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ANTIBACTERIAL ACTIVITY OF VITEX DONIANA (VERBENACEAE) BARK EXTRACTS AGAINST TWO BACTERIAL STRAINS PRODUCING OF β-LACTAMASES AT EXTENDED SPECTRUM (ESBL)

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ABSTRACT

Vitex doniana has been reported to be used in treatment of various diseases. *The aim of our study was to evaluate the antibacterial properties of various extracts from the bark of V. doniana on Escherichia coli and Klebsiella pneumoniae, two strains of beta-lactamase producing extended spectrum (ESBL). The method of wells in the agar was used to test the sensitivity of bacterial strains while the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by the dilution method in liquid medium* associated with the spread on gelose. *The methanolic, and ethyl acetate extracts gave inhibition zone diameters between 11 and 19 mm on the strains tested. Moreover, these extracts showed bactericidal powers on E. coli ESBL and K. pneumoniae ESBL.* Methanolic extract recorded an MBC of 142.84 mg/mL on both germs. As for ethyl acetate extract, the MBCs obtained were 6.25 mg/mL (*E.coli* ESBL) and 12.5 mg/mL (*K. pneumoniae* ESBL). These results justify certain ethnopharmarcological uses of *V. doniana*.

KEYWORDS: *Vitex doniana*, bactericidal, *Escherichia coli* ESBL, *Klebsiella pneumoniae*, ESBL.

INTRODUCTION

Vitex doniana is commonly called black plum. The genus Vitex has 250 species.[1] This plant has been reported to be used in treatment of various diseases.[2] The authors further quoted the work done on the *V. doniana*, such as hot aqueous extracts of the leaves being used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhea and dysentery.[3,4] The roots and leaves are also used in curing nausea, colic and epilepsy.[5,6] The use of *V. doniana* stem bark extract in treating hypertension has also been reported.[7] The extracts of the stem bark have also shown effectiveness in trypanocidal activity against *Trypanosoma bruceiin* vitro.[8] Again, aqueous methanol extract has been documented to demonstrate anti-diarrhoeal activity.[9] The bark extract of this plant has been used in treating stomach complaints and kidney troubles.[10] Locally, dried and fresh fruits are eaten against diarrhea and as source of vitamin A and B.[11, 12] Therefore, our first work on this plant on methicillin-resistant S. aureus (SRM) has shown that it has anti-staphylococcus properties.[13] The purpose of this study was to investigate the effect of *Vitex doniana* used by the population to cure various diseases by screening their potential to inhibit *two strains producing of β- lactamases at extended spectrum* (*E. coli* ESBL and *K. pneumonia* ESBL).

MATERIALS AND METHODS

Plant material

The barks of *Vitex doniana* were collected in January 2010 in the north of Côte d'Ivoire in the village of Lataha (Korhogo). The plant was identified and authenticated by the Professor Ake-Assi, National Center Floristic (CNF) University Félix Houphouët-Boigny of Cocody-Abidjan where a sample is retained.

Bacterial strains

The bacterial carrier used in this study is composed of a strain of *Escherichia coli* (No 150C/12) and a strain of *Klebsiella pneumoniae* (No 141C/12), all both beta-lactamase producing extended spectrum (ESBL). They were provided by the Department of Bacteriology and Virology, Institut Pasteur de Côte d'Ivoire (IPCI).

Extract preparation

The *Vitex doniana* s bark harvested were washed, cut, dried in sunlight for two weeks and made into powder using a grinder type IKAMAG. These powders were used to prepare various extracts. Indeed, according to the methods described by Guede-Guina et al.[14], 100 g of plant powder were macerated in 1 L of distilled water and homogenized under magnetic stirring for 24 hours at 25°C using a magnetic stirrer RCT-type IKAMAG. The homogenate obtained was filtered successively twice cotton wool and once on Whatman paper No 2. The volume of the filtrate obtained is first reduced by means of a rotary evaporator Büchi type with the temperature of 60°C. Then the rest of the filtrate is evaporated using an oven type Med Center Venticell at 50°C to give a powder which is the total aqueous extract (Etaq). The same operation was performed using in place of distilled water a 70% ethanol or methanol or ethyl acetate to obtain respectively 70% ethanolic extract (Eeth70%), methanolic extract (Emet) or ethyl acetate extract (Eace).[15] All plant crude extracts thus formed are kept refrigerated until used for testing antibacterial.

Antibacterial assay

Antibacterial activity of different extracts was performed with Mueller Hinton (Biorad, France) agar by well plate method.[16] Nutrient agar plates were seeded with overnight cultures of the test organisms. Wells, 6 mm wide, were cut in the agar plates with cork borer and 80 µL of extracts at 200 mg/mL were pipetted and carefully added. A control well for each bacterial strain was added with 80 µL of mixture containing DMSO/sterile distilled water (V/V). The nutrient agar plates were incubated right side up at 37°C for 24 hours. Oxacillin (5 µg) and Cefoxitin (30 µg) were used as standards antibiotics. After incubation, the action of the extracts is determined by measuring an area of growth inhibition (lack of colonies) around the well.The zones of inhibition were then measured and the mean of two replicates recorded.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Dilution method in liquid medium was used to determine these antimicrobials parameters. Thus, in a series of 10 hemolysis tubes numbered C1 to C10 was introduced 1 mL of the bacterial inoculum containing 5.10^6 cfu. Then 1 mL of a plant extract well known concentration as the concentration range was added prepared in the same tubes. This distribution of plant extract is made so that 1 mL of plant extract 100 mg/mL is transferred to

