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*RESEARCH ARTICLE*

# COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITY OF ROOTS, BARKS AND LEAVES OF JATROPHA MULTIFIDA, JATROPHA CURCAS AND ZANTHOXYLUM ZANTHOXYLOIDES

**Djaboutou Kafuyemon Ismanth[1,2], Lauris Fah[1,2], Phénix Assogba[1], Eric Agbodjento[1], Esther Deguenon[1], Edna Hounsa[1], Hans Ohouko[1], Hornel Koudokpon[1], Boris Legba[1], Arnaud Soha[1], Jean Robert Klotoe[1] and Victorien Dougnon[1]**

1. Research Unit in Applied Microbiology and Pharmacology of Natural Substances, Laboratory Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Benin.
2. Department of Continuing Education, National Medical and Health Institute, University of Abomey-Calavi, Benin.

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## *Abstract*

Antimicrobial resistance is a major public health issue. This study compares the antibacterial activity of leaves, bark, and roots of *Jatropha curcas*, *Jatropha multifida*, and *Zanthoxylum zanthoxyloides*. Extracts (water, ethanolic, and hydro-ethanolic) were tested against *Staphylococcus aureus* and *Klebsiella pneumoniae*, and their antioxidant activity was assessed using the DPPH test. Polyphenol and flavonoid levels were quantified spectrophotometrically.Hydro-ethanolic extracts of *J. multifida* bark and *Z. zanthoxyloides* leaves showed strong activity against K. pneumoniae (MIC, MBC: 6.25mg for *J. curcas*, *J. multifida*; 3.12mg for *Z. zanthoxyloides*). Against *S. aureus*, the best were *J. curcas*, *J.multifida* (3.12mg), and *Z. zanthoxyloides*(1.5mg). Leaves and bark were richer in flavonoids and polyphenols (p=0.001) and exhibited superior antioxidant activity. Using leaves and bark over roots enhances sustainability.



## Introduction:-

Bacterial resistance to antibiotics is one of the most important challenges for anti-infective treatments for healthcare professionals around the world and for the pharmaceutical industry(Onzo et al., 2016). The inappropriate and often exaggerated use of antibiotics, particularly in human and animal health, is at the origin of an evolutionary selection pressure on microbial populations (Bihari et al., 2011). The latter results in the growth and spread of resistant and multidrug-resistant strains, leading to increasingly common treatment failure (Bihari et al., 2011).In order to mitigate this phenomenon, a lot of scientific research is directed towards the identification of new antimicrobial agents of natural origin such as bioactive molecules of plants (Monciardini et al., 2014). African flora is known for its richness, which is the basis for frequent exploitation with the aim of developing therapeutic recipes(Gurib-Fakim et al., 2010; Veilleux et al., 1996). Akoègninou et al.(2006) estimated the Beninese flora at more than 2807 plant species. This flora consists of several medicinal plants with antimicrobial properties that are used in the treatment of infections (Djego et al., 2012; Dougnon et al., 2017; Koudokpon et al., 2017). The roots of some medicinal essences are widely used in recipes to treat various infections (Arekemase et al., 2011).*Jatropha curcas*, *Zanthoxylum Zanthoxyloides*and *Jatropha multifida*, are among these plants whose roots are commonly used in Benin in the treatment of several microbial infections(Dougnon et al., 2017). These plants have the potential to act on several

**Corresponding Author:-Victorien Dougnon**
Address**:-**Research Unit in Applied Microbiology and Pharmacology of Natural Substances, Laboratory Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Benin.

bacterial strains such as S*taphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes, Staphylococcus epidermidis, Staphylococcus saprophyticus, Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae, Salmonella Typhimurium* and *Proteus mirabilis*(Aiyelaagbe et al., 2007; Arekemase et al., 2011; Ynalvez et al., 2012). The biological properties of these plants are much more attributed to the roots than to the other organs (leaves and bark) of these plant species, which then proves the frequent use of these roots in traditional medicine(Agbulu et al., 2015; Aiyelaagbe et al., 2008; Arekemase et al., 2011). However, the frequent use of the roots of these plants can contribute to their rarity or even the total extinction of these species. In addition, other plant organs (leaves and roots) can have biological properties similar to those of their roots. But very few studies have been done on the antibacterial activity of extracts from the renewable organs (leaves and bark) of these plants(Agban et al., 2020; Sharma et al., 2012; Tine et al., 2020). In addition, the mechanism of action of the antibacterial effect of extracts of these three plants has not been specified in these studies. The same applies to the resistance profile of the bacterial strains used. It is therefore wise to determine the biological properties as well as the mechanism of action of the renewable organs of these plants in order to limit the use of roots in traditional medicinal recipes. This is why our study aims to promote the use of the renewable organs of these three plants of the Beninese flora.

