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Study of the antibacterial activity of bark extracts from *Terminalia mantaly* (Combretaceae) on the *in vitro* growth of eight (8) clinical enterobacteria strains

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Abstract
Therapeutic failures due to bacterial resistance to antibiotics are becoming more common and require other alternatives care. The aim of this study is to evaluate antibacterial activity of extracts of the bark of *Terminalia mantaly* on Enterobacteria. The determination of the MIC and MBC values of aqueous and hydroethanolic 70% extracts on 08 enterobacteria was made according to the double dilution method in a liquid medium. MIC and MBC values of the aqueous extract ranged from 3.125 mg/ml to 50 mg/ml while for hydroethanolic extract these values were ranged from 1.562 mg/ml to 25 mg/ml. All bacteria strains were sensitive to aqueous and hydroethanolic extracts in a dose-response relationship. Moreover, these extracts were bactericidal on all the strains tested.

Keywords: *Terminalia mantaly;* aqueous extract, hydroethanolic extract, enterobacteriaceae, bactericidal

1. Introduction
Medicinal plants are valuable resources for the majority of people. In fact, according to a World Health Organization (WHO) report in 2002, over 80% of the population use plants to provide primary health care. This great interest is due to the fact that herbal remedies have made and continue to prove effective. Their use is also linked to other factors such as population poverty and the lack of health infrastructure in some developing countries [1, 2].
Work on medicinal plants with antibacterial properties should be at the heart of research topics in infectiology in view of the emergence of neglected infectious diseases and especially the multi-resistance observed in many bacteria including Enterobacteriaceae due to pressure drug (long-term antibiotic therapy) [3, 4]. Indeed, antimicrobial compounds derived from plants could be a very good therapeutic alternative because they are able to inhibit bacterial growth by acting on several cell targets different from those targeted by conventionally used antibiotics such as penicillins, macrolides or tetracyclines [5].
In Côte d'Ivoire, several studies have focused on the biological, pharmacological and phytochemical properties of plants used in traditional medicine. These data on medicinal plants have on the one hand explained their therapeutic action and on the other hand confirmed their use in traditional medicine [6-8]. *Terminalia mantaly* H. perrier is one of the plants commonly used in traditional Ivorian medicine to treat various infections including dysentery, diarrhea, oral and digestive candidiasis, and some parasitic infections [9-11]. In view of its many ethnomedical uses, this plant has been targeted to study its efficacy against 8 enterobacterial clinical strains.

2 Materials and Methods
2.1 Biological materials
The microbial material consists on the one hand of *Escherischia Coli* 290, *Escherischia Coli* 356, *Klebseilla* sp 225, *Klebsiella* sp 290 bacterial strains which have been isolated from patients in consultation with the Regional Hospital Center (HRC) of Daloa, and other part of the *Enterobacter* sp 254, *Proteus* sp 255, *Salmonella* sp 253 and *Escherischia Coli* 256 strains that were provided by the Bacteriology-Virology Unit of the Pasteur Institute of Côte d'Ivoire. These bacteria have been preserved and kept alive in deep agar plates all the time.

2.2 Methods

2.2.1 Preparation of plant extracts

The bark was harvested from a tree in the town of Daloa (Ivory Coast), washed and dried under the sun, exposed to the open air for two weeks and then made into a fine powder with the help of a mechanical grinder type Retsch SK100. From this powder, the total aqueous and hydroethanol extracts of *T. mantaly* were prepared according to the method described by Zirihi *et al.*[11] and Okou[12] For the preparation of these extracts, 2 X 100g of powder of this plant were extracted separately in 1L of distilled water and 1L of an ethanol-water mixture (70/30 v / v) using a blinder (LX-300). The homogenates obtained for each solvent used are first dewatered in a square of clean fabric, then filtered successively twice on hydrophilic cotton and once whatman paper 3 mm. The hydroethanol and aqueous filtrates obtained were evaporated to dryness in an oven at 50°C. respectively for 3 days and 5 days. The dried extracts obtained from *T. mantaly* are codified X_0 for the total aqueous extract and X_1 for the hydroethanolic extract 70%.

The yields of the different extracts were calculated according to the relation below[13]:

$$R\ (\%) = (M_{obt}/M_{init}) \times 100$$

R: Yield in (%), M_{obt}: Obtained mass from the extract (g), M_{init}: Initial mass (g).

