any unit. In practice, it is determined by the ratio of weight of the solids content after evaporation by the weight of the dry powder of the plant material used for the extraction, multiplied by 100. This gives the following formula: Yd = (m x 100) / M. (Yd: Extraction yield in percentage, m: mass in grams of the dry extract, M: mass in grams of the drug powder).

3.3 Sterility test of ATE

This test verified that the extract contained no germ. For this, 0.1 g of the extract was enriched by adding in 10 ml of thioglycholate broth and incubated at 37°C for 24 h. After that, the broth was plated on a Petri dish containing ordinary agar and incubated under the same conditions. The extract is declared sterile, if no colony is visible on the agar box.

3.4 Effectiveness test

The strain sensitivity to aqueous total extract of *Bersama* was performed using the agar diffusion method. Mueller Hinton agar were inoculated with a swab. A total of 4 wells of 6 mm in diameter were then made in the agar, of which 1 served as control well in the center of the agar and containing only sterile distilled water (TS). Each of the three wells received 50 µl of the test substance into the concentrations of 100, 50 and 25 mg/ml (C_1, C_2 and C_3 respectively). After 30 min diffusion at laboratory temperature, the plates were incubated at 37°C for 24 h. The presence or absenceof inhibition zone was observed and measured with a caliper or ruler in millimeters (mm). The results are expressed as the diameter of inhibition zone. Therefore, according to the sensitivity of strain, we have strains that are[16]:

- Not susceptible or resistant: diameter less than 8 mm;
- Susceptible: diameter between 9 and 14 mm;
- Very sensitive: diameter between 15 and 19 mm,
- Extremely sensitive: diameter greater than 20 mm.

3.5 Preparation of the concentration range of plant extracts

The range of concentration of plant extract was prepared in twelve test tubesnumbered T_1 to T_{13} by the method of double dilution in geometrical ratio 1/2. The concentrations ranged from $C_1 = 100$ to $C_{13} = 0.0244$ mg/ml.

3.6 Preparation of the inoculum

The bacterial inoculum was prepared from colonies of less than 18-24 h in Mueller Hinton broth (BMH). A single colony of the bacterial culture was removed using a platinum loop and homogenized in 10 ml broth and incubated for 3 h at 37°C for a pre-culture. After incubation, a volume of 0.3 ml was taken and was added to 10 ml of sterile BMH. This made up bacterial suspension valued at approximately 10^6 cells/ml and constituted 10^0dilusion or the pure inoculum.

3.7 Counting of bacterial inoculum

The counting of the inoculum was performed by dilution from 10 to 10 from the pure inoculum (10^0) until the 10^{-4} dilution. We obtained 4 dilutions 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. These various dilutions and the pure inoculum were inoculated with a calibrated loop of 2 µl per striae of 5 cm long on Mueller Hinton agar and incubated at 37°C for 24 h. This preparation constituted box A.

3.8 Determination of the minimum inhibitory concentration (MIC)

The MIC determination was made using two 96 well microplates for each series. In a series of wells numbered from C_1 to C_7 (first microplate) and C_8 to C_{13} (second microplate) and each of the six strains (S_1-S_6) were added 0.2 ml of pure inoculum of each bacterial strain of *Staphylococcus aureus*. The TS (Sterility Control), contains 0.4 ml of sterile BMH. Then it was added to the wells (S_1 to S_6), 0.2 ml of plant extract according to the prepared concentration range. This distribution of plant extract was made such that 0.2 ml of plant extract (ATE) of 200 mg/ml was transferred into the wells C_1; C_2 wells received 0.2 ml of 100 mg/ml and so on until C_{13} well where 0.2 ml of the solution of 0.0244 mg/ml was added. TC well (Control well) received 0.2 ml of sterile BMH and 0.2 ml of sterile distilled water. Due to the volume / volume dilutionthus formed, the concentration in the wells were halved. Thus, the final concentrations in the wells were evolving C_1 = 100 mg/ml to C_7 = 1.563 mg/ml in the first microplate and C_8 = 0.781 to C_{13} = 0.0244 mg/ml in the second microplate. These plates were incubated at 37 ° C for 24 h.

The MIC (Minimum Inhibitory Concentration) is the lowest concentration of extract for which no bacterial growth was observed after 24 hours of incubation time. It's determination was made by observing the turbidity induced by the growth of studied germs in each tube. The MIC was the lowest concentration value for which there was no germs growth visible to the naked eyes.

3.9 Determination of minimal bactericidal concentration (MBC)

The minimal bactericidal concentration (MBC) is the lowest concentration of antibactrial agent that leaves at most 0.01% of surviving germs. Using a calibrated loop 2 µl, the tube contents in which no germs was observed were collected and seeded on Mueller-Hinton agar starting with the MIC tube. Seeding was done by parallel stripes of 5 cm long on the surface of the agar. This constitute the box B.

