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

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**ABSTRACT**

One of the health problems in recent decades is the emergence of multi-antibiotic resistant bacteria. Among the solutions to 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 methicillin resistant *S. aureus* and on imipenem and ceftazidime resistant *P. aeruginosa*. The phytochemical study shows that bark of *Ceiba pentandra* harvested in the center of Côte d'Ivoire contains alkaloids, flavonoids, saponins, tannins, steroids, terpenoids and cardiac glycosides. Microbiological testing showed that in liquid medium, ethyl acetate extract is active on all studied bacteria. Minimum Inhibitory Concentration (MIC) range from 0.78 to 6.25 mg/mL and Minimum Bactericidal Concentration (MBC) from 1.04 to 8.33 mg/mL. Purification of ethyl acetate extract had no influence on its activity against the studied bacteria. In fact, the most active fraction has MIC ranging from 0.52 to 6.25 mg/mL and MBC from 1.04 to 10.42 mg /mL. According to the ratio MBC /MIC, which was nearly 2, ethyl acetate extract and his active fraction are 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 service units, especially in rural areas. Moreover, in hospitals the control of bacterial infections is complex due to the emergence of bacteria resistance to many conventional antibiotics. In the literature, many cases of multidrug-resistant bacteria are reported in Côte d'Ivoire [2, 3, 4] as well as in other countries in Sub-Saharan Africa [5]. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are major concern in hospitals because of their epidemic spread [6] and unfortunately, they are a part of microorganisms that acquire the multidrug resistance. Furthermore, a lack of new efficient antibiotics together with the growth of multidrug resistant bacteria may lead, in the future, to increased therapeutic impasses. Therefore, new treatment methods are likely to be developed to deal with these threats.

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

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

## 2. MATERIAL AND METHODS

Fresh barks of *Ceiba pentandra* were collected in December 2010 around the Institut National Polytechnique Felix Houphouet-Boigny in Yamoussoukro (central Côte d'Ivoire).

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 Antibiotics, Natural Substances and Bacterial Anti-Infectious Resistance Monitoring Unit (ASSURMI) of Bacteriology Department at the Institut Pasteur in Côte d'Ivoire (IPCI). They consist of a methicillin susceptible strain of *Staphylococcus aureus* (*S. aureus Meti S*); methicillin resistant

*Staphylococcus aureus* (*S. aureus Meti R*); ceftazidime and imipenem susceptible *Pseudomonas aeruginosa* (*P. aeruginosa Cefta S / Imp S*); and ceftazidime and imipenem resistant *Pseudomonas aeruginosa* (*P. aeruginosa Cefta R / Imp R*). Two reference strains including *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were tested as well. All the different bacterial strains were subcultured twice before testing.

### 2.1. Extraction and purification of secondary metabolites

In a 3L flask, away from light, 1500 grams of crushed bark was macerated at room temperature using 2.5L of hexane for 12 hours. This operation aimed at dewaxing the powder and it was repeated twice. After filtration, the dried residual powder was exhaustively extracted with ethyl acetate under the same conditions. At each extraction stage, 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 dry at 40°C under reduced pressure using a rotavapor Buchi 161. 12 g of brown powder was obtained corresponding to 0.80% yield.

A mass of 10 g of ethyl acetate extract was fractionated on a silica gel column [Merck-Silica gel 60 (0.063-0.200 mm)] with eluent mixture of hexane, 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 phytochemical and antimicrobial tests.

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

### 2.2. Evaluation of antibacterial activity

#### 2.2.1. Efficiency of substances

Efficiency tests were carried out 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 diameter, purchased from Polychimie were used. The tests were performed on bacterial inocula of $5.10^6$ CFU/ mL.

Each disc was impregnated with 40 µL of extract or fraction solution at 200 mg/mL concentration. The choice of 200 mg/mL concentration for this test was literature guided.

After drying, the discs were placed on the agar previously seeded with 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 40 µL 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 et *al* [13] and Golly et *al* [12]. The tests were performed in a series of hemolysis tubes 1 mL of the solution of plant extract and 1 mL of bacterial inoculums as described by Moroh et *al* [14]. At the same time, in the 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 inocula 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 showed its ineffectiveness (a sign of bacterial growth). The Minimum Inhibitory Concentration (MIC) is the lowest concentration of extract in the tube where there is no visible growth observable with the naked eye.

