Phytochemical study and antimicrobial activity of bark extracts of *Ceiba pentandra* (L.) Gaertn. (Bombacaceae) from Côte d'Ivoire on resistant bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*

ABSTRACT

One of the health problems in recent decades is the emergence of multi-antibiotic resistant bacteria. Among there solution tracks of this concern, the secondary metabolites of medicinal plants seem to be privileged. In this context, our work is devoted to the phytochemical study and evaluation of antibacterial activity of bark extracts of *Ceiba pentandra* from Ivorian Pharmacopoeia on *S. aureus* methicill in resistant and *P. aeruginosa*imipenem and ceftazidime resistant. The phytochemical study shows that bark of *Ceiba pentandra* harvested in center of Ivory Coastcontain alkaloids, flavonoids, saponins, tannins, steroids,terpenoids and cardiac glycosides. Microbiological testing showed that in liquid medium,ethylacetate (AcOEt)extracted is active on all studies germs. MIC range from 0.78 to6.25 mg /mL and CMB from1.04 to 8.33mg /mL. Purification of AcOEt extract had no influence on his activityagainst studied germs. In fact the most active fraction(F8)has MIC ranging from 0.52 to6.25 mg/ mLandCMB1.04 to 10.42mg /mL. According to the ratio CMB /CMI which was nearly 2, AcOEt extract and fractionF8are bactericidal.

Keywords: *Ceiba pentandra*, Phytochemistry, *Staphylococcus aureus, Pseudomonas aeruginosa,*resistant

1. INTRODUCTION

According to WHO [1], over 80% of the population in Africa still use traditional medicine to meet their health care needs. This appears to be linked to the high cost of modern drugs, distance and / or insufficiency of health units especially in rural areas. Moreover, in hospitals control of bacterial infections is complex due to the emergence of bacteria resistant to many conventional antibiotics. In the literature, many cases of multidrug-resistant bacteria are reported as well in Ivory Coast[2, 3, 4] than in other countries in sub-Saharan Africa [5].*Staphylococcus aureus* and *Pseudomonas aeruginosa*area major concern in hospitals because of their epidemic spread [6]and unfortunately they are a part of microorganisms that acquire the multidrug resistance. Furthermore, the conjunction of the lack of new efficient antibiotics and the growth of multidrug resistance bacteria may lead, in the future, to increase therapeutic impasses. So, it is more than appropriate to promote the development and diffusion of treatments which are able to handle with these threats.

Cote d'Ivoire, with its 761 medicinal species and 1421 drug recipes that have been identified [7] provides investigative options still poorly exploited. Thus, among the plants of the Ivorian Pharmacopoeia, *Ceiba pentandra* (Bombacaceae) widely used at several levels of traditional medicine. Indeed this plant is use to relieve painful conditions such as fevers, abscesses, paronychia, mental illness, conjunctivitis, dizziness, headache etc [8], as well as in the treatment of wounds and ulcers. In Nigeria, Adebayo-Tayo[9] confirmed the antimicrobial activities of *Ceiba pentandra* local.

The present work aims to establish the phytochemical profile of *Ceiba pentandra*from savannah of Côte d'Ivoire and to evaluate the antimicrobial activity of the ethyl acetate extract of his bark against resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

2. MATERIALS AND METHODS

Fresh bark of *Ceiba pentandra* were collected in December 2010 around the Institut National Polytechnique Felix Houphouet-Boigny in Yamoussoukro (central Côte d'Ivoire). This plant was identified by Mr. Amani N'Guessan botanist from the Institut National Polytechnique Felix Houphouet-Boigny. A sample was deposited in the Herbarium of the Institute.

The barks were dried for three weeks out of the sun and then crushed with an electric grinder. The obtained powder was stored in polyethylene bags at 4°C until extraction.

