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Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACTS OF *ABRUS PRECATORIUS* LINN (FABACEAE) ON BACTERIA RESPONSIBLE FOR NOSOCOMIAL INFECTIONS

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Abstract: In this study we evaluated the *in vitro* antimicrobial activity of ethanol extract of the seeds of *Abrus precatorius*Linn, an herb used traditionally to treat infectious diseases. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used for this work. By the diffusion method in solid, our extract was found active in all the selected strains. *S. aaureus* was the most sensitive of the three strains tested with an inhibition zone of 15 mm.Moreover, by the macro method of broth dilution, the plant extract showed bactericidal effects against all the pathogens studied. The greatest activity was manifested on *S. aureus* with a Minimum Inhibitory Concentration (MIC) of 0.75 mg/mL. The sensitivity of the bacteria tested justifies the use of this plant in the treatment of infectious diseases.
Key words: *Abrus precatorius*, ethanolic extract, bactericidal, nosocomial infections

INTRODUCTION

Infectious diseases are a real public health problem. According to Iqbal and Arina[1], they are responsible for 50,000 deaths per day.The discovery of antibiotics in the twentieth century was a revolution in the fight against infectious diseases. Because the antibiotherapy succeeded in saving a very big number of lives to such sign that one believed that the infectious illnesses would be one day all suppressed. But inappropriate use of antibiotics commonly used to treat infectious diseases has led to consequences, the emergence and spread of bacteria resistant to several groups of antibiotics[2,3,4]. The situation is particularly serious concern in hospital where staphylococci and some Gram negative bacilli including *Pseudomonas* are frequently involved in nosocomial infections[5]. The increasing number of treatment failures observed can be justified largely by a loss of effectiveness of available antibiotics[6]. According to these authors, diseases that were thought to have mastered re-emerged in recent decades.
Facing this situation, it is urgent to find new antibacterial substances and broad spectrum of action.Several studies have identified compounds with antimicrobial activity from plants[7,8,9,10]. The use of plants could therefore be an alternative in the search for new compounds to deal with the phenomenon of bacterial resistance[11] especially since they are widely used in the treatment of infectious diseases. Among the plants used in Côte d'Ivoire for their antimicrobial property appears *Abrus precatorius*Linn, a Fabaceae used as an ointment to treat certain skin diseases and cough in infants[12].

This work concerns the evaluation of the antibacterial activity of ethanol extract of *Abrus precatorius* on the *in vitro* growth of three bacteria responsible for nosocomial infections, including *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Plant material
The plant material consists of seeds of *Abrus precatorius*, harvested in the region of Beoumi (central Côte d'Ivoire) between December 2007 and January 2008. The authentication was performed by Professor Ake-Assi, Flora National Centre (CNF) from the University of Cocody-Abidjan Côte d'Ivoire.These seeds are then roasted and then reduced to a fine powder.

Microorganisms
The support consists of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.All these hospital strains were provided by the National Institute of Public Health (INSP).

Preparation of ethanol extract
This extract was obtained using the method described by Zirihi et *al.*[13]. So, 100 g of plant powder were subjected to magnetic stirring for 24 h in one liter (1L) of a solvent mixture of ethanol-water (70%, 30%, v/v) and then filtered through cotton wool. The filtrate obtained is subjected to decantation.After separation of the residue, the aqueous phase is recovered and then dried in an oven at 50°C.The powder obtained after drying is the ethanol extract.

Evaluation of the antibacterial activity
The test of sensitivity of bacteria in our plant extract is produced by the diffusion method in solid medium using Mueller-Hinton medium[14,15,16]. Thus, disks of Whatman paper No 1 (9 mm diameter) previously impregnated with 50 L of the extract at 48 mg/mL were deposited gently on

the surface of an agar medium inoculated with a bacterial suspension of 18 to 24 hours.By the diffusion method, an extract is considered active when it induces a zone of inhibition greater or equal to 10 mm[17].In the same way to the plant extract, a commercial antibiotic gentamicin (100 µg), chosen because of its large spectrum, was used as positive control.After 30 minutes of pre-release, the dishes are incubated at 37°C for 24 hours and then the diameters of any zones of inhibition observed around the disks are measured.

The Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined by the macro method of broth dilution[18].To this end, concentrations of the extract ranging from 48 to 0.18 mg/mL was prepared by the technique of double dilution and then sterilized in an autoclave at 121°C during 15 minutes.Then, in hemolysis tubes, 1 mL of each concentration of the extract is inoculated with 1 mL of an inoculum from 18 to 24 hours with 5.10^6 densities.Two tubes, one of which, without extract, used as growth control and the other without a germ, a witness of infertility, are prepared. All tubes are incubated at 37°C for 24 hours and then we determine the MIC (defined as the lowest concentration that inhibits any growth visible to the naked eye within 24 hours).From the MIC, the lowest concentration that leash to survive more than 0.01% of bacteria suspension within 24 hours of departure is the CMB. It is determined by plating on solid medium with 0.1 ml of each tube concentration greater or equal to the CMI.

Finally, the MBC/MIC ratio was used to determine the antibacterial powers of our extract. According to Berche et *al*.[19],when this ratio is greater than 4, the extract is considered bacteriostatic and bactericidal when it is greater or equal to 4.

Determination of phytochemical constituents

The extract was subjeted to standard phytochemical analyzes for different constituents such as alkaloids, tannins, flavonoids, triterpenoids, anthraquinones, glycosides, steroids and sterol as described by Rahmi et *al*.[20].

Statistical analysis

The tests were performed in triplicate and data were analyzed by analysis of variance test (ANOVA) followed by least significant difference test (LSD)

RESULTS AND DISCUSSION

In this study, we evaluated the antibacterial activity of ethanol extract of *Abrus precatorius* on the *in vitro* growth of three bacteria potentially involved in nosocomial infections. Analysis of the obtained results shows significant antibacterial activity of our extract of all strains tested. Based on the diameters of inhibition zones, there is a greater sensitivity of *S. aureus* (15±0.3 mm) compared to *P. aeroginosa* (11±0.2 mm) and *E. coli* (11±0.1 mm) (Table 1).Similarly, considering the CMI, *S. aureus* appears to be the most sensitive strain with a MIC value equal to 0.75 mg/mL. *E. coli* and *P. aeroginosa* have similar sensitivities opposite to our extract because they have identical MIC values (3 mg/mL) (Table 2).

Table 1: Diameter of zones of growth inhibition of tested strains (mean ± S.E.M., n = 3)

Inhibition zone diameter (mm)		
Strains tested	Plant extract	Gentamicin (100 µg)
E. coli	11±0.1	23±0.2
S. aureus	15±0.3	27±0.1
P.aeruginosa	11±0.1	24±0.1

Table 2: Parameter values of antibacterial ethanol extract of *Abrus precatorius* on the strains tested

Bacterial germs	Antibacterial parameters (mg /L)			
	MIC	MBC	MBC/MIC	antibacterial effect
E. coli	3,00	12,00	4	Bactericidal
S. aureus	0,75	0,75	1	Bactericidal
P.aeruginosa	3,00	6,00	2	Bactericidal

MIC: Minimum Inhibitory Concentration, MBC: minimal bactericidal concentration

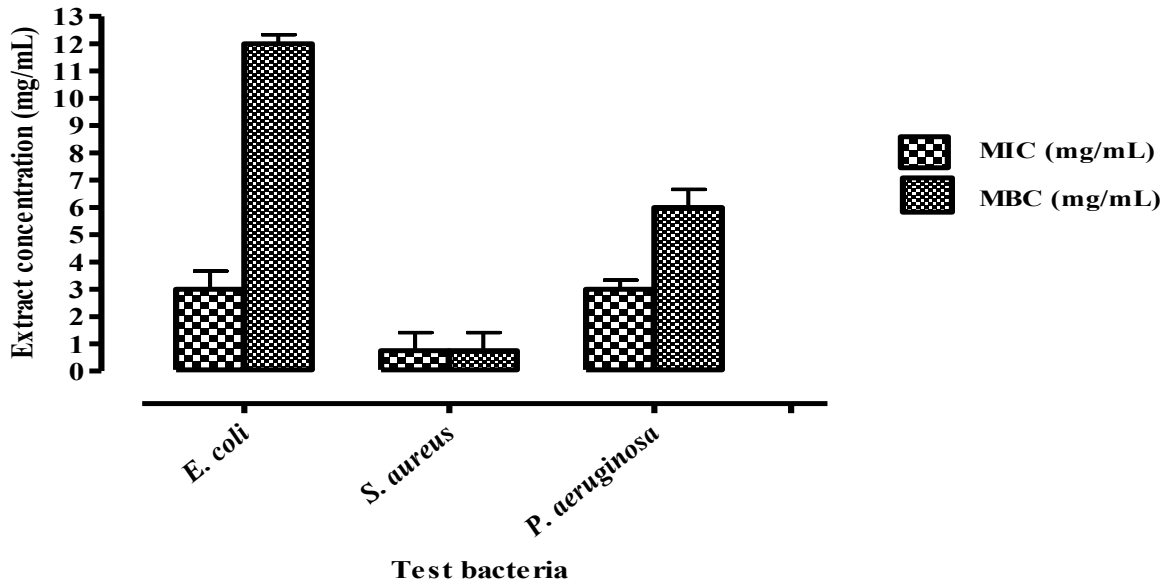

Figure 1: Minimal Inhibition concentration (MIC) and minimal bactericidal concentration (MBC) of ethanolic extract of *Abrus precatorius* against the test bacteria