After 24 h of incubation in an incubator at 37 ° C the number of colonies on the streaks was compared to those of the box of the inoculum. Thus, the first experimental tube, of which germs count on the streak is less than or equal to 10^{-4} dilution represent the MBC.

3.10 Modalityof ATE action

The MBC / MICration is used to specify the modality of a substance [17].
If the result:

- MBC / MIC ≤ 2, the substance is said to be bactericidal
- MBC / MIC > 2, the substance is said to be bacteriostatic.

4. Results

4.1 Yield for aqueous total extract (ATE)

For hundred grams (100g) of *Bersama abyssinica*powder, 25.3±1.0 grams of ATE was obtained,that is a yield = 25.3±1.0%.

4.2 Sterility test

Sterility tests showed that ATE of stem bark of B. *abyssinica* shows no signs of contamination.

4.3 Effectiveness test

The diameters of the inhibition zones are reported in Table 2. It is noted that ATE had a good inhibitory activity, with different concentrations tested on bacterial strains. Having diameter of inhibition ranging from 11.1±0.1 to 18,0±0.3 mg/ml.

Table 2: Diameters of the zones of inhibition in millimeters (mm)

		Concentrations (mg/ml)			*TC*
Strain	*Codes*	C_1=100	C_2=50	C_3=25	
Staphylococcus aureus	ATCC 25923	15,1±0,1	13,1±0,1	11,2±0,2	6±0,0
	039C/11	15,1±0,2	13,1±0,1	11,1±0,1	6±0,0
	237YO/14	16,1±0,1	14,1±0,0	12,0±0,0	6±0,0
	446UB/15	18,0±0,3	16,0±0,1	14,1±0,1	6±0,0
	524YO/14	15,2±0,3	14,0±0,1	12,1±0,1	6±0,0
	833UB/14	16,0±0,0	14,1±0,2	12,0±0,1	6±0,0

TC witness control

4 Effect of ATE of *B. abyssinica* on multi-resistant strains.
After the incubation time at 37 ° C, increasing concentrations of aqueous total extract have led to a gradual reduction of bacterial growth and a dose-dependent turbidity of the culture medium and that for each bacterial strain studied. The antibacterial parameters values obtained for each bacterial strain are reported in Table 3.

Table 3: Antifungal Parameters

Strain	*Codes*	*MIC (mg/ml)*	*MBC (mg/ml)*	*MBC/MIC*	*Action*
Staphylococcus aureus	ATCC 25923	1,563 ± 0,0	1,563 ± 0,0	1	Bactericidal
	039C/11	0,391 ± 0,0	0,098 ± 0,0	0,251	Bactericidal
	237YO/14	0,195 ± 0,0	0,195 ± 0,0	1	Bactericidal
	446UB/15	1,563 ± 0,0	0,391 ± 0,0	0,250	Bactericidal
	524YO/14	1,563 ± 0,0	0,781 ± 0,0	0,500	Bactericidal
	833UB/14	1,563 ± 0,0	1,563 ± 0,0	1	Bactericidal

5. Discussion

Of antibacterial activity is apparent that all multi-resistant strains of *Staphylococcus aureus* are sensitive to aqueous total extract ofstem bark of *Bersama abyssinica* compared to controls in a dose-response relationship.

We observed progressive increase of the inhibition zone as the concentration of the aqueous total extract increases. The diameters of the zones of inhibition ranged from 11.1±0.1 to 18.0±0.3 mm for multi-resistant strains and from 11.2±0.2 mm to 15.1±0.1 mm for the reference strain ATCC 25923.

An extract is considered to be active when induced an inhibition zone higher or equal to 10 mm[18]. The inhibition zone diameters are all greater than 10 mm, it could be said that the aqueous total extract of the stem bark of *B. abyssinica* is active. The MICs found between 1.563±0.0 and 0.195±0.0mg/ml and MBC varies from 1.563±0.0and 0.098±0.0 mg/ml.

The phytochemical study of the composition of the leaves, stem bark and root bark of *B. abyssinica* revealed the presence of 21 chemical compounds in the stem bark of which 1,2,3-benzenetriol (Pyrogallol) and 2 3-dimethylfumaric acid (Fatty acid) they possessed antibacterial activities[19]. This would justify the antibacterial activity found on multidrug resistant *S. aureus* strains in our study.

Finally, the aqueous total extract of stem bark of *B. abyssinica* showed bactericidal activity against all bacterial strains tested, as the ratio MBC/MIC was less than 2.

6. Conclusion

Our study has shown that the aqueous total extract of the stem bark of *B. abyssinica* hasantibacterial activity. All multi-resistant strains of *Staphylococcus aureus* as well as the reference strain studied were susceptible to the aqueous total extract of the leaves of *B. abyssinica*. ATE exhibit a bactericidal activity on various strains. This study justifies the traditional use of stem bark of *Bersama* in treating skin diseases. From the outcome of our study, the ATEof *B. abyssinica* opens a new path with respect to the search for new natural substances that can neutralize multi-resistant strains. *Bersama abyssinica* is a natural antibiotic.

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