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Original Research Article

Study of the antibacterial activity of aqueous and ethanolic extracts of the stem bark of *Erythroxylum emarginatum* (Erythroxylaceae)

ABSTRACT

The aim of our work was to evaluate the *in vitro* antibacterial activity of aqueous and ethanolic extracts of the stem bark of *Erythroxylum emarginatum*, a plant used by the Ivorian population to treat various infections, it effects on the following bacteria (5) strains, *Escherichia coli, Klebsilla pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Streptococcus pneumoniae,* were assesed. The choice of these bacterial strains was motivated by their frequency in Community-Acquired Pneumonia (CAP) in Africa and their natural resistance to various types of antimicrobial agents. The methods used for the evaluation of the antibacterial activity were the method of macrodilution in a liquid medium and the diffusion method in agar medium. At the end of our study we determined the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts tested. This study allowed us to demonstrate the bactericidal action of the aqueous and ethanolic extracts of the stem bark of *Erythroxylum emarginatum* and to confirm their use in treating bacterial infections.

Key words: *Erythroxylum emarginatum*, MIC, MBC, infections, bacteria

1. INTRODUCTION

The aim of our study was to evaluate the *in vitro* antibacterial activity of the aqueous and ethanolic extracts of *Erythroxylum emarginatum* on five bacterial strains, they are *Escherichia coli, Klebsilla pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pneumoniae*. To evaluate the antibacterial activity of the aqueous and ethanolic extracts of *Erythroxylum emarginatum* we determined the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts tested. Each experiment was repeated three times.

2. MATERIAL AND METHODS

Material

Plant material

UNDER PEER REVIEW

The plant material consisted of bark of stems of *Erythroxylum emarginatum* harvested in Kami in 2013 in the Bouaflé region, a town in the central west of Cote d'Ivoire, 302 kilometers from Abidjan and identified by the National Floristic Center of the Félix Houphouët Boigny University.

The bacterial strains:

The bacterial strains used were provided by the Center for Research on AIDS and opportunistic Infections (CEDReS) at the University Teaching Hospital of Treichville in Côte d'Ivoire (Table 1). These strains are all clinical strains.

Method

Preparation of extracts

We have used the extraction method developed by Guédé-Guina *et al.* on 1993 [7], the plant parts were dried under the shadeat ambient temperature for two weeks and then sprayed using an electric grinder IKA-MAG® model. Then one hundred (100) grams of this powder was added to one (1) liter of distilled water. The mixture obtained was homogenized in a magnetic stirrer for 24 hours. The homogenate obtained was filtered twice on hydrophilic cotton wool and then once on wattman paper successively. The collected filtrate was then heated in an oven at 500 ° C. The powder obtained was the total aqueous extract used in the preparation of the various concentrations of the drug. The ethanolic extract was prepared using the same procedure as that of aqueous extract but with700 ml of ethanol and 300 ml of water.

Evaluation of antibacterial activity

The antibacterial activity of the aqueous and ethanolic extracts of *Erythroxylum emarginatum* was evaluated using the agar disk diffusion method as described by Bauer *et al. on* 1966 [2] and taken up by Barry *et al.* on 1985 [3].

From young colonies of 18 to 24 hours, a bacterial suspension was made with the sterilized distill water for each strain. The turbidity of this suspension was adjusted to 0.5 Mcfarland and then diluted to 1/100. An inoculum estimated at 10^6 colony forming units per milliliter (cfu / ml) was then obtained. This inoculum was seeded by flooding on Petri dishes containing Mueller-Hinton agar [8]. The discs impregnated with the various extracts were then delicately deposited on the surface of the agar. The same applies to ciprofloxacin discs (reference antibiotic). The Petri dishes were first left at room temperature for pre-diffusion of the substances and then incubated at 37 ° C. in an oven for 24 hours [1].

Strain Confirmation:

For each of the strains a pre-enrichment was performed on the selective isolation medium and then a Gram staining was performed [6].

Antibacterial susceptibility activity:

UNDER PEER REVIEW

The macrodilution method in liquid medium was used to determine the parameters of inhibition of bacterial growth (MIC, MBC) in active extracts. Antibacterial activity is determined by measuring the diameter of the inhibition zone around each disc.

