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Chemical Characterization and Evaluation of the Antioxidant and Antibacterial Activities of Methanolic Extracts of the Root Bark of *Terminalia Avicennioides* (Combretaceae)

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Abstract *Terminalia avicennioides* is a plant widely used in the Central African Republic for the treatment of numerous pathologies with anti-inflammatory components. The main objective of this study was to identify the chemical constituents and evaluate the antioxidant and antibacterial activities of *Terminalia avicennioides* root bark extracts. The phytochemical study showed that the methanolic extract was the best extraction solvent with a yield of 21.78%. Qualitative analysis revealed the presence of flavonoids, tannins, saponins, sterols and triterpenes. Quantitative analysis showed that the methanolic extract had a high total phenolic content, i.e. 2149 ± 1391,10 mg EqAG/g Ms. Evaluation of the free radical scavenging capacity of methanolic extracts of *Terminala. avicennioides* using the DPPH method gave a moderate IC_{50} value with an R^2 = 0,75. The antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was studied using the diffusion method. The zone of inhibition, inhibitory concentration and minimum bactericidal concentration of the extracts on the organisms were evaluated. The methanolic extract inhibited the growth of *E. coli* and *S. aureus* with MICs between 0,45-4,00 µg/mL and MBCs between 4,50-22,2 µg/mL. The root bark of T. avicennioides showed significant antibacterial activity due to its high content of polyphenolic compounds.

Keywords: *Terminalia avicennioides, chemical characterization, antioxidant activity, antibacterial activity*

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1. Introduction

According to the World Health Organization, approximately 81% of the population relies on traditional herbal preparations for primary health care [1].

Currently, more than 120 plant compounds are used in modern medicine, and almost 75% of these are used according to their traditional use [2]. From the 25 best-selling pharmaceutical compounds in the world, 12 are derived from natural products [3], demonstrating that the number of drugs derived from combinatorial chemistry, where more than 10,000 molecules must be synthesized and then tested, has led to the development of one drug.

Compounds derived from natural substances have the advantage of having a very large diversity of chemical structures and a very wide range of biological activities [4].

Antibiotic resistance among pathogenic microorganisms has become an increasingly important public health problem worldwide. Plant-derived antimicrobial compounds are capable of inhibiting bacterial growth by acting on cellular targets different from those targeted by currently used antibiotics such as penicillins, macrolides or tetracyclines. They could also be of significant clinical value in the treatment of infections with resistant microbial strains [5]. With antibacterial treatments, we are seeing more and more strains that are resistant to common antibiotics [6].

In Africa, medicinal plants generally play a very

important role in therapy due to their relatively low cost and availability. For this reason, the World Health Organization encourages the inclusion of controlled and effective medicinal plants in primary health care in developing countries [7].

In Central African Republic, recent political crises have destroyed the entire health system. In remote areas, poor people are forced to use medicinal plants for primary treatment, not to mention the fact that these medicinal plants have secondary metabolites that have not yet been evaluated.

One such plant is *Terminalia avicennioides*, a savannah plant known by different ethnic groups in Central Africa under different popular names. Why *Terminalia avicennioides*?

Terminalia. avicennoiides has been selected for its uses in traditional medicine both in Africa and in Asia for the treatment of microbial infection. *Terminalia avicennioides* is indicated in the treatment of certain diseases such as gastrointestinal disorders, urinary tract infections, ulcers moebic dysentery, hemorrhoids, cavities and malaria [8]. Many of the species from combretaceae family are reputed to contain antimicrobial constituents [9] in the treatment of a range of conditions, including syphilis, wounds gastric ulcer [10]

Pharmacological sutides have shown that the stem barks of *Terminalia avicennioides* are used in traditional medicine in Nigeria in the treatment of painful menstruation, menorrhagia, trypanosomiasis, diarrhea, tuberculosis, cough, wounds especially for a better healing of skin infections, dental caries [11,12].