## Methods:-

### Collection of plant material and preparation of extracts

The organs (leaves, roots and fresh bark) were dried in the laboratory at a temperature varying between 16°C and 22°C. After drying, they were crushed in the mill. The powders were then used for aqueous and ethanolic extractions of each plant species by the methodology described by Fah et al.(2015).Fifty (50g) grams of powder from each plant part were macerated in 500 ml of solvent (water and ethanol). The mixtures were left for 72 hours under continuous stirring at room temperature. The homogenate obtained was filtered three times on hydrophilic cotton and once on Wattman No. 1 paper. The filtrate obtained was evaporated at a temperature of 40°C in an oven to a dry mass which is the extract. The extract obtained was stored in the refrigerator at 4°C. The hydro-ethanolic extraction was carried out based on the methodology used by Klotoé et al.(2020). Briefly modified, 50g of powder was macerated in 500 ml of the 50% v/v ethanol and water mixture. After 72 hours of stirring at room temperature, the homogenate obtained was filtered three times on cotton wool and once on Wattman paper.

### Determination of total polyphenols and flavonoids

The amount of polyphenols and flavonoids contained in the extracts was determined by quantitative phytochemical analysis following the methodology applied byKlotoé et al.(2020).

Total phenols were determined using Folin Ciocalteu reagent (FCR). 50 µL of the extract was mixed with 250 µL of the RCF (10 times diluted in distilled water) and 750 µL of an aqueous solution of sodium carbonate $Na_2CO_3$ (7.5%). After 8 min of incubation, 950 µL of distilled water was added and mixed with the vortex and incubated for 2 hours. Optical densities (ODs) were read at 760 nm using a spectrophotometer. The reading was taken against a blank composed of a mixture of 250 µL of FCR, 750 µL of Na2CO3 and 1 ml of distilled water. The total polyphenol content in the different extracts was calculated from a linear calibration curve (y = ax + b), established with precise gallic acid concentrations as the reference standard (0-200 µg/ml). Total phenolic content was determined as mg gallic acid equivalent/g extract (mg GAE/g) using the formula below:

**TFC = (X xV) / m**

With: TFC is a total phenolic content, X is the concentration of gallic acid in mg/ml; V is the extraction volume used in ml and m is the weight of the extract in grams.

Flavonoid content was measured using aluminum trichloride ($AlCl_3$) as a reagent. 500 µl $AlCl_3$ (2%), 500 µl of the extract and 3 ml of methanol were mixed. The white was composed of 500 µl of $AlCl_3$ and 3.5 ml of methanol. The reading was made with a spectrophotometer at 415 nm after 10 min of incubation. Samples were prepared three times for each assay and average values were taken. The amounts of flavonoids in the extracts were calculated from the calibration curve of a standard flavonoid (Rutin) as the reference molecule (0-1 mg/ml). The total flavonoid content was determined using the formula below:

**TFC = (X xV) / m**

With: TFC is the content of total flavonoids, X is the concentration of rutin in mg/ml; V is the extraction volume used in ml and m is the weight of the extract in grams.

**Antioxidant activity of plant extracts studied by DPPH test**

The method adopted in this study is that of Klotoé et al.(2020)that 100 µL volume of different concentrations of each extract was added to 1900 µL of the ethanolic solution of DPPH (0.4 mg/mL). The white was prepared by mixing 100 µl of the extraction solvent with 1900 µl of the DPPH solution. After incubation in the dark for 1 hour at room temperature, absorbance readings were performed at 517 nm using a spectrophotometer. These measured absorbances were used to calculate the percentage of DPPH radical scavenging that is proportional to the antioxidant potency of the sample. Vitamin C was used as the gold standard. Antioxidant activity is expressed as the percentage of trapping determined by the formula:

**P = (Ab- Ae) / Ab x 100**

With P: Percentage of trapping; Ab: absorption of white; Ae: Absorption of the sample.