2.2.2 Antibacterial activity

A perfectly isolated colony from 18 to 24 hours on Mueller-Hinton Agar (MHA) was removed using a platinum loop and emulsified in 10 ml Mueller-Hinton Broth (MHB) and incubated for 3 to 4 hours at 37°C to have a pre-culture. 0.1 ml of the broth of this pre-culture was introduced into a tube containing 10 ml of MHB concentrated twice. This bacterial suspension constituted the inoculum was evaluated at approximately 10^6 cells/ml [14, 15].

The study of the antibacterial activity of the two prepared extracts was carried out by the macrodilution method in a liquid medium [15, 16]. A concentration range of the extracts was prepared in hemolysis tubes by the double dilution method in a 1/2 reason geometric progression with concentrations ranging from 200 mg/ml to 0.781 mg/ml. A series of 9 hemolysis tubes numbered from T_1 to T_9 were inoculated with 1ml of MHB twice-concentrated already contaminated with the target germ, and then the plant extracts prepared according to the previously established concentration range were added to these same tubes because 1 ml of plant extract to be tested as follows: 1 ml of plant extract of 200.00 mg/ml is transferred into the T_9 tube, that of 100.00 mg/ml in the T_8 tube, that of 50 mg/ml ml in the T_7 tube so on to the T_1 tube which will receive 1ml of plant extract of 0.781 mg/ml. The T_C tube received instead of plant extract, 1 ml of sterile distilled water to serve as a growth control. This distribution of plant extracts of well-known concentrations in each of the tubes already containing 1 ml of inoculum allowed to reduce the initial concentrations of plant extracts by half and to obtain the new range of concentrations of 100 mg/ml to 0.390 mg/ml of the tube T_9 to the tube T_1 according to a geometric connection of reason 1/2. The first nine (9) tubes (from T_9 to T_1) are called "experimental tubes" and the last tube (T_C) is labeled "growth control tube or T_C". All tubes were incubated at 37 ° C for 18 hours. The experiment was repeated three (3) times for each test.

2.2.3 Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the lowest concentration of antimicrobial that can inhibit any visible growth after an incubation time of 18 to 24 hours. Its determination was made from the observation of the turbidity induced by the growth of the studied germs[15,16]. The MIC is therefore the smallest concentration for which there is no turbidity. The turbidity induced by bacterial growth is observed with the naked eye through the hemolysis tubes. The tubes from the experimental series (T_1 to T_9) that received inoculated Mueller-Hinton broth plus a known extract concentration will be compared to another series of tubes that received uninoculated Mueller-Hinton broth (Reference tubes showing no turbidity). In fact, the content of each experimental test tube is observed simultaneously with sunlight with its counterpart in the reference series in order to detect the difference in appearance (turbidity) due to bacterial growth in the test tube [12].

2.2.4 Determination of Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration (MBC) is the concentration of the antimicrobial that leaves at most 0.01% of surviving germs. For its determination, the control tube (T_C) was diluted to 10^{-4}. This dilution represented 0.01% survival. It is subcultured by 5 cm streaks on a Mueller-Hinton agar and incubated at 37°C for 18 hours. The number of cells obtained on the streak of the 10^{-4} dilution is compared to that of each experimental tube also transplanted by 5 cm streaks. The experimental tube whose number of germs present on its streak is less than or equal to that of the 10^{-4} dilution corresponds to the MBC [15, 17].

3 Results

3.1 Extraction yields

The yield of the aqueous extract (X_0) was 12.08% whereas that of the hydroethanolic extract (X_1) was 15.41% (Table 1).

3.2 Antibacterial activity

Figure 1 shows the MIC and MBC values obtained with the aqueous extract. On the *E. coli* 290 and *E. coli* 356 strains, the recorded MIC values were 25 mg/ml with a MBC of 50 mg/ml. However, with the strain of *E. coli* 256, the MIC and the MBC were identical (MIC = MBC = 25 mg/ml). Also, on the *Klebsiella* sp 225 and *Klebsiella* sp 290 strains, the MIC and MBC values of the X_0 extract were respectively 25 mg/ml and 50 mg/ml. As for the strains of *Proteus sp*, *Salmonella sp* and *Enterobacter sp*, the MIC values determined were respectively 3.125 mg/ml; 12.5 mg/ml and 25 mg/ml while the MBC values were 12.5 mg/ml, 50 mg/ml and 50 mg/ml.