The frequent use of this species in the treatment of these various pathologies in the Central African Republic has shown interesting antioxidant, antibacterial and even antifungal activities. *Terminalia avicennioides* has shown remarkable antioxidant activity and significant antibacterial activity against certain bacteria and micro-organisms. The aim of this study is to evaluate the antioxidant and antibacterial activities of the methanolic extract of *Terminalia avicennioides* on microbial strains.

2. Materials and Methods

2.1. Plant Material

Origin of the plant: The study was carried out on the root bark of *Terminalia avicennioides*, collected in September 2023 in the village of Yassara, about 15 km north of Bangui (Central African Republic) (figure 1).

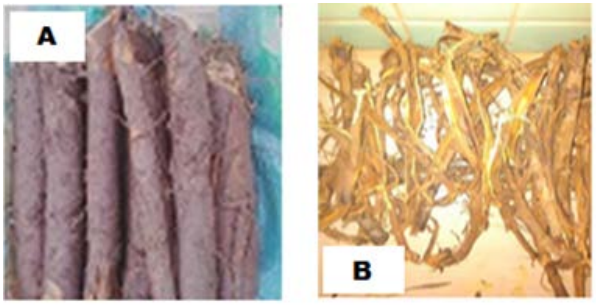

Figure 1. (**A**) Roots and (**B**) Stem bark

2.2. Drying Technique

Roots were harvested at 8 am on 18 September 2024 in the village of Yassara, 15 km from Bangui in the Begoua region, and taken to the laboratory the following day. One week later, the stem bark was removed from the root and dried in a dark, dry place for 24 days before being ground into a powder and stored in a jar in the laboratory for extraction.

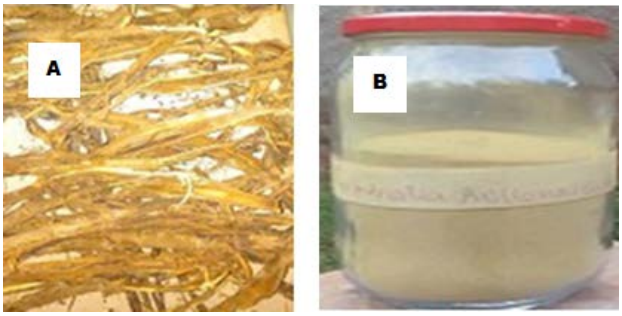

Figure 2. (**A**) Dried stem bark and (**B**) Powder

2.3. Biological Material

To evaluate the antibacterial activity of the plant studied, two important pathogenic bacterial species were selected for the study, namely *Staphylococus aureus* and *Escherichia coli*. These bacterial strains were isolated at the National Laboratory of Clinical Biology and Public Health (LNBCSP) in Bangui (Central African Republic).

2.4. Chemical Characterization

2.4.1. Preparation of Extracts

The cold maceration method was used to extract the plant under study. 100 g of *Terminalia avicennioides* powder was extracted successively with four solvents of increasing polarity (dichloromethane, acetone, cyclohexane and methanol). 400 mL of cyclohexane was added to the plant powder and stirred for 4 hours at room temperature. After filtration, the extract was evaporated with a Rotavapor at 25°C and the residue was taken up with the next solvent.

2.4.2. Qualitative Analysis

The presence of major chemical groups in the extracts was investigated using the tests described by [13,14]: flavonoids (cyanidine reaction), alkaloids (Dragendorff and Mayer test), triterpenes and sterols (Libermann-Burchard test), saponosides (foam index), tannins (ferric chloride test and Stiasny reaction).

2.5. Quantitative Analysis

2.5.1. Determination of Total Polyphenols

Total polyphenols were determined using the method described by [15,16] with slight modifications.

A solution of gallic acid (So solution) with a concentration of 2,3 mg/mL was prepared and diluted 20 times in water. This solution was used to prepare a series of gallic acid solutions at different concentrations. We then prepared a 3 mg/mL solution of each extract in DMSO.