**Determination of the antimicrobial activity of *Jatropha curcas, Jatropha multifida*and *Zanthoxylum zanthoxyloides* extracts**

**Characteristics of the selected bacterial strains**

For the evaluation of antibacterial properties, the bacterial strains used came from the strain collection of the Research Unit in Applied Microbiology and Pharmacology of Natural Substances (U.R.M.A.Pha). The criterion for choosing Strains was based on species. The bacterial species chosen are the most predominant in cases of clinical infection(Agbulu et al., 2015; Arekemase et al., 2011; Igbinosa et al., 2009).

1. ***Staphylococcus aureus*:** Golden-yellow colonies, characteristic of Staphylococcus aureus, from the transplanting of isolates on Chapman medium were selected. Then the Gram staining of these colonies was carried out followed by their identification using catalase, freestaphylocoagulase and D-nase.
2. ***Klebsiella pneumoniae*:** the brownish colonies, mucous membranes characteristic of the Klebsiella from the transplanting of isolates on EMB medium were selected. Then the Gram staining of these colonies was carried out followed by their identifications thanks to the Leminor gallery.

**Control of the resistance profile of selected strains**

The control of the resistance profile of the selected strains was done according to the method described by the Antibiogram Committee of the French Society of Microbiology, (2020) (Table 1).

**Determination of antimicrobial activity in liquid and solid media**

It was performed in microplates from 96 wells following the method described by Agbankpe et al.(2016). A stock solution sample was prepared at a concentration of 100mg/ml in distilled water. In fact, 100 µl of MH broth and 100 µl of the stock solution of each extract concentrated at 100 mg/ml were deposited in the 1st well. After homogenization, 200 µl of a 50mg/ml extract solution was obtained. A volume of 100 µl of this new solution was then taken and mixed with the MH broth contained in the 2nd well and this series of dilutions was carried out up to the 10th well. Then 100 µL of the bacterial suspension was added to each well. The positive (100µL Mueller Hinton Broth + 100µL bacterial suspension) and negative (100µL Mueller Hinton Broth + 100µL test extract stock solution) controls were taken in the 11th and 12th wells, respectively. The microplates were covered with parafilm and then placed for 18 hours in the oven at 37°C. Bacterial growth was revealed by tetrazolium.

**Determination of the Minimum Bactericidal Concentration**

To determine the Minimum Bactericidal Concentration, all IJC wells with higher concentrations were inoculated on HD agar and incubated at 37°C for 18 to 24 hours. This made it possible to assess the minimum bactericidal concentration, which corresponded to the lowest concentration of extract that did not reveal bacterial colonies. The antibiotic potency (Pa) of each extract was then calculated with the MBC/MIC formula. The antibacterial effect or potency is judged to be bactericidal or bacteriostatic on the basis of Pa = MBC/MIC. If $1 \leq Pa \leq 2$, the effect is bactericidal and if $4 \leq Pa \geq 16$, the effect is bacteriostatic.

**Mode of action of the antibacterial effect plant extracts**

The mode of action of the antibacterial effect was evaluated for the extracts that were Improved antibacterial activity on the bacterial strains tested. Thus, the external membrane permeability test was performed**.** It was determined according to the method described by Dougnon et al. (2021), with some modifications. In a 96-well microplate, concentrations of MIC and 2MIC of each extract were prepared as a triplicate by dilution in series of four. One hundred microliters (100 µl) of suspension of each strain were added and the plate was incubated at 37℃ for 24 hours. The optical densities were read at 405 nm and the percent destabilization was calculated using the formula below:

**%D = [(AB – AE) ÷ Ao] x 100**
With %D: Percentage of destabilization; AB: Absorbance of White; AE: absorbance of the samples tested.

Imipenem was used as a positive control and was prepared equally at the MIC (0.0781 µg/mL) and 2MIC (0.1562 µg/mL). The HD broth and bacterial suspension constituted the negative control while the sterility control contained the HD broth alone. The wells containing the MH broth and the extracts alone made up the white. A graph of the %D in relation to the concentration of the extract was plotted in order to determine the concentration that causes maximum destabilization of the bacterial membrane.