In comparison with the hydroethanolic extract (X_1), the two strains of *Klebsiella sp* (225; 290) and *E. coli* 290 recorded identical MIC and MBC values (MIC = MBC = 25 mg/ml). On the *E. coli* 256 and *Enterobacter sp* 254 strains, the MIC (12.5 mg / ml) was half of the MBC (25 mg/ml). Also, on the *Proteus sp*, *Salmonella sp* and *E.coli* 356 strains, the MIC values ranged from 1.562 mg/ml to 12.5 mg/ml for MBC values ranged from 3.125 mg/ml to 25 mg/ml (Figure 2).

Table 1: Yield of differents extracts

Extracts	Initial mass (M_{init}) (g)	Obtained mass (M_{obt}) (g)	Yield (%)
Aqueous extract (X_0)	100	12.08	12.08
Hydroethanolic extract 70% (X_1)	100	15.41	15.41

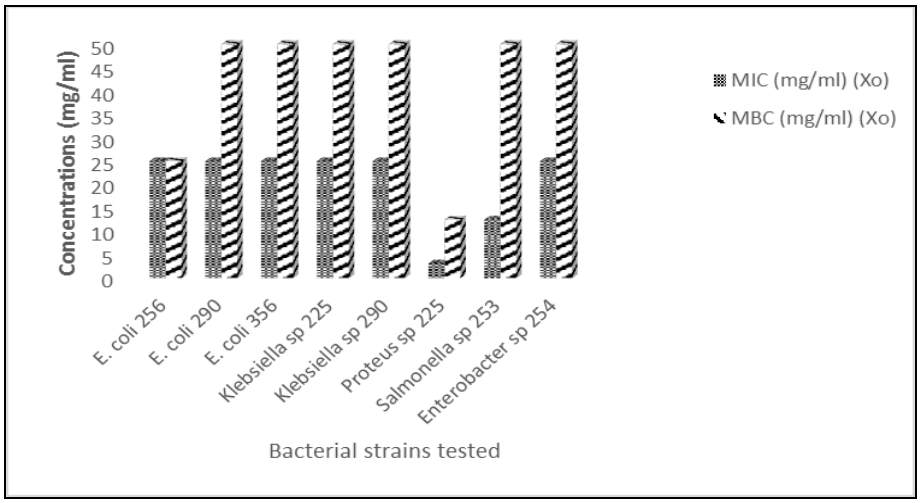

Fig 1: Comparative antibacterial parameters of the total aqueous extract (X_0) of *T. mentaly*

In this figure, MIC values were determined at 25 mg/ml on each target bacterium with the exception of Proteus sp 255 and Salmonella sp 253 for which the MIC values were 3.125 mg/ml and 12.5 mg/ml, respectively. mg/ml. As for the MBC values, they were determined at 50 mg/ml on all the target bacteria with the exception of E. Coli 256 and Proteus sp 255 for which these values were respectively determined at 25 mg / ml and at 12, 5 mg/ml.

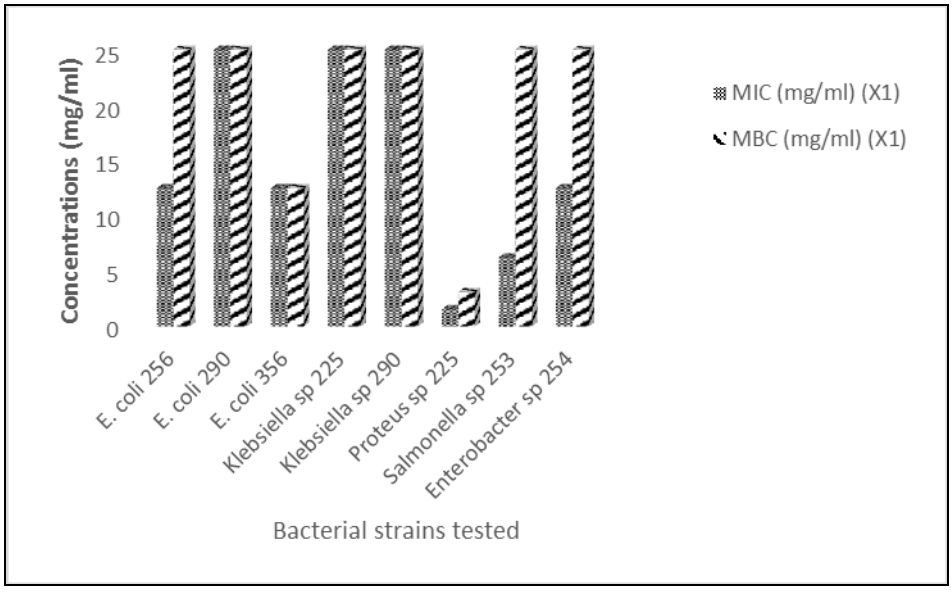

Fig 2: Comparative antibacterial parameters of hydroethanolic extract (X_1) of *T. mentaly*