We added 20 mL of the extract solution to the cells of a 96-well microplate and then added 100 mL of Folin-Ciocalteu reagent. For the extract blank, Folin was replaced by water. The mixture was shaken for 30 seconds and then incubated in the dark for 5 minutes, to which 80 mL of Na_2CO_3 solution (75 g/l) was added, shaken again for 30 seconds and then incubated for 15 minutes. The absorbance was read at a wavelength of 620nm. Three tests were performed for each product concentration tested.

2.5.2. Determination of Total Flavonoids

Total flavonoid content was determined according to the method described by [15,16] with some modifications.

Briefly, 100 mL of the extracts at 3 mg/mL were added to 96-well microplates, followed by 100 mL of $AlCl_3$ solution prepared at 0,02 g in 50 mL methanol. The blank was prepared by replacing the $AlCl_3$ solution with MeOH. For the control blank, DMSO was used instead of the extracts. The mixture was vortexed for 30 seconds and incubated for 15 minutes. Absorbance was measured at a wavelength of 415 nm against the blank. Quercetin was used as a reference standard to establish the calibration curve and to quantify the total flavonoid content, expressed as milligrams of quercetin equivalent per gram of extract (mg Eq Quer/g extract). Assays were performed in triplicate for each sample.

2.5.3. Determination of Condensed Tannins

The condensed tannin content was determined according to the method described by [15,16]

A 1% vanillin solution was prepared in H_2SO_4 (7M). 50 mL of the extracts (3 mg/mL) were added to 96-well microplates. 150 mL vanillin solution was then added. The mixture was vortexed for 30 seconds and incubated for 15 minutes at room temperature. The absorbance was measured at a wavelength of 500 nm. Tests were performed in triplicate for each sample. A stock solution of catechin was used as a reference standard to establish the calibration curve and to quantify the condensed tannin content, expressed as milligrams of catechin equivalent per gram of dry matter (mg Cat Eq/g dry matter).

2.5.4. Determination of Anthocyanins

The determination of anthocyanins was carried out according to the method described by [15] with a slight modification.

Two solutions were prepared. A solution at pH = 1 containing KCl (0,2M) and HCl (0,2M). The mixture of CH_3COOH, CH_3COONa with concentrations (0,2M) and water (H2O). For anthocyanin quantification, 100 µL of extract is added to 100 mL of pH = 1 solution in four wells, then the other four wells contain the mixture of 100 µL of extract + 100 µL of pH = 4,5 solution. The absorbance of the extract is measured at 450 and 620 nm after incubation for 15 minutes at room temperature. The change in absorbance is calculated using the following expression:

$$Abs = \left(Abs_{510} - Abs_{700}\right) pH\ 1{,}0 - \left(Abs_{510} - Abs_{700}\right) pH\ 4{,}5$$

The total anthocyanins in the samples are expressed as milligram equivalents of cyanidin-3-glucoside per gram of dry matter (mg Eq C3GE/g Ms).

2.6. Biological Activity

2.6.1. Antioxidant Activity

This method is based on the use of a free radical: DPPH, as described by [15,17] with a slight modification. The reduction of DPPH is accompanied by a change in the colour of the solution from purple to yellow, which can be measured spectrophotometrically at 750 nm. This indicates antioxidant activity. The intensity of the colour change, measured with a spectrophotometer, is inversely proportional to the antioxidant activity of the extracts whose activity is being determined.

For our extract, we prepared a solution of DPPH at 0.35 mg/ml in methanol. This solution was diluted 10 times and stored in a refrigerator at 4°C, protected from light. Four dilutions were prepared from the stock solution of each extract (MeOH, acetone, dichloromethane and cyclohexane) at different concentrations (60, 150, 240 and 300 mg/L).

A pasteurized pipette was used to add 20 µL of each extract and 180 µL of DPPH to each microplate well. The blank was 20 µL DMSO and 180 µL MeOH. Readings were taken at 750 nm after 25 minutes incubation in the dark. Ascorbic acid was used as a standard, prepared under the same conditions as the extracts. All extracts were reproduced at least 4 times to minimize error. The results were expressed as percentage inhibition (%I).

$$\%\ Inhibition = \left[\frac{Abs\ blanc - Abs\ extraits}{Abs\ blanc}\right] \times 100$$

2.6.2. Antibacterial Activity

2.6.2.1. Microorganisms

Microorganisms were provided by the National Laboratory of Clinical Biology and Public Health. Two strains of bacteria were isolated and provided for the test.