**Data processing and analysis**
Data were collected and saved in the Excel 2019 spreadsheet and graphs were made with Graph Pad Prism 7 software. Descriptive statistics were produced using SPSS 20 software. The ANOVA test was used for the comparison of the means. Comparisons were made at the 5% significance level.

## Results and Discussion:-

## Results:-

**Determination of total polyphenols and flavonoids**

**Total polyphenol content**

The comparison of the different types of extracts according to the organs of *Jatropha curcas* is presented in Table 2. This table shows that for the aqueous extract, the leaves and bark are significantly richer in polyphenols than the roots ($p < 0.05$). For the ethanolic extract, the leaves and bark are significantly richer in polyphenols than the roots ($p < 0.05$). In addition, for the hydro-ethanolic extract, the leaves and bark are significantly richer in polyphenols than the roots ($p < 0.05$). Therefore, the extracts obtained from the leaves and bark showed a better polyphenol content compared to the roots.

Table 3 shows the comparison of the different types of extracts according to the organs of *Jatropha multifida*. From this table it appears that the aqueous extract of the bark showed a richness in polyphenols compared to the leaves ($p < 0.05$). Also, with regard to the hydro-ethanolic extract, the bark exhibited a significantly better content than the leaves ($p < 0.05$). While there is no significant difference between the polyphenol content of the ethanolic extract of the different organs. Hence the best contents were obtained for the bark extracts while the low contents were noted for the leaves.

Table 4 shows the comparison of the different types of extracts according to the organs of *Zanthoxylum zanthoxyloides*. From the analysis of this table it can be seen that there is no significant difference between the polyphenol content of the aqueous extract of the different organs. For the bark, the ethanolic extract was rich in polyphenols compared to the roots. While for the hydro-ethanolic extract, the leaves and bark showed a significantly better polyphenol content compared to the roots ($p < 0.05$). Generally speaking, all extracts of extracts from roots are less rich in polyphenols than those of other organs.

**Total flavonoid content**

The comparison of the different types of extracts according to the organs of *Jatropha curcas* is presented in Table 5.

This table shows that for the aqueous extract, the leaves were significantly richer in flavonoids compared to the bark ($p< 0.05$) and the roots ($p< 0.05$). Similarly,to the hydro-ethanolic extract, the leaves were richer in flavonoid than the bark and roots. While for the ethanolic extract, the bark was richer in flavonoids compared to the leaves ($p < 0.05$). In general, the extracts obtained from the leaves and bark were richer in flavonoids than those from the roots.

Table 6 shows the comparison of the different types of extracts according to the organs of *Jatropha multifida*. From this table it can be seen that for the aqueous extracts, the bark followed by those of the leaves showed a significantly high flavonoid richness compared to the roots ($p < 0.05$). While for the ethanolic extract, the leaves are significantly richer in flavonoids than the bark and roots ($p < 0.05$).

The same is true for the hydro-ethanol extract. In short, for this plant, the extracts of the bark followed by those of the leaves are richer in flavonoids than those of the roots.

Table 7 illustrates the comparison of the different types of extracts according to the organs of *Zanthoxylum zanthoxyloides*. From the analysis of this table it appears that for the aqueous extract, the leaves are richer in flavonoids than the other plant organs ($p < 0.05$). Similarly, for the ethanolic extract, the leaves are richer in flavonoids than the other plant organs ($p < 0.05$). In addition, for the hydro-ethanolic extract, the leaves showed a significantly better flavonoid richness than the bark ($p < 0.05$) and the roots ($p < 0.05$). Thus, extracts from the leaves of this plant are particularly richer in flavonoids than other organs.

**Antioxidant activity**

All the extracts tested reduced the DPPH radical to varying proportions. This inhibition of the DPPH radical showed that the antioxidant activity of the different extracts was proportional to the increase in the concentration of the extracts. The comparison of the different types of extracts according to the organs of *Jatropha curcas* is presented in Table 8. This table shows that for the aqueous extract, the roots significantly inhibited the DPPH radical compared to the leaves and bark ($p < 0.05$). On the other hand, for the ethanolic extract, the bark significantly inhibited the DPPH radical compared to the leaves ($p < 0.05$) and roots ($p < 0.05$). Concerning the hydro-ethanolic extract, the leaves significantly inhibited the DPPH radical compared to the roots ($p < 0.05$). In short, with the exception of the aqueous extract, all the other extracts (hydro-ethanolic and ethanolic) of the leaves and bark were more antioxidant than those of the roots.

