SCREENING PHYTOCHEMICAL AND ANTI-METHICILLIN RESISTANT (MRSA) ACTIVITY OF 70 % ETHANOLIC EXTRACT FROM THE STEM BARK OF ALBIZIA LEBBECK (L.) BENTH.(FABACEAE)

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ABSTRACT

The emergence of multi-drug resistant strains and limitations of present antimicrobial drugs have led to continuous search for natural products as curative agents for Anti-methicillin resistant infections. The aim of this study was to evaluate antibacterial activity of an ethanolic extract from Albizia lebbeckstem bark against Anti-methicillin resistant. Methods and Results : The methods of dissemination swab on muller-hinton agar and double dilution were used to evaluate the antibacterial activity of 70 % ethanolic extract of stem bark of Albizia lebbeck.All multi-resistant strains of Staphylococcus aureus and the reference strain (ATCC 25923) were sensitive to 70 % ethanolic extract of the stem bark of Albizia lebbeck. The MBC vary from 0,49 mg/mL to 2mg/mL. Also, the phytochemical screening of this extract revealed the presence of Polyphenols, Gallic tannins, Catechin tannins and Flavonoids. These findings confirm that an 70 % ethanolic extract from Albizia lebbeck stem bark inhibited growth of Anti-methicillin resistant at low concentration and could be utilised as an alternative Anti-methicillin resistant agent.

Keywords: Albizia lebbeck, Anti-methicillin resistant, 70 % ethanolic extract, MBC

INTRODUCTION

In the 1950s, methicillin was introduced to treat S. aureus infections. Unfortunately, after several years, resistance of S. aureus to methicillin was discovered. Methicillin is a β-lactam antibiotic that interferes the penicillin-binding proteins needed for synthesis of peptidoglycans for S. aureus. The emergence of MRSA infections cannot be underestimated, as treatments are ineffective and it is associated with increased morbidity, mortality, hospital admissions and healthcare costs. MRSA also shows high resistance rates against tetracycline, clindamycin, cotrimoxazole, rifampicin, macrolides and fluoroquinolones. This antibiotic resistance in pathogenic microorganisms has caused a lot of premature deaths and has become a public health problem worldwide. Many cases of multidrugresistance have been reported in Ivory Coast. Therefore, various studies have been carried out to identify alternative treatments to curb the problem of MRSA resistance, especially the use of natural products. Plants offer a diverse reservoir of biologically active components as potential therapeutic agents. They could also have a significant clinical value in the treatment of infections caused by microbial resistant strains. Thus an ethnobotanical sturdy was conducted in the Haut-Sassandra Region (Ivory Coast), we found Albizia lebbeck, a plant widely used in the treatment of skin diseases. Previous work has shown that Albizia lebbeck is used in the treatment of various disessel: Furuncle,
cough, conjunctivitis, influenza, abdominal tumor, depression. The main objective of this study is to perform a phytochemical screening and to evaluate the anti-methicillin resistant activity of *Albizia lebbeck* stem bark on the in vitro growth of five clinical strains of *Staphylococcus aureus*.

**MATERIAL AND METHODS**

![Image](image1.png)

**Figure 1: Albizia lebbeck (L) Benth. (Fabaceae)**

A: Pod; B: Leaf; C: Flower

**Plant Material**

Going by our ethnobotanical investigation in Haut-Sassandra Region (Ivory Coast), it appears that *Albizia lebbeck* plant is widely used in the treatment of microbial diseases. The stem bar kwas harvested, cut, washed with water and dried under the shade. These dried plant part, using a grinder were then reduced to a fine powder 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 Ivory Coast (IPCI).