2.6.2.2. Determination of Biological Activity

For the evaluation of antibacterial activity, the methanolic extract of *Terminalia avicennioides* was used.

The microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

2.6.2.3. Preparation of Culture Media

We prepared a series of antibiotics of decreasing concentration by successive dilutions (0,5; 0,1 and 0,01 mg/mL) from a solution of 0,5 mg/mL of antibiotic. We prepared a series with the same volume of liquid culture, and then the same amount of bacteria was added to each

tube (inoculum). The prepared tubes were incubated at 37ºC for 24 hours and examined. The MIC is determined from the cloudiness of the tubes: a cloudy medium means that the culture has grown and the concentration is not high enough to block bacterial growth. Conversely, a clear medium indicates that the culture has not grown.

2.6.2.4. Determination of the Minimum Inhibitory Concentration (MIC)

A slightly modified serial micro-dilution test [18,19] was used to determine the value of the minimum inhibitory concentration of the methanolic extract of Terùinalia avicennioides.

Final test concentration ranges of 0,5; 0,1 and 0,01 mg/mL were used from an aqueous solution of Terùinalia avicennioides from a 0,5 mg/mL solution of the antibiotic. Each tube was covered and incubated at 37°C for 24 hours. Clear staining of the well was interpreted as absence of growth and wells with a turbid appearance were considered positive due to bacterial growth. Each experiment was replicated twice.

2.6.2.5. Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration is the concentration of antibiotic required to achieve 0.01% viable bacteria after 24 hours incubation at 37°C [20]. It was determined according to the method of [18].

It can be determined using a broth dilution minimum inhibitory concentration (MIC) test by subculturing bacteria that do not contain the antibacterial agent being tested. The susceptibility of the bacterial strains was assessed on the basis of macroscopic and microscopic aspects. The concentration of the tube containing less than 0.01% viable bacteria with respect to the initial inoculum constitutes the MBC [21]. The results are interpreted using the ratio (MBC/MIC).

3. Results and Discussions

3.1. Extraction and Fractionation Yields

The yields of the cyclohexane extract and the different fractions are shown in Figure 3, which shows that the yield of the cyclohexane extract of *Terminalia avicennioides* root bark was 0,22%. The yields of the fractions varied from 1,17% for the dichloromethane fraction, 7,.42% for the acetone fraction and 21,78% for the methanol fraction. The best yield was obtained with the methanol fraction, with a value of 21,78%.

The yield obtained with the methanol fraction, with a value of 21.78%, indicates the greater presence of moderately polar secondary metabolites in the methanol extract of the root bark of *Terùinalia vicennioides*. Our yields are significantly lower than those obtained by [22], with a value of 0,8% for the cyclohexane extract and 2,10% for the dichloromethane extract, and higher than those obtained by [23].

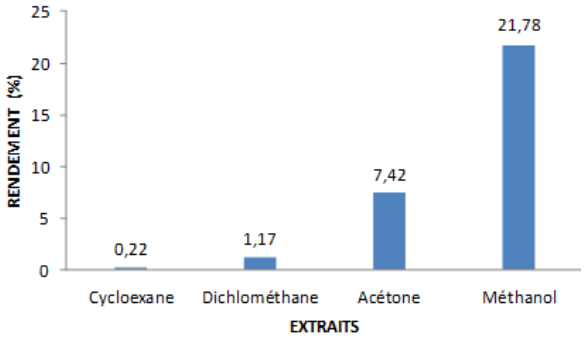

Figure 3. Yield of *T. avicennioides* extracts

3.2. Chemical Characterization

3.2.1. Qualitative Analysis Using Tube Reactions

The qualitative analysis by tube reactions carried out on extracts of the root bark of *Terminalia. avicennioides* is presented in Table 1.