Table 9 provides information on the comparison of the different types of extracts according to the organs of *Jatropha multifida*. This table shows that for the aqueous extract, the bark significantly inhibited the DPPH radical compared to the leaves ($p < 0.05$) and the roots ($p < 0.05$). Similarly, for the ethanolic extract, the bark inhibited the DPPH radical compared to the leaves ($p < 0.05$). The same is true for the hydro-ethanol extract. From all of the above, it can be said that the extracts of the bark exhibited the best inhibitory powers of the DPPH radical.

The comparison of the different types of extracts according to the organs of *Zanthoxylum zanthoxyloides* is presented in Table 10. From the analysis in this table, we can see that for the aqueous extract, the roots showed a better inhibitory potential of the DPPH radical compared to the leaves ($p < 0.05$) and the bark ($p < 0.05$). On the other hand, for the ethanolic extract, the leaves showed a better inhibitory potential of the DPPH radical compared to the roots ($p < 0.05$). The same observation is made for the hydro-ethanolic extract. Finally, apart from the aqueous extract, the leaves followed by the bark showed a better inhibitory power of the DPPH radical for the other extracts (ethanolic and hydro-ethanolic)

**Effect of extracts from different plant organs on bacterial strains**

**Determination of the antibiotic power of plant extracts**

Table 11 shows the results of the MIC, MBC and antibiotic potency of extracts of *J. curcas*, *J. multifida*and *Z. zanthoxyloides*tested on *S. aureus* strain ATCC 25923. From this table it appears that the aqueous extract of the leaves of *J. curcas*had a lower Minimum Inhibitory Concentration (MIC) than that of the bark at the level of the *S. aureus* strain ATCC 25923. In contrast, the ethanolic extract of the roots showed a smaller MIC than that of the bark and leaves. Similarly, the hydro-ethanolic extract of the roots showed a smaller MIC than that of the leaves and bark. The aqueous extracts of the leaves and bark showed the same bacteriostatic powers on *S. aureus* strain ATCC 25923. On the other hand, the hydro-ethanolic extract of the roots showed a lower bactericidal power than that of the bark on the same strain. As for *J. multifida*, the aqueous extract of the bark showed a lower Minimum Inhibitory Concentration than that of the roots and leaves for S. aureus strain ATCC 25923. Similarly, the hydro-ethanolic extract of the bark showed a lower Minimum Inhibitory Concentration than that of the roots and leaves at the level of *S. aureus* strain ATCC 25923. Ethanolic extracts from the bark and roots showed a lower Minimum Inhibitory Concentration than that of the leaf at the level of the *S. aureus* strain. The extracts (aqueous, ethanolic and hydroethanolic) of the bark and roots showed the same bacteriostatic powers on *S. aureus* strain ATCC 25923. On the other hand, only the aqueous extract of the leaves showed bactericidal potential (MBC/MIC ratio equal to 2) on this same strain. With regard to *Z. zanthoxyloides*, the aqueous extract of the leaves showed a lower Minimum Inhibitory Concentration than that of the roots on *S. aureus* ATCC 25923. Similarly, hydro-ethanolic extracts from the leaves and bark showed smaller MICs than those from the roots. In contrast, the ethanolic extract of the roots had a smaller MIC than that of the bark at the level of *S. aureus* strain ATCC 25923. Ethanolic extracts from the bark and roots showed the same bacteriostatic properties on *S. aureus* strain ATCC 25923.On the other hand, only the aqueous extract of the leaves showed bactericidal potential (MBC/MIC ratio equal to 1) on this same strain.

Table 12 shows the results of the MIC, MBC and antibiotic potency of *Jatropha curcas*, *J. multifida*and *Zanthoxylum zanthoxyloides*extractstested on the *K. pneumoniae* strain. For *J. curcas*, the strain of *K. pneumoniae*, the aqueous extract of the bark had a MIC of 25. The ethanolic extract of the leaves showed a smaller MIC than that of the bark. Hydro-ethanolic extracts from the bark and roots, on the other hand, showed lower MICs than those of the leaves.