**Bacterial Material**

Made up of a reference strain (ATCC 25923) and five multiresistant strains of *Staphylococcus aureus* obtained from biological products (Table 1). 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 Ivory Coast (IPCI).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Codes</th>
<th>Profile</th>
<th>Biological products</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 25923</td>
<td>Sensitive to methicillin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>377 CA/15</td>
<td>Methicillin resistant</td>
<td>Pus</td>
</tr>
<tr>
<td></td>
<td>310 CA/15</td>
<td>Methicillin resistant</td>
<td>Urine</td>
</tr>
<tr>
<td></td>
<td>1541 C/14</td>
<td>Methicillin resistant</td>
<td>Urine</td>
</tr>
<tr>
<td></td>
<td>412 C/14</td>
<td>Methicillin resistant</td>
<td>Pus</td>
</tr>
<tr>
<td></td>
<td>505 PP/15</td>
<td>Methicillin resistant</td>
<td>Pus</td>
</tr>
</tbody>
</table>

**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) 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 Ivory Coast (IPCI).

**Preparation of 70% Ethanolic Extract (70% FE)**

The extract was obtained by dissolving 5 g of TAE in 100 mL of 70% ethanolic (67.2 mL of pure 96% ethanolic for 28.8 mL of distilled water) solution and then homogenised. After decantation and filtration of the alcoholic fraction on hydrophilic cotton and on Whatman filter paper (n°3), the filtrate collected is evaporated in an oven at 50°C. The powder obtained constitutes 70% ethanolic extract (70% EE) [11].

**Effectiveness test**

The strain sensitivity to 70 % ethanolic extract of *Albizia lebbeck* 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 (C₁, C₂, and C₃ respectively). After 30 min diffusion at laboratory temperature, the plates were incubated at 37°C for 24 h. The presence or absence of 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...
• 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.

**Preparation of the concentration range of plant extracts**

The range of concentration of plant extract was prepared in twelve test tubes numbered T₁ to T₁₂ by the method of double dilution in geometrical ratio 1/2. The concentrations ranged from C₁ = 100 to C₁₂ = 1.56 mg/mL.

**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 10⁶ cells/mL and constituted 100 dilution or the pure inoculum.

**Counting of bacterial inoculum**

The counting of the inoculum was performed by dilution from 10 to 10 from the pure inoculum (100) 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.

**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 C₁ to C₁₂ (in microplate) and each of the six strains (S₁-S₆) 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 (S₁ to S₆), 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 (70% EE) of 200 mg/mL was transferred into the wells C₁; C₁₂ wells received 0.2 mL of 100 mg/mL. TC well (Control well) received 0.2 ml of sterile BMH and 0.2 ml of sterile distilled water. Due to the volume / volume dilution thus formed, the concentration in the wells were halved. Thus, the final concentrations in the wells were evolving C₁ = 100 mg/mL to C₁₂ = 1.563 mg/mL in the microplate. The plate was 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.

**Determination of minimal bactericidal concentration (MBC)**

The minimal bactericidal concentration (MBC) is the lowest concentration of antibacterial 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.

**Modality of 70% ethanolic extract action**

The MIC / MBC ration is used to specify the modality of a substance 13.

If the result:
- MBC / MIC ≤ 2, the substance is said to be bacteriosis
- MBC / MIC > 2, the substance is said to be bactericidal

**Phytochemical Screening**

The identification of different chemical compounds in 70% ethanolic was done by tubes-characterisation reactions. This method consists of detecting the different families of chemical compounds that may exist in plant extracts on the basis of characteristic colourations or precipitation reactions 14.

- **Alkaloids characterisation**

The characterisation of alkaloids was made using Bouchard (iodo-iodide) and Dragendorff (tetraiodo potassium bismuthate) reagent. 6 mL of 70% ethanolic extract solution was evaporated to dryness. The residue was taken up in 6 mL of alcohol at 60°C. The filtrate thus obtained was divided into two test tubes. In the first tube, two drops Dragendorff reagent were added. The presence of alkaloids was characterised by observing orange-coloured precipitates. In the second tube, two drops of Bouchard reagents was added. The appearance of reddish-brown colour indicates the presence of alkaloids. A control test was made with quinine.