The bacterial strains used for testing were provided by the unit Antibiotics, Natural Substances and Surveillance of Resistance of microorganisms to anti-Infective (ASSURMI) of Bacteriology Department at the Institut Pasteur in Côte d'Ivoire (IPCI). It consists of a strain of:

- *Staphylococcus aureus* methicillin susceptible (*S. aureus Meti S*);
- *Staphylococcus aureus* resistant to methicillin (*S. aureus Meti R*);
- *Pseudomonas aeruginosa* ceftazidime and imipenem susceptible (*P. aeruginosa Cefta S / Imp S*);
- *Pseudomonas aeruginosa* ceftazidime and imipenem resistant (*P. aeruginosa Cefta R / Imp R*).

Referenced Strains of *Staphylococcus aureus*(ATCC 25923) and *Pseudomonas aeruginosa*(ATCC 27853) were also tested.

Before testing, the different bacterial strains were cultured by the method of streaking and incubated in an oven at 37 ° C for 18 to 24 hours to obtain young colonies.

2.1. Extraction and purification of secondary metabolites

In a flask of 3L capacity, protected from light, 1500 grams of crushed bark is macerated at room temperature using 2.5L of hexane for 12 hours. This operation aimed to dewax the powder and it have been repeated twice. After filtration the dried residual powder was exhaustively extracted with ethyl acetate under the same conditions. At each cycle of extraction, the ratio plant material - solvent was maintained at 1: 5 (w / v). The organic phases were filtered through cotton wool and then on Whatman paper N°3. The filtrates were evaporated to dryness at 40°C under reduced pressure using a rotavapor Buchi 161. A 12 g of brown powder was obtained corresponding to 0.80% yield.

A mass of 10 g of extract in ethyl acetate was fractionated on a silica gel column [Merck-Silica gel 60 (0.063-0.200 mm)] eluting with hexane, to ethyl acetate, and methanol in the proportions 100/0/0; 50/50/0; 0/100/0; 0/50/50 (v / v / v). Fractions were collected following the chromatographic profiles and after solvent evaporation, dry products were stored at 4°C under nitrogen until achievement of phytochemicals and antimicrobial tests.

Chemicals used: All chemicals and drugs used were obtained commercially and of analytical grade.

2.2. Evaluation of antibacterial activity

2.2.1. Efficacy of substances

Efficiency testswere used to detect the antimicrobial activity of the substances. For these tests, the Mueller Hinton agar was the main culture medium [10, 11, 12]. For preparing extracts a mixture of DMSO / distilled water in the ratio 1: 1 (v / v) was used. Non-impregnated discs of 6 mm of diameter, purchased from Polychimie were used. The tests were performed on bacterial inoculums of 5.10^6 CFU/ mL.

Each disc was impregnated with 40µL of extract or fractions solutions at 200 mg/mL concentration. The choice of 200mg/mL concentration for this test was literature guided. After drying, the discs were placed on the agar previously seeded with micro bacterial strains and incubated at 37 ° C for 18 to 24 hours [10]. The observation of an inhibition zone reflected the existence of antimicrobial activity. Observation of an inhibition zone can be used to judge the efficiency of substances in extract or fractions. Control tests were carried out using discs impregnated with 40µL of appropriate solvent used to prepare extract or fractions.

To confirm the resistance of bacteria, tests on young colonies using oxacillin (OX-5µg) and cefoxitin (FOX-30µg) for *S. aureus* and the ceftazidime (CAZ-30µg) and imipenem (IMP 10 µg) for *P. aeruginosa* were made under the same conditions.

2.2.2. Antimicrobial screening

A concentration range of plant extract was prepared by the method of double dilution with concentrations ranging from 100.00 to 0.39 mg / mL for ethyl acetate extract and from 50.00 to 0.19 mg/mL for its fractions.

The antimicrobial screening was performed using the method proposed by Oussou [13] and Golly [12]. The tests were performed by introducing into a series of hemolysis tubes 1 mL of the solution of plant extract and 1 mL of bacterial inoculums as described by Moroh [14]. At the same time, in control tube, 1 mL of the solvent used to solve the extract (DMSO / distilled water to 1: 13 v / v) and 1 mL of bacterial inoculums were introduced. All the tubes were incubated at 37°C for 18 to 24 hours.