The results of the chemical screening showed the presence of secondary metabolites: flavonoids, tannins, saponosides, triterpenes and sterols. Alkaloids and anthocyanins were not found in the plant organ studied.

Phytochemical analysis of the extract showed that it was rich in flavonoids, tannins, saponins, triterpenes and sterols. The results obtained are identical to those obtained by [24] on the same species. The presence of flavonoids, tannins and saponosides would demonstrate the potential of the extract for antibacterial activities [25].

Table 1. Results of tube reactions of *T. avicennioides* root bark

Echantillon	Alcaloids	Tanins	Anthocyanes	Saponins		flavonoids	Triterpens et Stérols
T. avicennioides					Foam index		
Results	-	++	-	++	1525	++	++

++: Abundant –: Absent

Table 2. Content of total polyphenols (mg Eq AG/g Ms), flavonoids (mg Eq Quer/g Ms), condensed tannins (mg Eq Cat/g Ms) and anthocyanins (mg Eq C3GE/g Ms) extracted from *Termnalia avicennioides*

Extract	Polyphénols	Flavonoids	Tanins	Anthocyanins
MeOH	2149,90 ± 13	0,02 ± 0,00	ND	ND
Acétone	1980, 40 ± 14	0,02 ± 0,00	ND	ND
DCM	830,20 ± 11	0,02 ± 0,00	ND	0,48 ± 0,88
Cyclohexane	100,70 ± 0,90	0,02 ± 0,00	ND	7,87 ± 0,38

Values are the mean of three replicates ± Sem, ND: Not determined.

3.2.2. Content of Polyphenols, Total Flavonoids, Condensed Tannins and Anthocyanins

The contents of total polyphenols, total flavonoids, condensed tannins and anthocyanins in *Terminalia avicennioides* extracts were determined separately by colorimetric methods (Folin-Ciocalteu, aluminium chloride, sulphuric vanillin and the change in anthocyanin colour as a function of pH (differential pH method)). The quantitative analyses of total phenolics, total flavonoids, condensed tannins and anthocyanins were determined from the linear regression equations of the calibration curves, expressed as mg gallic acid equivalent, mg quercetin equivalent, mg catechin equivalent and mg cyanidin-3-glucoside equivalent per gram of dry matter, respectively.

The results of the quantitative analyses are given in Table 2.

The results of quantitative analyses by UV-visible spectrophotometry of the various *Terminalia avicennioides* root bark extracts studied are shown in Table 2 above.

We note that the methanolic extract of the root bark of this species is quantitatively richer in phenolic compounds with a content of 2149,90 ± 13 mg Eq AG/g Ms, followed by the acetone extract with a content of 1980,4 ± 145,6 mg Eq AG/g Ms. The dichloromethane and cyclohexane extracts have low contents ranging from 830,20 ± 119,8 to 100,7 ± 0,90 mg Eq AG/g Ms. Flavonoids were present but at very low levels in the different extracts. Anthocyanins were also present in the dichloromethane and cyclohexane extracts. We found that this plant has high levels of total polyphenols compared to flavonoids in the different extracts.

These results confirm the high level of phenolic substances present in *Terminalia. avicennioides*, as shown by the tube reactions.

3.3. DPPH radical Reduction Test on a 96-Well Microplate

The four (04) *Terminalia avicennioides* extracts studied showed high inhibition percentages in the root bark (Table 3).

Table 3. Results of percentage inhibition of antioxidant activity of different extracts of the *Terminalia avicennioides* plant

Extracts	Percentage inhibition	IC_{50} (mg/mL)
Methanol	90,76 ± 02,81	0,016
Acetone	92,94 ± 00,42	0,016
Dichloromethane	82,44 ± 00,53	0,020
Cyclohexane	52,70 ± 05,66	0,22
Vitamin C	94,43 ± 00,42	0,025

Values are the average of three replicates ± sem.

