

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

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ABSTRACT

The aim of our work was to evaluate the *in vitro* antibacterial activity of aqueous and hydroethanolic extracts of the stem bark of *Erythroxylum emarginatum*, a plant used by the Ivorian population to treat various infections, its effects on the following bacteria (5) strains, *Escherichia coli*, *Klebsilla pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*, were assessed. 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 hydroethanolic extracts of the stem bark of *Erythroxylum emarginatum* and to confirm their use in treating bacterial infections.

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

1. INTRODUCTION

Erythroxylum 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 *Erythroxylum 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. *Erythroxylum 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 [14]. *Erythroxylum 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 Erythroxylum, and the Species of emarginatum [5,14].

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 [8]. To evaluate the antibacterial activity of the aqueous and hydroethanolic 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

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.



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65 **Figure 1:** Stems bark powder of *Erythroxylum emarginatum* (Kouakou, 2015)

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71 **Figure 2 :** Aqueous extract of *Erythroxylum emarginatum* (Kouakou, 2015)

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Figure 3 : Hydroethanolic extract of *Erythroxylum emarginatum* (Kouakou, 2015)

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 [11], the plant parts were dried under the 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 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 using the same procedure as that of aqueous extract but with 700 ml of ethanol and 300 ml of water.

96 **Evaluation of antibacterial activity**

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

100 From young colonies of 18 to 24 hours, a bacterial suspension was made with the sterilized
101 distill water for each strain. The turbidity of this suspension was adjusted to 0.5 Mcfarland
102 and then diluted to 1/100. An inoculum estimated at 10^6 colony forming units per milliliter (cfu
103 / ml) was then obtained. This inoculum was seeded by flooding on Petri dishes containing
104 Mueller-Hinton agar [10, 12, 15]. The discs impregnated with the various extracts were then
105 delicately deposited on the surface of the agar. The same applies to ciprofloxacin discs
106 (reference antibiotic). The Petri dishes were first left at room temperature for pre-diffusion of
107 the substances and then incubated at 37 ° C. in an oven for 24 hours [1].

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109 **Strain Confirmation:**

110 For each of the strains a pre-enrichment was performed on the selective isolation medium
111 and then a Gram staining was performed [9].

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113 **Antibacterial susceptibility activity:**

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

117 **Minimum Inhibitory Concentration (MIC):** dilutions were made progressively as follow 1/2,
118 1/4, 1/8, 1/16, 1/32, 1/50, 1 / 64, 1 / 80, 1/128.

119 **Minimum Bactericidal Concentration (MBC):** The nutrient agar in Petri dishes was
120 streaked seeded with 100 µl of the contents of the tubes having a concentration $\geq$ MIC in the
121 preceding dilution series.

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

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

126 If the result of the ratio MBC / MIC = 1 to 2, the effect is bactericidal and if the ratio MBC /
127 MIC = 4 to 16, the effect is bacteriostatic [4,12].

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3. RESULTS AND DISCUSSION

Our results showed that the aqueous and hydroethanolic 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 hydroethanolic extracts of *Erythroxylum emarginatum* had bactericidal effect on all the bacteria studied (Table 3 and 4).

Our results agree with those of De wet on 2012 [8] that methanolic, acetonic, 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 *Erythroxylum emarginatum*.

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

TABLE 1: The selected bacterial strains

<i>Microbial groupe</i>	<i>Strains tested</i>	<i>Source of the strains</i>
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

155 **TABLE 2 :** Determination of the inhibition diameters of the aqueous and hydroethanolic
 156 extracts of *Erythroxylum emarginatum*

Bacteria strain studied	Inhibition diameter (mm)		
	Aqueuos extract (200mg/ml)	Hydroethanolic extract (200mg/ml)	Ciprofloxacin (250mg/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

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159 **TABLE 3 :** Determination of MIC and MBC of Aqueous extracts Of *Erythroxylum*
 160 *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

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165 **TABLE 4 :** Determination of MIC and MBC Hydroethanolic extracts Of *Erythroxylum*
 166 *emarginatum*
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<i>Studied bacteria strains</i>	<i>Hydroethanolic extract</i>			
	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

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171 **CONCLUSION**

172 The aqueous and hydroethanolic extracts of *Erythroxylum emarginatum* both have good
 173 antibacterial activity. The two extracts have a bactericidal effect on the bacteria studied:
 174 *Escherichia coli*, *Klebsilla pneumoniae*, *Pseudomonas aeruginos*, *Staphylococcus aureus*
 175 and *Streptococcus pneumoniae*. These extracts have an efficacy comparable to
 176 ciprofloxacin (reference molecule). This study justifies the uses of aqueous and
 177 hydroethanolic extracts of *Erythroxylum emarginatum* to treat infections by the populations.

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179 **Ethical Approval**

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

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