All the extracts (aqueous, ethanolic and hydro-ethanolic) from the bark as well as the ethanolic and hydro-ethanolic extracts from the leaves showed the same bacteriostatic powers; the same for the hydro-ethanolic extract of the roots. Tan disk for *J. multifida*, the aqueous extract of the leaves showed a smaller MIC than that of the bark and roots on *K. pneumoniae*. Hydro-ethanolic extracts of the leaves and bark showed a smaller MIC than that of the root. In contrast, the ethanolic extract of the bark had a smaller MIC than that of the leaf. All extracts (aqueous, ethanolic and hydroethanolic) from the bark and leaf showed the same bacteriostatic properties, while only the hydro-ethanolic extract of the roots showed bactericidal potential (MBC/MIC ratio equal to 1). For *Z. zanthoxyloides*, the aqueous extract of the roots showed a smaller MIC than that of the bark on *K. pneumoniae*. In contrast, the ethanolic extract of the bark showed a smaller MIC than that of the roots and leaves. The hydro-ethanolic extract of the leaves showed a smaller MIC than that of the bark and roots. All extracts (ethanolic and hydroethanolic) from the leaves, bark and roots showed the same bacteriostatic properties (MBC/MIC ratio equal to 1).

**Determination of the percentage of destabilization of extracts on the membrane of the stumps**
The ability of plant extracts to penetrate the membrane of resistant bacterial strains was evaluated for 24 hours at two different concentrations (MIC and 2MIC). It appears from the figures below that the extracts showed variable capacities of variable destabilization depending on the strain tested. Figures 1 and 2 illustrate the destabilization rates of the active plant extracts on the membrane of the different bacterial strains used.

Figure 1 shows that the destabilization varies with the concentration of extracts (aqueous and ethanolic) from the leaves and roots on the membrane the *S. aureus* strain ATCC 25923. The percentage of destabilization of aqueous and ethanolic extracts of leaves and roots at the IJC was the same with the reference molecule (imipenem). For 2MIC, membrane destabilization of *S. aureus* ATCC 25923 by leaf and root extracts was better compared to imipenem. On the other hand, hydro-ethanolic extracts from the leaves and roots showed a percentage of destabilization higher than the reference molecule (imipenem) at the MIC. The best percentages of destabilization were noted with aqueous extracts from the leaves (MIC: 64.11 ± 0.11%; 2MIC: 81.11 ± 0.02%); Ethanol (MIC: 64.11 ± 0.08%; 2MIC: 81.11 ± 0.57%) and hydro-ethanolic (MIC: 91.22 ± 0.12%; 2MIC: 88.62 ± 0.12%) of the root of J. multifidaon the membrane of *S. aureus* ATCC

Figure 2 illustrates the membrane destabilization capabilities of extracts (aqueous, ethanolic and hydroethanolic) from leaves and roots on the *K. pneumoniae* strain. It varies according to the concentrations of extracts used. The percentage of destabilization of the ethanolic and hydro-ethanolic extracts of the *J. multifida* leaf at the IJC is higher than that of the root and reference molecule (imipenem) extracts. As for 2MIC, the destabilization of the *K. pneumoniae* membrane by leaf and root extracts was better compared to imipenem. The best percentages of destabilization at MIC and 2MIC concentrations were noted with aqueous extracts (MIC: 50.02 ± 0.07%; 2MIC: 62.20 ± 3.25%); ethanolic (MIC: 50.02 ± 0.03%; 2MIC: 62.20 ± 0.04%) and hydro-ethanolic (MIC: 71.88 ± 0.04%; 2MIC: 79.57 ± 1.35%) extracts from *J. multifida*leaveson the membrane of the *K. pneumoniae* strain.

## Discussion:-

The present study aimed to compare the antibacterial activity of aqueous, ethanolic and hydro-ethanolic extracts from the organs (roots, leaves and bark) of *Jatropha curcas, J. multifida*and *Zanthoxylum zanthoxyloides*. These plants are species whose roots are traditionally used in Benin in the treatment of infections. Antibacterial activity was assessed on *S. aureus* strains ATCC 25923 and *K. pneumoniae*. The present study shows that aqueous, ethanolic and hydro-ethanolic extracts from plant organs showed variable yields at extraction. This variation in yields can be explained by the solvents used and the extraction capacity of each solvent (Akinmoladun et al., 2022).