- **Characterisation of polyphenols**

The polyphenols colorimetry forms coloured precipitate with a solution of ferric chloride(FeCl₃). Thus, one drop of alcoholic solution of 2% ferric chloride and 2 mL of solution of 70% ethanolic extract was added. The formation of blue-black or green colouring more or less darkconfirms to the presence of polyphenols. A control test was performed with a solution ofphenol.

- **Characterisation of flavonoids**

Flavonoids have been characterised by the reaction to cyanidin. Thus, 2 mL of 70% ethanol solution was evaporated to the dry sand bath. The residue thus obtained was mixed with 5 mL dilute hydrochloric acid 2 times. The mixture was collected in a test tube, in which pink-orange or violet colouration will appear. The addition of 3drops of isooamyl alcohol intensifies this colouring and confirms the presence of flavonoids. Analcoholic solution of quercetin was used as a control.
• Tannins characterisation

The Stiasny reagent (Formalin 30%, concentrated HCl 1/0.5) helped to distinguish the catechin tannins (by precipitation) of gallicitannins (by saturation). Tannins cathéchiques: to 10 mg of 70% ethanolic extract, were added 10 mL of Stiasny reagent. The mixture was heated in a water bath at 80°C for 30 minutes. After cooling in a stream of water, observation of precipitate in the form of clear-brown flakes characterises catechin tannins. An alcoholic solution of catechin was used as a control. Gallictannins: For this test, the filtrate obtained from the reaction of catechol tannins characterisation was saturated with sodium acetate. To this mixture was added a few drops of a dilute aqueous solution of FeCl₃ at 1% (approximately 1 mL). The appearance of an intense blue-black colouration indicates the presence of gallictannins not precipitated by Stiasny reagent. An alcoholic solution of gallic acid was used as a control.

• Terpenes characterisation

Sterols and terpenes characterisation was made by the Liebermann-Burchard reaction. To 0.2 g of 70% ethanolic extract, were added 5 mL of ethyl ether, then the mixture was macerated for 30 minutes. The solution obtained after the maceration was filtered and then evaporated to dryness. The residue was then dissolved in 0.5 mL of acetic anhydride. Using a pipette, 2 mL of concentrated sulfuric acid were laid down at the bottom of the test tube without stirring. The appearance of brownish red or purple ring reflects the two liquid contact zone. The upper liquid turns green or purple indicating the presence of sterols and terpenes. A control test was performed with progesterone.

• Coumarins characterisation

For the detection of coumarins, 2 mg of 70% ethanolic extract was added to 2 mL of warm water and then homogenised. The homogenate thus obtained was divided into two test tubes. Thereafter, 0.5 mL of dilute ammonia at 25% was added to the contents of one of the tubes. After observation under UV 365 nm, the presence of fluorescence in the tube where ammoniac was added indicates the presence of coumarins.

RESULTS

Effectiveness test

The diameters of the inhibition zones are reported in figure 2, figure 3 and figure 4. It is noted that 70% ethanolic extract had a good inhibitory activity, with different concentrations tested on bacterial strains. Having diameter of inhibition ranging from 9 ±0.57 to 17.0±0.57 mg/mL.

Figure 2: Dose-response action of 70%ethanolic extract of Albizia lebbeck stem bark on Staphylococcus aureus. Data expressed as mean ± ecart-type (n=3)

Figure 3: Dose-response action of 70%ethanolic extract of Albizia lebbeck stem bark on Staphylococcus aureus. Data expressed as mean ± ecart-type (n=3)

TC: witness control

Effect of 70% ethanolic extract of Albizia lebbeck on multi-resistant strains.

After the incubation time at 37 °C, increasing concentrations of 70% ethanolic 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 2.