Table 3: Phytochemical constituents of ethanolic extract of *Abrus precatorius* seeds

Phytochemical constituents	Ethanolic extract
Alkaloids	++
Glycosides	-
Steroids and sterols	+
Triterpenoids	+++
Flavonoids	++
Tannins and phenolic compound	+
antraquinone	-

Key : - (absent) ; + (present in low concentration) ; ++ (present in moderate concentration) ; +++ (present in high concentration).

The choice of the ethanol extract was prompted by the work of Bolou et *al.* that proved that this extract is more active than the aqueous total extract[12]. The diameters of inhibition zones obtained ranged from 11 to 15 mm. An extract is active when it induces a zone of inhibition greater or equal to 10 mm[17]. We can assert that all tested germs are sensitive to our extract. The greatest sensitivity was observed on *S. aureus* with 15±0.3 mm diameter. However, for all strains studied, the diameters of inhibition induced by this ethanolic extract of *Abrus precatorius* are inferior to those of the reference antibiotic, gentamicin, which varied between 23 and 24 mm.

Moreover, the evolution of the sensitivity of germs according to the diameters of inhibition zones is almost similar to the one determined with the help of the CMI and CMB. Indeed, the lowest values of bacterial parameters were obtained with *S. aureus* (MIC=0.75 mg/mL, MBC=0.75 mg/mL), which makes this strain, the most sensitive.Work achieved by Aibinu *et al.* with various extracts of *A. precatorius* has reached the same conclusion[21].

The greatest resistance is presented by *E. coli* with MIC values and MBC respectively 3.00 mg/mL and 12.00 mg/mL. Between these two extreme values, we obtained intermediate values with *P. aeruginosa* (MIC = 3.00 mg/mL, MBC = 6.00 mg/mL). Thus, the sensitivity of our extract decreases in the following order: *S. aureus* > *P. aeroginosa* > *E. coli* (Figure 1). These results agree with those of Parekh and Chanda[22] who showed that bacteria Gram positive are more sensitive to plant extracts than Gram negative bacteria. According to these authors, the difference in sensitivity between strains could be explained by differences in the structure and chemical composition of their walls and at the membrane permeability. The results of chemical analysis of our sample are given in Table III. They reveal the presence of compounds such as alkaloids, tannins, flavonoids whose antimicrobial properties have been demonstrated by several researchers[17,23,24].Also considered molecules and antistaphylococcal antibacterial such as pectin, cycloartenol and gallic acid were extracted from seeds of *Abrus precatorius*[25].Thus, the antibacterial activity observed in our sample could be due to a combined action of these molecules revealed.

The table of parameter values obtained with antibacterial ethanol extract of *Abrus precatorius* shows activity reports MBC/MIC less or equal to 4 for all strains studied. According Berche et *al.* [19], when the activity report MBC/MIC of an antibacterial substance is less or equal to four (≤4), the latter is described as bactericidal substance and if greater than four (>4), then it is called bacteriostatic.

Thus, *Abrus precatorius* has a bactericidal action on all the strains studied. This work confirms the antibacterial activity of *Abrus precatorius* seeds already described by other authors like Desai and Sirsi[26] and Saxena and Vyas [27].

In addition to nosocomial infections, *S. aureus*, *E. coli* and *P. aeroginosa*, bacteria are responsible for various other diseases including diarrhea, urogenital infections and dysentery syndromes[17.] Inhibition of growth of these bacteria can justify the different traditional uses of *Abrus precatorius*.

CONCLUSION

This work allowed us to demonstrate the antibacterial properties of the ethanol extract of *Abrus precatorius* on bacteria involved in many infections such as diarrhea, urogenital infections and nosocomial infections. This extract has a bactericidal effect on all strains tested.

These results justify certain ethnopharmarcological uses of *Abrus precatorius*. They show that this plant could be used as phytomedicin to treat the pathologies in which the tested germs are implied.

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