



Study of the Antibacterial Activity of Aqueous and Hydroethanolic Extracts of the Stem Bark of *Erythroxylum emarginatum* (Erythroxylaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Author KKV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FNBY, OAT, YHF and GKG managed the analyses of the study. Author GKG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this work was to evaluate the in vitro antibacterial activity of aqueous and hydroethanolic extracts of the stem bark of *Erythroxylum emarginatum* commonly used by the Ivorian population to treat various infections.

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Bactericidal efficacy evaluated on strains such as *Escherichia coli*, *Klebsilla pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumonia*.

The choice of selection of these bacterial strains due to high incidence as commonly acquired pneumonia in Africa and development of resistance. The methods used were the method of macrodilution in a liquid medium and the diffusion method in agar medium. Study results evinced minimum inhibitory concentration of aqueous and hydroethanolic extracts of *Erythroxyllum emarginatum* on bactericidal action.

This screening study demonstrated the use of stem bark extracts of *Erythroxyllum emarginatum* emphasize the alternate use for bacterial infections.

Keywords: *Erythroxyllum emarginatum*; hydroethanolic extract; MIC; MBC; infections; antibacterial.

1. INTRODUCTION

Erythroxyllum emarginatum is used to treat asthma and this disease is often associated with infections of the respiratory tract that is what motivated our study. The aim of our study was to evaluate the in vitro antibacterial activity of the aqueous and hydroethanolic extracts of *Erythroxyllum emarginatum* on five bacterial strains, they are *Escherichia coli*, *Klebsilla pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. The germs studied are the most commonly found in pneumonia in Africa and are generally resistant to the antibiotics used. The use of plant extracts could be a safe alternative. *Erythroxyllum emarginatum* is a shrub or small tree of six (6) meters high, distributed in tropical regions of South America, Africa, and Madagascar. The leaves are alternate, oblanceolate, and elliptical. The flowers are in small axillary clusters, white, fragrant. The fruit is a drupe ellipsoid, fleshy and red [1]. *Erythroxyllum emarginatum* belongs the vegetal reign, the Sub-reign of Tracheobionta (vascular plants), the Super-division of Spermatophyta (seed plants), the Division of Magnoliophyta (flowering plants), the Class of Magnoliopsida (dicotyledons), the Sub-Class of Rosidae, the Order of Linales, the Family of Erythroxylaceae, the Genus of *Erythroxyllum*, and the Species of *emarginatum* [2,1].

The decoction of leaves is used to treat asthma, arthritis and influenza and kidney problems and reproduction. The leaves and roots of the plant are used to relieve pain. In general, this plant is known for its anti-inflammatory properties and its use in the treatment of bronchial and respiratory affections [3]. To evaluate the antibacterial activity of the aqueous and hydroethanolic extracts of *Erythroxyllum 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. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

The plant material used was bark of stems of *Erythroxyllum 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.



Fig. 1. Stems bark powder of *Erythroxyllum emarginatum* (Kouakou, 2015)

2.1.2 The bacterial strains

The bacterial strains used were obtained by the Center for Research on AIDS and opportunistic Infections (CEDReS) at the University Teaching Hospital of Treichville in Côte d'Ivoire (Table 1) and isolated stains from clinical samples.

2.2 Methods

2.2.1 Preparation of extracts

We have used the extraction method developed by Guédé-Guina et al. on [4], the plant parts

were dried under the ambient temperature for two weeks and then sprayed using an electric grinder IKA-MAG® model. The weight of one hundred grams of this powder was added to volume of one liter 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 40°C. The powder obtained was the total aqueous extract used in the preparation of the various concentrations of the drug. The hydroethanolic extract was prepared by the similar procedure adapted for aqueous extract where volume of one litre of 70 percent ethanol used in place of distilled water.



Fig. 2. Aqueous extract of *Erythroxyllum emarginatum* (Kouakou, 2015)



Fig. 3. Hydroethanolic extract of *Erythroxyllum emarginatum* (Kouakou, 2015)

2.2.2 Evaluation of antibacterial activity

The antibacterial activity of the aqueous and hydroethanolic extracts of *Erythroxyllum emarginatum* was evaluated using the agar

disk diffusion method as described by Bauer et al. on [5] and adapted by Barry et al. on [6,7,8].

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 [9,10,11,12,13]. 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 incubator for 24 hours [14].