In this figure, the MIC values were determined at 25 mg/ml on E. coli 290, Klebsiella sp 225 and Klebsiella sp 290, at 12.5 mg/ml for E. coli. 256, E. coli 356, and Enterobacter sp 254, at 6.25 mg/ml for Salmonella sp 253 then at 1.562 mg/ml for Proteus sp 255. As for the MBC values, they were determined at 25 mg/ml over 6 target bacteria at 12.5 mg/ml for E. coli 356 and 3.125 mg/ml for Proteus sp 255.

4 Discussion

4.1 Yields

The highest yield was obtained with the hydroethanolic extract (X_1) is 15.41% and the lowest (12.08%) with the aqueous extract (X_0) per 100g of powder *T. mantaly*. The different yields have shown that the hydroalcoholic solvent extracts a large quantity of the compounds present in the plant. These performance results are consistent with the work of the authors Bolou *et al.*[18]. Indeed, these authors have shown that the extraction with a hydroalcoholic solvent increases the extraction yield compared to the extraction with water as was the case with the species *Terminalia Glaucescens* for which the yields of the aqueous decoction, aqueous macerate and hydroalcoholic extract were respectively 14.2%, 16.8% and 21.9%). The work of Kokora *et al.* [16] also showed that with 100 g of *T. Mantaly* bark powder the yield obtained with the hydroalcoholic solvent was 12.4% and 10.1% for the aqueous extract. The best yields obtained with the hydroalcoholic solvent could be explained by the ability of alcoholic solvents to penetrate cell walls by facilitating the extraction of a greater number of polar molecules, of medium and low polarity[19]. However, the

collection area, the nature of the soil, the stage of development of the plant and the organ used could also influence the extraction yield [20].

4.2 Antibacterial activity of aqueous extract (X_0)

T. mantaly extract X_0, prepared from water, showed inhibitory activity on all bacterial strains. The best activity (considering the lowest values of MBC) was 12.5 mg/ml on the *Proteus sp* 255 strain. On the other hand, the MBCs obtained for the other germs were 25 mg/ml or 50 mg/ml. The MBC_{X0}/ MIC_{X0} ratios made it possible to note that the X_0 extract is bactericidal on all the bacterial strains studied because the ratios obtained varied between 1 and 4[21]. The MBC values obtained with the extract X_0 on five (5) strains studied (*Enterobacter* sp 254, *E. Coli* 356, *E. Coli* 290, *Klebsiella sp* 225 and *Klebsiella sp* 290) are twice higher than the values of the corresponding MICs. A single strain (*E. coli* 256) has a MIC equal to the MBC. The inhibition is complete on this strain. The relative activity of the aqueous extract on these germs would justify its use in traditional environment for the treatment of bacterial infections.

In addition, all the bacterial strains tested showed different sensitivities to the aqueous extract of *T. mantaly* stem bark. The most sensitive strain for this extract was *Proteus sp* 255 (MBC = 12.5 mg/ml) whereas the least sensitive strains would be *E. Coli* 290, *E. Coli* 356, *Klebsiella sp* 225, *Klebsiella sp* 290, *Salmonella sp* 253 and *Enterobacter sp* 254 (MBC = 50 mg/ml). Previous studies by Kokora *et al.*[16] on the aqueous extract of *T. mantaly* bark had already shown that this extract also had a bactericidal action against strains of *E. coli* ATCC25922 (MBC = 0.625 mg/ml) and *E. coli* 27180 (MBC = 2.5 mg/ml). Similarly, Tizhe *et al.*[22] showed that the aqueous extract of *T. mantaly* leaves acted on *E. Coli* (MBC = 25mg/ml) and *Salmonella typhi* (MBC = 6.25mg / ml).

This activity of the aqueous extract of *T. mantaly* may be due to its richness in compounds with antimicrobial properties that would be soluble in water. This would explain its traditional use against diarrheal diseases[23].