Minimum Inhibitory Concentration (MIC): dilutions were made progressively as follow 1/2, 1/4, 1/8, 1/16, 1/32, 1/50, 1 / 64, 1 / 80, 1/128.

Minimum Bactericidal Concentration (MBC): The nutrient agar in Petri dishes was streaked seeded with 100 µl of the contents of the tubes having a concentration ≥ MIC in the preceding dilution series.

MBC was determined after incubation at 37 ° C. for 24 hours in an oven. It is the smallest concentration that totally inhibits bacteria growth.

The antibacterial effect was judged bactericidal or bacteriostatic based on the result of MBC / MIC ratio.

If the result of the ratio MBC / MIC = 1 to 2, the effect is bactericidal and if the ratio MBC / MIC = 4 to 16, the effect is bacteriostatic [4].

3. RESULTS AND DISCUSSION

Our results showed that the aqueous and ethanolic extracts of *Erythroxylum emarginatum* inhibited the growth of all the bacteria studied (Table 2). Ciprofloxacin (reference molecule) inhibited only three of the five bacteria studied (Table 2).

The MBC / MIC ratios showed that the aqueous and ethanolic extracts of *Erythroxylum emarginatum* had bactericidal effect on all the bacteria studied (Table 3).

Our results agree with those of De wet on 2012 [5] that methanolic, acetonic, and aqueous leaf and bark extracts tested on four pathogenic bacteria *Bacilus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* to demonstrate the anti-infectivity potential of *Erythroxylum emarginatum*.

However, the efficacy of the extracts varies depending on the bacteria studied, the ethanolic extract of *Erythroxylum emarginatum* is more active on *Escherichia coli* compared to the aqueous extract of *Erythroxylum emarginatum* (Table 3).

TABLE 1: The selected bacterial strains

Microbial groupe	***Strains tested***	***Source of the strains***
Gram-negative Bacteria	*Escherichia coli*	Urine
Gram-negative Bacteria	*Klebsilla pneumoniae*	Urine
Gram-negative Bacteria	*Pseudomonas aeruginosa*	Pus
Gram-positive Bacteria	*Staphylococcus aureus*	Cerebro spinal fluid
Gram-positive Bacteria	*Streptococcus pneumoniae*	Pus

UNDER PEER REVIEW

TABLE 2 : Determination of the inhibition diameters of the aqueous and ethanolic extracts of *Erythroxylum emarginatum*

Bacteria strain studied	***Inhibition diameter (mm)***		
	Aqueuos extract	Ethanolic extract	Ciprofloxacine
Escherichia coli	22	22	00
Klebsilla pneumoniae	10	12	00
Pseudomonas aeruginosa	19	18	23
Staphylococcus aureus	21	23	18
Streptococcus pneumoniae	20	25	10

TABLE 3 : Determination of MIC and MBC of Aqueous and Ethanolic extracts Of *Erythroxylum emarginatum*

Studied bacteria strains	***Aqueous extract***			***Ethanolic extract***		
	MIC (mg/ml)	**MBC (mg/ml)**	**Ratio MIC/MBC**	**MIC (mg/ml)**	**MBC (mg/ml)**	**Ratio MIC/MBC**
Escherichia coli	12.50	12.50	1	3.125	3.125	1
Klebsilla pneumoniae	12.25	12.25	1	12.50	12.50	1
Pseudomonas aeruginosa	6.25	6.25	1	6.25	6.25	1
Staphylococcus aureus	1.56	1.56	1	0.78	1.56	2
Streptococcus pneumoniae	25.00	25.00	1	25.00	25.00	1

CONCLUSION

The aqueous and ethanolic extracts of *Erythroxylum emarginatum* both have good antibacterial activity. The two extracts have a bactericidal effect on the bacteria studied: *Escherichia coli, Klebsilla pneumoniae, Pseudomonas aeruginos, Staphylococcus aureus* and *Streptococcus pneumoniae*. These

UNDER PEER REVIEW

extract sare more efficient than Ciprofloxacin (reference molecule). This study justifies the uses of aqueous and ethanolic extracts of *Erythroxylum emarginatum* to treat infections by the populations.

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