The results of the antioxidant activity of the four extracts carried out on 96-well microplates gave inhibition percentages of around 90,76 ± 02,81, 92,94 ± 00,42, 82,44 ± 00,53, 52,70 ± 05,66 for the methanol, acetone, dichloromethane and cyclohexane extracts respectively. Polar extracts had a higher inhibition percentage than apolar extracts. However, the four (04) extracts studied had a relatively high free radical scavenging capacity compared to the reference molecule, ascorbic acid, which had an inhibition percentage of 94,43 ± 00,42.

These results show that all the extracts have a better antioxidant activity, which is consistent with the total polyphenol content, which is high in the polar extracts and moderately low in the apolar extracts (Table 2). Our results are close to those obtained by [25]

3.4. Correlation between Total Phenolics and Antioxidant Activity (DPPH)

The results obtained show a good linear correlation (R^2 = 0.75) between the total phenolic content and the antiradical power of *Terminalia avicennioides* extracts (Figure 4).

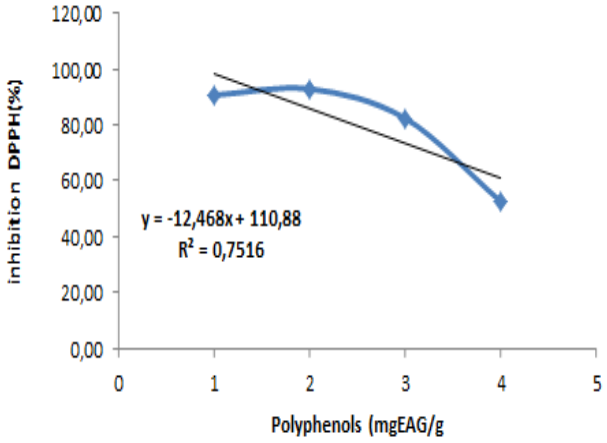

Figure 4. Correlation between total phenolic content and antioxidant activity

Looking at the results in Figure 4 above, a positive correlation was observed due to the fact that plants richer in phenolic compounds have the highest oxidative activity. These results are in line with what has been stated in the literature by some authors that the antioxidant activity potential of an extract depends on its phenolic compound content [26,27]. It is clear that the high antioxidant activity recorded in our different extracts (Table 2) is due to their richness in phenolic compounds.

3.5. Evaluation of Antibacterial Activity

The results of the in vitro antibacterial activity of the methanolic extracts of *Terminalia avicennioides* with the different concentrations on the bacterial strains, viz: *Staphylococus aureus* and *Escherichia coli* are presented in Table 4 below.

This table shows the macroscopic and microscopic aspects of *Terminalia. avicennioides* methanolic extract in liquid medium at different concentrations on *S. aureus* and *E. coli* strains.

In view of the results obtained (Table 4), the antibacterial activity was carried out up to a concentration of 0,25 mg/mL on *S. aureus* and 0,05 mg/mL on *E. coli*. The disturbances observed in the tubes between 0,05 mg/mL and 0,005 mg/mL for *S. aureus* and then at 0,005

mg/mL for *E. coli* explain the resistance of the bacteria at these concentrations.

Table 4. Macroscopic and microscopic aspects of methanolic extract on bacterial strains

Germs	Tube	Concentration (mg/mL)	Aspects	
			Macroscopic	Microscopic
S. aureus	1 Control tube	0	Nothing	Nothing
	2	0,25	Nothing	Nothing
	3	0,05	Trouble at the bottom of the pipe	presence of bacteria
	4	0.005	Trouble at the bottom of the pipe	presence of bacteria
E. coli	5	0,25	Nothing	Nothing
	6	0,05	Nothing	Nothing
	7	0.005	Trouble at the bottom of the pipe	presence of bacteria

3.5.1. Determination of MIC and MBC

The MIC is the lowest concentration of the substance at which no growth is visible to the naked eye after 24 hours incubation. It is determined by the absence of cloudiness in the tube. On the other hand, the minimum bactericidal concentration (MBC) is the lowest concentration of a substance that leaves no more than 0.01% of germs behind.