The results of antimicrobial screening were read looking through at daylight using human eye [15, 16]. The transparency of the tubes indicated the antimicrobial effect of the tested extract, while its turbidity shows its ineffectiveness (a sign of bacterial growth). The Minimum Inhibitory Concentration (MIC) will correspond to the concentration of the extract in the first tube with a clear content.

The minimum bactericidal concentration (MBC) is the lowest concentration of extract that kills at least 99.99% of bacteria in culture. For its determination, the content of control tube was diluted to 10^{-4}, corresponding to 0.01% of survival bacteria in culture. The experimental tubes sowed antimicrobial effect from the CMI's one are transplanted by streaks of 5cm on Mueller Hinton agar and incubated at 37°C for 24 hours. The first experimental tube in which the number of determined germs is less or equal to the dilution concentration (10^{-4}) corresponds to the CMB.

2.3. Phytochemical screening

The phytochemical study of the barks of *Ceiba pentandra*, based on color and / or precipitation tests was carried out on the powder of crushed bark, ethyl acetate extract and the active fraction [17, 18]. The target molecules groups of this screening were saponins, tannins, flavonoids, alkaloids, steroids, terpenoids and cardiac glycosides

3. RESULTS

The tests of efficiency conducted prior to the determination of microbiological parameters of extract at 200 mg/mL give the results summarized in Table 1. The results of the effectiveness tests confirm the resistant and sensitive characters received germs. Moreover, these tests indicate that the extract of the bark of *Ceiba pentandra*, and its fractions exert antibacterial actions on all *S. aureus strains*. According to the inhibitions diameters, fraction F8 appears more effective than the crude extract, fractions F6 and F7 but still less effective than antibiotics. However, no significant action is observed on *P. aeruginosa strains*.

These results highlight the presence in the crude extract and its fractions F6, F7 and F8 of bioactive substances which are able to inhibit the growth of *S. aureus* at 200 mg / mL in a solid medium.

Even if the efficacy tests showed a biological activity at the concentration of 200 mg / mL of crude extracts and fractions only on *S. aureus*, antibacterial parameters were investigated using all studied germs. However we limited the tests with AcOEt extract and fraction F8. The results of this determination are summarized in Tables 2. This Table shows different results from Table 1. In liquid medium crude ethyl acetate extract and fraction F8 have antibacterial activity on all studied germs. This results are conformed to those we have observed wile testing leaves extracts of *Vernonia colorata* on the same strains [12]. The results of this table shows that the purification of total extract does not affect positively the antibacterial activity. Indeed, depending on the bacterial strain, the AcOEt extract exhibits MIC values ranging from 0.78 to 6.25 mg / mL and those of CMB vary from 1.04 to 8.33 mg / mL. The most active fraction (F8) after purification has a substantially similar activity: CMI ranging between 0.52 to 6.25 mg / mL and CMB from 1.04 to 10.42 mg / mL.

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148 **Table 1 Results of effectiveness tests of the extract, fractions and antibiotics**

Bacterial strains	Tested substances and observed inhibition diameter (mm)													149
	Extrait AcOEt	F1	F2	F3	F4	F5	F6	F7	F8	F9	OX (5µg)	FOX (30µg)	CAZ (30µg)	IPM (10µg)
S. aureus Méti S	13,00±1,00	0	0	0	0	0	10,00±1,00	10,67 ±0,58	14,33 ±0,58	0	43	32	-	-
S .aureus Méti R	12,33±0,58	0	0	0	0	0	10,33±0,58	0,00 ±0,00	13,67 ±0,58	0	0	0	-	-
S. aureus ATCC 25923	12,00±1,00	0	0	0	0	0	11,00 ±00	0,67± 0,58	16,00 ±1,00	0	28	30	-	-
P. aeruginosa Cefta S/ImpS	0	0	0	0	0	0	0	0	0	0	-	-	30	30
P. aeruginosa Cefta R/ImpR	0	0	0	0	0	0	0	0	0	0	-	-	0	0
P. aeruginosa ATCC 27853	0	0	0	0	0	0	0	0	0	0	-	-	26	25