Determination of the content of polyphenols and total flavonoids on plant extracts. Indeed, the extracts (aqueous, ethanolic and hydro-ethanolic) obtained from the leaves and bark of Jatropha curcas are richer in flavonoids than those from the roots. Extracts from the bark of *Jatropha multifida* followed by those from the leaves are significantly richer in flavonoids than those from the roots. As for *Zanthoxylum zanthoxyloides*, the extracts of the leaves are particularly richer in flavonoids than the other organs. The polyphenol contents of the aqueous, ethanolic

and hydro-ethanolic extracts, leaves and bark of *J. curcas*are better. For *J. multifida*the best concentrations were obtained for the bark extracts while the low concentrations were noted for the leaves. Concerning *Z. zanthoxyloides*, all extracts from the roots are less rich in polyphenols than those of the other organs. The presence of phytochemicals such as saponin, steroids, tannins, glycosides, alkaloids, polyphenols and flavonoids in extracts from the leaves and bark of *J. curcas*, *J. multifida* and *Z. zanthoxyloides*has been reported in several studies (Carvalho et al., 2018; El Diwani et al., 2009; Igbinosa et al., 2009; Kosh-Komba et al., 2017). Based on the work of Igbinosa et al.(2011) and El Diwani et al.(2009), the determination of the total polyphenol and flavonoid content of *J. curcas*bark extracts showed lower polyphenol and flavonoid values compared to those found in the present study. This difference in content between the extracts obtained and other previous studies could be equated with the extraction conditions, the extraction process, and the quality of the solvents used (Akinmoladun et al., 2022). The presence of these chemical compounds in these plants is the basis of their antimicrobial powers. Indeed, the antimicrobial and antioxidant properties of these chemical compounds are well known (Agban et al., 2020; El Diwani et al., 2009; Hirota et al., 2012).

In terms of antioxidant activity, ethanolic and hydro-ethanolic extracts from the leaves and bark of *J. curcas* and *Z.zanthoxyloides*inhibited the DPPH radical at better concentrations compared to extracts from their roots. Also, all extracts of the bark of *J. multifida*have shown the best inhibitory powers of the DPPH radical. The results obtained are lower than those found by Hirota et al.(2012) in a study carried out on *J. multifida*. The difference observed in the results of antioxidant activity obtained and other previous studies could be due to the level of maturity of the leaves, bark and roots collected, the time of harvest and the drying conditions (Assefa et al., 2008; Chen et al., 2022). The antioxidant power of medicinal plants could be attributed to the presence of phenolic compounds and flavonoids(Carvalho et al., 2018; Garde-cerdán et al., 2017; Igbinosa et al., 2011).

For the antibacterial activity of the extracts of the different plant organs with regard to the bacterial strains, it appears that all the extracts showed a variable activity depending on the strains tested. All extracts from the leaves, bark and roots of *J. curcas*were active on *S. aureus* ATCC 25923with the exception of the aqueous extract of the roots. The hydro-ethanolic extract of the roots showed better activity compared to the other extracts. As for the bacterial strain of *K. pneumoniae*, only bark extracts and ethanolic and hydroethanolic extracts from the leaves as well as hydroethanolic extracts from the roots were active.

Hydro-ethanolic extracts from the bark and roots presented the best activities. According toIgbinosa et al.(2009), aqueous and ethanol extracts have antibacterial activities on *Staphylococcus aureus* ATCC 25923and *Klebsiella pneumoniae*. This study, consistent with the present study, demonstrated the ability of *J. curcas*extracts to inhibit bacterial strains.

All extracts from the leaves, bark and roots of *J. multifida* were active on *S. aureus* ATCC 25923but only the ethanolic extract from the bark was the most active. For the strain of *K. pneumoniae*, all extracts showed activity except the ethanolic extract of the roots; and only the hydro-ethanolic extracts of the leaves and bark showed better bactericidal activities. According to Fitria, (2018), extracts of *Jatropha multifida*bark showed antibacterial and antibiotic activity on methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus*. This antimicrobial potential of extracts against *Staphylococcus aureus* NCTC6571 has been shown with all aqueous and ethanolic extracts of leaves, bark and roots (Aiyelaagbe et al., 2008). These studies are in line with the results obtained in the present study by confirming the antibacterial properties of the extracts of the different organs of *J. multifida*on bacteria.