The minimum bactericidal concentration (MBC) is the lowest concentration of extract that kills at least 99.99% of bacteria in culture. For its determining, the content of the control tube was diluted to $10^{-4}$, corresponding to 0.01% of survival bacteria in culture. The experimental tubes limpid contents with MIC were 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 bacteria is less or equal to the dilution concentration ($10^{-4}$) corresponded to MBC.

### 2.3. Phytochemical screening

The phytochemical study of the barks of *Ceiba pentandra*, based on colour 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 gave the results summarized in Table 1. The results of the effectiveness tests confirm the resistant and sensitive characters of the stains for experiments. Moreover, these tests indicated 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 to be more efficient than the crude extract, fractions F6 and F7 but yet less efficient than antibiotics. However, no significant action was observed on *P. aeruginosa strains.*

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

Even if the efficiency 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 stains under study. In this case, the tests were limited to AcOEt extract and fraction F8 and the results are summarized in Table 2.

According to these results, in liquid medium crude ethyl acetate extract and fraction F8 have antibacterial activity on all studied stains. These results are conform to those observed while testing leaves extracts of *Vernonia colorata* on the same strains [12]. The date from this table show 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 MBC vary from 1.04 to 8.33 mg/mL. The most active fraction (F8) after purification has a substantially similar activity: MIC ranging between 0.52 to 6.25 mg/mL and MBC from 1.04 to 10.42 mg/mL.

**Table 1: Results of efficiency tests of the extract, fractions and antibiotics**

| Bacterial strains | Tested substances and observed inhibition diameter (mm) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 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 | 10.00 ±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 | 10.67± 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 |

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

These results can 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 MBC/MIC of all substances is less than or equal to two (≤ 2). According Marmonier [21], such of ratio indicates that the substances are bactericide. We can therefore conclude that ethyl acetate extract of *Ceiba pentandra* has bactericide activity on all studied stains.

Table 2: Antimicrobial parameters of AcOEt extract and active fraction (F8)

| | **Crude ethyl acetat extract (AcOEt)** | | | **Fraction F8** | | |
|---|---|---|---|---|---|---|
| **Bacterial strains** | **MIC (mg/mL)** | **MBC (mg/mL)** | **MBC/MIC** | **MIC (mg/mL)** | **MBC (mg/mL)** | **MBC/MIC** |
| ***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 analyses 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 contains alkaloids, tannins, flavonoids, saponins, steroids and terpenoids and cardiac glycosides. These results are almost conform to those reported by Adebayo-Tayo et *al* [9]. However, no tannin was found in Nigeria's *Ceiba pentandra.* The presence of these secondary metabolites in bark of *Ceiba pentandra* could justify its use in relieving symptoms such as fevers, abscess, paronychia, mental illness, conjunctivitis, vertigo, headache, wounds and some other skin diseases [8, 9].

In fact, ethyl acetate extract and the most active fraction contain flavonoids, tannins, terpenes and steroids. That suppose that the active substance is quite flavonoid, terpene or tannin.

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

| Molecule groups | Crushed powder | (AcOEt) extract | Fraction F8 |
|---|---|---|---|
| **Alkaloids** | + | - | - |
| **Flavonoids** | + | + | + |
| **Tannins** | + | + | + |
| **Saponins** | + | - | - |
| **Terpenoids and steroids** | + | + | + |
| **Cardiac glycosides** | + | - | - |

*Presence (+); Absence (-)*

## 4. CONCLUSION

Phytochemical study revealed that secondary metabolites composition the bark of *Ceiba pentandra* from Côte d'Ivoire is slightly different from Nigerian's one.

As a result of antimicrobial tests, ratio values of MBC/MIC indicate that the ethyl acetate extract and its active fraction have bactericidal properties on the studied strains. This activity could be attributed to flavonoids, tannins, terpenes or steroids.

Further research to identify the active components of this plant may help clarify the antimicrobial effect of its components.


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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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