Figure 4: Dose-response action of 70%ethanolic extract of Albizia lebbeck stem bark on Staphylococcus aureus. Data expressed as mean ± ecart-type (n=3)
Table 2: Antifungal Parameters

<table>
<thead>
<tr>
<th>Strain</th>
<th>Codes</th>
<th>70 % ethanolic extract (mg/mL)</th>
<th>action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 25923</td>
<td>3.12 ± 0.00, 0.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>377 CA/15</td>
<td>6.25 ± 0.00, 0.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>310 CA/15</td>
<td>3.12 ± 0.00, 0.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1541 C/14</td>
<td>6.25 ± 0.00, 0.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>412 C/14</td>
<td>3.12 ± 0.00, 0.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>505 PP/15</td>
<td>6.25 ± 0.00, 0.0</td>
<td>0.49</td>
</tr>
</tbody>
</table>

bc : Bactericidal

Phytochemical sorting

The phytochemical sorting performed with the extracts of 70% ethanolic extraclass allowed to detect the presence of various chemical groups (Table 3). They are the polyphenols, tannins, and flavonoids, in both 70% ethanol extract.

<table>
<thead>
<tr>
<th>Molecules sought</th>
<th>Sample tested (70 % EE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>Gallic tannins</td>
<td>+</td>
</tr>
<tr>
<td>Catechin tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Terpene / sterol</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
</tr>
</tbody>
</table>

+: presence of the chemical group.; -: absence of the chemical group.70%EE: 70%ethanolic extract

DISCUSSION

Recently, a number of plants have been reported for antimicrobial properties across the world. Several workers have reported that many plants possess antimicrobial properties. In this study all multi-resistant strains of *Staphylococcus aureus* are sensitive to 70% ethanolic extract of the stem bark of *Albizia lebbeck* compared to controls in a dose-response relationship. We observed progressive increase of the inhibition zone as the concentration of the 70% ethanolic extract increases. The diameters of the zones of inhibition ranged from 9 ± 0.57 to 17.0 ± 0.57 mm for multi-resistant strains. A bacterial strain is said to be sensitive to an extract when the inhibition diameter of the extract is between 9 and 14 mm. The inhibition zone diameters are all greater than 9 mm, it could be said that the 70% ethanolic extract of the stem bark of *Albizia lebbeck* is active. The MBC / MIC ratio gives values that are less than or equal to two, so it can be said that the 70% ethanolic extract of *Albizia lebbeck* is bactericidal on the five clinical strains of *Staphylococcus aureus*. This property might be due to direct action of bioactive compounds on membrane resulting in its lysis and cell death. Our results are comparable to those of which showed that *Albizia bernieri*, another species of *Albizia*, had a bactericidal activity on *Staphylococcus aureus*. The phytochemical composition of the stem bark of *Albizia lebbeck* revealed the presence of Polyphenols, Flavonoids and tannins whose antibacterial properties are known.

The antibacterial activity of 70% ethanolic extract of *Albizia lebbeck* is therefore linked to the presence of these molecules. Tannins for example, are known for their ability to inhibit the growth of many microorganisms including bacteria. In addition to this property, the tannins are endowed with astringent and healing power. This provides a scientific rational for the use of this plant in the treatment of skin diseases. The biological properties of these compound justify the antimicrobial activities expressed in this study and to link these activities and the traditional use of *Albizia lebbeck* in the treatment of bacterial diseases.

CONCLUSION

Our study has shown that the 70% ethanolic extract of the stem bark of *Albizia lebbeck* has antibacterial activity. All multi-resistant strains of *Staphylococcus aureus* as well as the reference strain studied were susceptible to the 70% ethanolic extract of the stem bark of *Albizia lebbeck*. 70% ethanolic extract exhibit a bactericidal activity on various strains. This study justifies the traditional use of stem bark of *Albizia lebbeck* in treating skin diseases. From the outcome of
our study, the 70% ethanolic extract of *Albizia lebbeck* opens a new path with respect to the search for new natural substances that can neutralize multi-resistant strains.

**REFERENCES**


**ACKNOWLEDGMENTS**

We thank the Department of Bacteriology and Virology of the Pasteur Institute of Ivory Coast (IPCI), for antibacterial activity analysis and the traditional health practitioners of the Haut-Sassandra Region.