For *Z. zanthoxyloides*, all extracts of the leaves, bark and roots were active on *S. aureus* ATCC 25923 with the exception of ethanolic leaf extracts and aqueous bark extract. Only the ethanolic extract of the roots showed good bactericidal activity. For *K. pneumoniae*, all extracts from the leaves, bark and roots were active with the exception of the aqueous extract from the leaves. Only the hydro-ethanolic extract of the leaves showed the best bactericidal activity. According to Kosh-Komba et al. (2017), hydro-ethanolic extracts from the leaves, bark and roots of *Z. zanthoxyloides* showed an antibacterial effect on *Klebsiella pneumonia* and *Staphylococcus aureus*.

The work carried out by Agbulu et al.(2015) showed the antibacterial potential of *Z. zanthoxyloides* extracts on *Staphylococcus aureus* ATCC 25923 and *Klebsiella pneumoniae*. The results obtained in this work are consistent with those of Agbulu et al.(2015)andKosh-Komba et al.(2017), who demonstrated that extracts from the leaves and bark of *Z. zanthoxyloides*have activity on bacterial strains. Only ethanolic and hydro-ethanolic extracts from plant

organs (leaves, bark and roots) (*J. curcas, J. multifida*and *Z. zanthoxyloides*) showed the best antibacterial activities depending on the plants, organs and strains used compared to aqueous extracts. This is due to the strong polarity of ethanol (Akinmoladun et al., 2022). Several bacterial strains have been used to evaluate the mode of action of the active extracts.

In the present study, the bacterial external membrane permeability test was chosen to assess the mode of action of aqueous, ethanolic and hydro-ethanolic extracts of the leaves and roots of *J. multifida* on *K. pneumoniae* and *S.aureus* ATCC 25923. The results obtained revealed the membrane-destabilizing power of the bacterial strains of the tested extracts. These extracts showed a superior destabilizing power compared to the Imipenem used here as a reference molecule. These results show that the extracts have a consequent mode of action on the destabilization of the outer membrane of the bacterial strains tested. In addition, regardless of the concentration of leaf extracts used, the destabilization of the membrane of *S. aureus* ATCC 25923 strainsdoes not change. However, destabilization is dependent on the concentration on the membrane of *K. pneumoniae* strains. In the case of *S. aureus* ATCC 25923, destabilization increased as the concentration of leaf extracts increased. While it is independent on the membrane of the *K. pneumoniae* strains. Therefore, the administration of leaf extracts could vary between MIC and 2 MIC on *S. aureus* ATCC 25923strains.But, it could be administered at the IJC on strains of *K. pneumoniae*. The phytochemical composition of plant extracts could be at the origin of their high potential for membrane destabilization (Frirdich and Whitfield, 2005). In addition, it has been reported that the destabilization and dysregulation of interactions between lipopolysaccharide molecules are caused by phenolic compounds (flavonoids, tannins, etc.) and terpenoids (Frirdich and Whitfield, 2005; Vaara, 1992). The explosion of the cytoplasmic membrane and the dysregulation of ionic homeostasis between the intracellular and extracellular compartments of Gram-negative bacteria are responsible for the antibacterial effect of plant extracts (Kumar et al., 2013; Yala et al., 2016). The results of this study therefore confirm the previous results.

## Conclusion:-

The present study was carried out as part of a comparison of the antibacterial activity of renewable organs (leaves, bark) of 03 medicinal plants of the Beninese flora whose roots are used in the treatment of infections. The present study showed that hydro-ethanolic extracts from the bark and leaves of *J. curcas* and *Z. zanthoxyloides*, respectively, showed good antibacterial activities. Similarly, hydro-ethanolic extracts from the leaves and bark of *J. multifida*have shown strong antibacterial potential. The other plant organs expressed great therapeutic potential in the present study and should be advocated in the treatment of infections instead of roots in order to preserve biodiversity. The results obtained on the mechanism of action of plant extracts give them potential to destabilize the membrane of bacteria. It is therefore very important to carry out research on the toxicity of these plant organs in order to minimize health risks and propose them as candidates for ATMs.

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**Competing Interests**

The authorsdeclarethat they have no competinginterestconcerningthis article.

**Authors' Contributions**

All authorscontributed to the realization of the work and to the manuscript preparation.

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