150 *Not tested (-); Oxacillin (Ox); Cefoxitin (Fox); Ceftazidime (Caz); Imipenem (Imp).*

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These results could be due to a specific nature, concentration of the actives ingredients, modes of action and the test mode [19]. In addition, the antibacterial activity observed in the liquid medium seems to indicate a poor dissemination of the extract in solid medium [20]. The ratio CMB / CMI of all substances is less than or equal to two (≤ 2). According Marmonier [21], such of ratio indicate that the substances are bactericide. We can therefore conclude that bark extracts of *Ceiba pentandra* have bactericidal activity on all studied germs.

Table 2: Antimicrobial parameters of AcOEt extract and fraction F8

	Crude ethyl acetat extract (AcOEt)			**Fraction F8**		
Bacterial strains	**CMI (mg/mL)**	**CMB (mg/mL)**	**CMB/CMI**	**CMI (mg/mL)**	**CMB (mg/mL)**	**CMB/CMI**
S.aureus Méti S	1,04±0,45	1,04±0,45	1	1,04 ± 0,45	1,56 ± 00	2
S.a ureus Méti R	0,78±00	1,56±00	2	0,78 ± 00	1,56 ± 00	2
***S.aureus ATCC* 25923**	1,04±0,45	1,04±0,45	1	0,52 ± 0,23	1,04 ± 0,45	2
P.aeruginosa Cefta S	6,25±00	8,33±3,61	1	6,25 ± 00	10,42 ± 3,61	2
P. aeruginosa Cefta R	6,25±00	8,33±3,61	1	6,25 ± 00	10,42 ± 3,61	2
***P. aeruginosa ATCC* 27853**	6,25±00	8,33±3,61	1	6,25 ± 00	10,42 ± 3,61	2

To establish the molecular family of active substance against bacterial strains phytochemical screening was undertaken. The results of these analyzes of the crushed dried bark, AcOEt extract and fraction F8 are summarized in Table 3. From this table it is apparent that the bark of this plant contain alkaloids, tannins, flavonoids, saponins, steroids and terpenoids and cardiac glycosides. These results are almost consistent with those reported by Adebayo-Tayo [9]. However, no tannin were found in Nigeria's *Ceiba pentandra.* The presence of these secondary metabolites in bark of *Ceiba pentandra* could justify its use in relieving ailments such as fevers, abscesses, paronychia, mental illness, conjunctivitis, vertigo, headaches, wounds and other skin diseases [8, 9].

If the ethyl acetate extract contains flavonoids, tannins, terpenoids and steroids, the most active fraction (F8) contains only flavonoids, tannins, steroids and terpenes. Therefore, we may apply the antimicrobial activity on the microorganisms studied flavonoids,

tannins and terpenes and steroids. That support that the active substance is quite flavonoid, terpene or annin

Table 3: Phytochemical screening of crushed bark, extract and active fraction of *Ceiba pentandra*

Groupes chimiques	Crushed powder	(AcOEt) extract	Fraction F8
Alcaloïdes	+	-	-
Flavonoïdes	+	+	+
Tanins	+	+	+
Saponines	+	-	-
Stéroïdes et Terpénoïdes	+	+	+
Glycosides cardiaques	+	-	-

Presence (+); Absence (-)

4. CONCLUSION

This microbiological and phytochemical study revealed that the bark of *Ceiba pentandra*of Côte d'Ivoire have a slightly different composition secondary metabolites that of the Nigerian plant.

Ratio values of CMB / CMI indicate that the ethyl acetate extract and its fraction F8 have bactericidal properties on the studies strains. This activity could be attributed to flavonoids, tannins, terpenes or steroids. In future, we plan to identify the actives components of this plant.

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