The results giving MIC and BMC values in mg/ml and the ratio of BMC to MIC for the methanolic extract of *Terminalia. avicennioides* are given in Table 5 below.

Table 5. Minimum inhibitory concentrations, minimum bactericidal concentrations and their ratios for *S. aureus* and *E. coli* strains

Germs	MIC (µg/ml)	BMC (µg/ml)	CMB/CMI	Plant extract and antibiotic
E. coli	0,45	4,5	10	*T. avicennioides*
	2,5	2,5	1	Gentamicin
S. aureus	4,5	22,72	5	*T. avicennioides*
	2	2,5	1,5	Gentamicin
	1,5	2	0,75	Gentamicin

In view of the results in Table 5, we note that the methanolic extract of *Terminalia avicennioides* root bark inhibits the growth of *S. aureus* and *E. coli* with a minimum inhibitory concentration of 0,25 mg/L for *S. aureus* and 0,05 mg/L for *E. coli* respectively.

These results suggest that this plant could be a potential source of compounds with significant antibacterial activity. The antibacterial activity in the methanolic extract would therefore be linked to a synergistic effect between the different phytochemical groups present, such as tannins, flavonoids, saponins, triterpenes and sterols, all of which have antibacterial activity according to the literature [28,29].

This antibacterial activity is due to its high tannin and flavonoid content according to [19]. Similarly, [30] found a MIC between 0,06 and 0,512 mg/mL, while [31] found an MIC between 0,1 and 10 mg/mL in the methanolic extract of different organs of the same species (leaves, stems and roots) against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis* and *Escherichia coli*. These differences have been explained by the parts of the plant used, the germs of the bacteria tested and the solvents used for the extractions.

The ratio of MBC to MIC has made it possible to specify the mode of action of the substance [32]. According to [33], the extract is bactericidal if its MBC is equal to its MIC or if the ratio of MBC to MIC is less than or equal to 4. It is bacteriostatic if its MBC is greater than or equal to its MIC or if the ratio of MBC to MIC is greater than 4.

With regard to the MBC to MIC activity ratio, *S. aureus* gives a value of 5, which is more than 4 times the MIC [19], so *S. aureus* is considered to be an antibacterial agent. The MBC to MIC ratio for *Escherichia coli* gives a value of 10, which is more than 4 times the MIC, so *E. coli* is considered to be a bactericidal agent. This result shows and confirms that the plant *Terminalia avicennioides* is highly active in the treatment of digestive disorders, abdominal pain and diarrhea. The effect of the methanolic extract on S. aureus is also confirmed by the work carried out by [34] with ethanol and acetone extracts on the same strains.

The evaluation of antibacterial activity of *Terminali avicennioides* on the strains responsible of dysentery disorder is realized from the methanolic extract. The results show that the extract is confirmed by the results obtained by [30] that shows that the methanolic extract of *Terminalia avicennioides* inhibited the Growth of diverse bacteria. The methanolic extract is then more active and concentrate better antibacterial components. The fact that the methanolic extract is more active shows that the bioactive molecules solubilize better in methanol and concentrate better the active components. The antibacterial activity of the *Terminalia avicennioides* would be attributed to secondary metabolites such polyphenols and flavonoids [35] which would justify the significant activity of the metabolic extract.

4. Conclusion

This study identified several major chemical families in the root bark of *Terminalia avicennioides*. Phenolic assays showed that the different extracts of *Terminalia avicennioides* were rich in total phenolics. DPPH methods on 96-well microplates revealed the antioxidant activity of the different extracts of this plant. The results showed a good linear correlation between total phenolic content and antioxidant activity. The methanolic extract of *Terminalia avicennioides* also showed antibacterial activity against *S. aureus* and *E. coli*. *Terminalia avicennioides* could be used in the treatment of infectious diseases of bacterial origin. Supplementary in vivo studies could be envisaged to confirm the antibacterial activity, as well as acute toxicity tests using the methanolic extract of *Terminalia. avicennioides*, and the highlighted compounds could be isolated and identified.

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Conflicts of Interest

The authors declare that they have no competing interests.

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