4.3 Antibacterial activity of hydroethanolic extract (X_1)

The MICs obtained for these eight (8) strains studied ranged from 1.562 mg/ml to 25 mg/ml. The MBC values for them ranged from 3.125 mg/ml to 25 mg/ml. These values were better than the values obtained with the aqueous extract. Indeed, considering the MBC_{X0}/ MBC_{X1} ratios, the hydroethanolic extract was 2 times more active than the aqueous extract on *E. coli* 290; *Enterobacter sp* 254; *Klebsiella sp* 225; *Klebsiella sp* 290 and *Salmonella sp* 253, 4 times more active than X_0 on *E. coli* 356 and *Proteus sp* 255. On *E. coli* 256, extracts X_0 and X_1 would have the same efficacy. The calculation of the MBC_{X1}/MIC_{X1} ratios made it possible to note that on the strains: *E. coli* 256, *E. coli* 290, *E. coli* 356, *Klebsiella sp* 225, *Klebsiella sp* 290, *Enterobacter sp* 254, *Proteus sp* 255 and *Samonella sp* 253, the extract X_1 would be bactericidal because the calculated ratios were of the order 1, 2 and 4. This bactericide is more marked with the *E. coli* strains. *E. coli* 356 and *Proteus sp* 255 (X_1 is 4 times more bactericidal than X_0) followed by *Enterobacter sp* 254 strains; *E. coli* 290; *Klebsiella sp* 225; *Klebsiella sp* 290 and *Salmonella sp* 253 (X_1 is 2 times more bactericidal than X_0). Extracts X_0 and X_1 have an identical bactericidal effect on *E. coli* 256. The most sensitive strain was *Proteus sp* 255 (MBC = 3.125 mg/ml). *E. Coli* 256; *E. coli* 290; *Klebsiella sp* 225; *Klebsiella sp* 290; *Enterobacter sp* 254 and *Samonella sp* 253 would be the least sensitive (MBC = 25 mg/ml). The values of the MBC/MIC ratio obtained with the hydroethanolic extract on *E. Coli* 290; *E. Coli* 356, *Klebsiella sp* 225 and *Klebsiella sp* 290 are equal to 1. This means that the inhibition was complete on these strains. These results are consistent with those of Kokora *et al.* [16]. In fact, these authors obtained with the hydroethanolic extract the antibacterial parameters (MIC and MBC = 0.3125 mg/ml on *E. coli* ATCC25922) and on *E. coli* 27180 a MIC and MBC = 1.25 mg/ml. The sensitivity of each strain tested is related to the concentration of the extract used. This means that the extracts have been active in a dose-dependent relationship.

The antibacterial potential of the X_0 and X_1 extracts of *T. mantaly* is related to the presence of secondary metabolites with antimicrobial activity. Indeed, the authors Yayé *et al.* [13] and Kokora *et al.*[16], have shown through a phytochemical screening that these extracts contain phenolic compounds, terpenoids, alkaloids and glycosides. Phenolic compounds are known for their antimicrobial properties. They cause the toxicity of microorganisms and in some cases they inhibit their growth[24].

Other studies have confirmed the antimicrobial potency of *T. mantaly* bark. Indeed, the authors [23] showed that the decoction of leaves and stems of *T. mantaly* inhibited the *in vitro* growth of *Shigella dysenteriae* at concentrations between 600 µg/ml and 800 µg/ml. The work of Yaye *et al.*[13,25] and Zirihi *et al.*[26], showed that *T. mantaly* also possessed antifungal properties on the *in vitro* growth of *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Trichophyton mentagrophytes* for which the MFC values ranged between 97.5 µg/ml and 500 µg/ml.

5 Conclusion

The aqueous and hydroethanolic extracts of *T. Mantaly* (Combretaceae) prepared made it possible to note that, with respect to the water solvent, the hydroethanolic solvent made it possible to extract a large quantity of bioactive substances. The aqueous (X_0) and hydroethanolic (X_1) extracts of *T. mantaly* had an inhibitory action on the *in vitro* growth of the eight clinical strains of enterobacteria responsible for human pathologies encountered in hospitals. In addition, the antibacterial activity of the hydroethanolic extract is significantly better than that of the aqueous extract on the bacterial strains studied. Also, the *Proteus sp* strain would be the most sensitive to the two extracts tested. On each of the target strains, the extracts tested were bactericidal.

This study provides scientific data that can justify the use of this plant in traditional medicine in the treatment of various infectious diseases caused by these bacteria. In addition, studies on molecular sequencing are currently being conducted to improve the characterization of these clinical strains.

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