the tube C1, the 50 mg/mL in the tube so C2 to C9 tube receive 1 mL of plant extract 0.39 mg/mL. The C10 has been tube, instead of plant extract, 1 mL of DMSO/distilled water (1/13, V/V) was used as control. This distribution of plant extract concentration is well known to each tube containing 1 mL of inoculum already reduced the concentration of the plant extract in the middle half. Tube and the concentration of C1 increased from 100.00 mg/mL to 50 mg/mL, 50 mg/mL to 25 mg/mL for C2 so on until a concentration of 0.19 mg / mL for T9. This experiment was performed identically for each extract tested. The nine (9) First tubes (C1 to C9) are called "experimental tubes" and the last tube (C10) is rated "growth control tube or TC." These loaded tubes are incubated at 37°C for 24 hours. The experiment was done three times. The MIC is the concentration of the first tube where it finds no trouble visible to the naked eye. From the MIC, the lowest concentration that leaves no more than 0.01% survival of bacteria suspended starting 24 hours corresponds to the MBC. It is determined by plating on solid medium 0.1 mL of each tube at a concentration greater than or equal to the MIC.[17]

RESULTS AND DISCUSSION

Table 1 shows that aqueous and hydroalcoholic extracts (Eeth 70%) were not active on the bacterial strains tested for an extract concentration at 200 mg/mL. Indeed, no inhibition diameter was observed at this concentration. It could be said that these two extracts could not extract the essential active ingredients that can inhibit these bacteria. In addition, standard reference antibiotics also had no effect on these β-lactamase producing strains.

Only, the methanolic extract and the ethyl acetate extract induced inhibition diameters on the bacterial germs studied. On E. coli strain ESBL, the inhibition diameters were 13 mm (Emet) and 19 mm (Eace). *K. pneumoniae* recorded inhibition diameters of 11 mm and 15 mm respectively with Emet and Eace. The bactericidal activity of Emet and Eace is related to the nature of these chemical compounds. Several studies have already revealed several groups of secondary metabolites in the various extracts of Vitex doniana.[13,18,19]

Table 1: Diameters of inhibition of total extracts of stem bark of *Pericopsis laxiflora.*

bacterial strains	Diameter of the inhibition zones (mm)						
	Etaq	$Eeth_{70\%}$	Emet	Eace	Ox	Fox	C
E. coli ESBL	-	-	13	19	-	-	-
K. pneumoniae ESBL	-	-	11	15	-	-	-

Etaq: aqueous total extract, Eeth70%: 70 % ethanolic extract, Emet: methanolic extract, Eace: ethyl acetate extract, C: Control (DMSO/distilled water 0.5: 0.5; V/V). Ox: oxacillin (5 µg), Fox: cefoxitin (30 µg);- : No zone of inhibition.

The antbacterial parameters of the extracts of *Vitex doniana* studied against the different strains tested are presented in table 2. No antibacterial parameters were determined with aqueous extracts (Etaq) and hydroalcoholic extracts (Eeth70%) on both bacteria up to a concentration of 200 mg / mL. Also, the values of the MIC agree with that of the diameters of the inhibition zone growth because the extracts that have induced the largest diameter of inhibition have presented the smallest values of MIC on the corresponding strains.

Emet recorded an MBC of 142.84 mg / mL on both germs. Concerning Eace, the MBC obtained was 6.25 mg / mL (E. coli ESBL) and 12.5 mg / mL (K. pneumoniae ESBL). In addition, these two extracts were bactericidal for these strains for MIC / MBC ratios between 1 and 2.

Indeed, the MBC/MIC ratio was used to determine the bactericidal or bacteriostatic different extracts. According, Berche et al.[20], when this ratio is less than or equal to 4, the extract has a bactericidal and bacteriostatic when thisratio is greater than 4.

Similar results were obtained with Kouadio et al.[21] These authors demonstrated that the metanolic extract of the leaves of Spondias mombin (Anacardiaceae) had bactericidal power against two strains of *E. coli* (*E. coli* 478C/14, *E. coli* 533C/14) with MBCs between 0.78 mg/mL and 1.56 mg/mL.

The methanolic extract obtained by these same authors was bactericidal at 1.56 mg/mL against the strains of *K. pneumoniae* 421C/14 and *K. pneumoniae* 486Y/14, two germs also producing β-lactamase. These results confirm that the antimicrobial activities of some secondary metabolites of some plants depend on many factors such as the extract origin, the extraction method, the solvent nature, the concentration in active compounds, the nature of the tests applied as well as the strain tested.

Table 2: Antibacterial parameters comparing total extracts of stem bark of *Pericopsis laxiflora* on the in vitro growth of the tested germs.

bacterial strains	antibacterial parameters (mg/mL)				
	Extracts	**MIC**	**MBC**	**MBC/MIC**	**Antibacterial effect**
E. coli ESBL	Etaq	>200	nd	nd	nd
	Eeth $_{70\%}$	>200	nd	nd	nd
	Emet	71,42	142,84	2	Bactericidal
	Eace	6,25	6,25	1	Bactericidal
K. pneumoniae ESBL	Etaq	>200	nd	nd	nd
	Eeth $_{70\%}$	>200	nd	nd	nd
	Emet	71,42	142,84	2	Bactericidal
	Eace	12.5	12.5	1	Bactericidal

Etaq: aqueous total extract, Eeth 70%: 70% ethanolic extract, Emet: methanolic extract, Eace: ethyl acetate extract, nd: not determited.

CONCLUSION

This work allowed us to demonstrate the antibacterial properties of different extracts of *V. doniana* on bacteria involved in many infections such as diarrhea, urogenital infections and nosocomial infections.

These results justify certain ethnopharmarcological uses of *V. doniana*. 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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