2.2.3 Strain confirmation

For each of the strains a pre-enrichment was performed on the selective isolation medium and then a Gram staining was performed [15].

2.2.4 Antibacterial susceptibility activity

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.

2.2.4.1 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.

2.2.4.2 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 \geq 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 [16,10,11,13].

3. RESULTS AND DISCUSSION

Our results showed that the aqueous and hydroethanolic extracts of *Erythroxyllum emarginatum* inhibited the growth of all the bacteria studied and Ciprofloxacin inhibited only three of the five bacteria studied as shown in Table 2.

The MBC / MIC ratios showed that the aqueous and hydroethanolic extracts of *Erythroxyllum*

emarginatum had bactericidal effect on all the bacteria studied as indicated in Table 3 and 4.

Our results agree with those of De wet on 2012 [3] that methanolic, acetonc, and aqueous leaf and bark extracts tested on four pathogenic bacteria *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* to demonstrate the anti-infectivity potential of *Erythroxyllum emarginatum*.

However, the efficacy of the extracts varies depending on the bacteria studied, the hydroethanolic extract of *Erythroxyllum emarginatum* is more active on *Escherichia coli* compared to the aqueous extract of *Erythroxyllum emarginatum* as shown in Table 3 and 4.

Table 1. The selected bacterial strains

Microbial group	Strains tested	Source of the strains
Gram-negative Bacteria	<i>Escherichia coli</i>	Urine
Gram-negative Bacteria	<i>Klebsilla pneumoniae</i>	Urine
Gram-negative Bacteria	<i>Pseudomonas aeruginosa</i>	Pus
Gram-positive Bacteria	<i>Staphylococcus aureus</i>	Cerebro spinal fluid
Gram-positive Bacteria	<i>Streptococcus pneumoniae</i>	Pus

Table 2. Determination of the inhibition diameters of the aqueous and hydroethanolic extracts of *Erythroxyllum emarginatum*

Bacteria strain studied	Inhibition diameter (mm)		
	Aqueuos extract (200 mg/ml)	Hydroethanolic extract (200 mg/ml)	Ciprofloxacin (250 mg/ml)
<i>Escherichia coli</i>	22±0.5	22±0.5	00
<i>Klebsilla pneumoniae</i>	10±0	12±0.5	00
<i>Pseudomonas aeruginosa</i>	19±0.5	18±0	23±0.5
<i>Staphylococcus aureus</i>	21±0.5	23±0.5	18±0.5
<i>Streptococcus pneumoniae</i>	20±0	25±0.5	10±0

Table 3. Determination of MIC and MBC of aqueous extracts of *Erythroxyllum emarginatum*

Studied bacteria strains	Aqueous extract			
	MIC (mg/ml)	MBC (mg/ml)	Ratio MIC/MBC	Antibacterial power
<i>Escherichia coli</i>	12.50	12.50	1	bactericidal
<i>Klebsilla pneumoniae</i>	12.25	12.25	1	bactericidal
<i>Pseudomonas aeruginosa</i>	6.25	6.25	1	bactericidal
<i>Staphylococcus aureus</i>	1.56	1.56	1	bactericidal
<i>Streptococcus pneumoniae</i>	25.00	25.00	1	bactericidal

Table 4. Determination of MIC and MBC hydroethanolic extracts of *Erythroxyllum emarginatum*

Studied bacteria strains	Hydroethanolic extract			
	MIC (mg/ml)	MBC (mg/ml)	Ratio MIC/MBC	Antibacterial power
<i>Escherichia coli</i>	3.125	3.125	1	bactericidal
<i>Klebsilla pneumoniae</i>	12.50	12.50	1	bactericidal
<i>Pseudomonas aeruginosa</i>	6.25	6.25	1	bactericidal
<i>Staphylococcus aureus</i>	0.78	1.56	2	bactericidal
<i>Streptococcus pneumoniae</i>	25.00	25.00	1	bactericidal

4. CONCLUSION

The study concludes that both aqueous and hydroethanolic extracts of *Erythroxylum emarginatum* has effective antibacterial activity on the bacteria of clinical isolates used. Both the extracts have potent inhibition on *Streptococcus pneumoniae* compare to other strains. In vitro study justify the use of these extracts against bacterial infection. However it laid foundation for the further research to study the specific ingredient involved in antibacterial activity.

ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, Felix Houphouët-Boigny University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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