EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 13
Issue: 11
Year: 2026

ANTIBACTERIAL ACTIVITY OF THE 70% HYDRO-ETHANOLIC EXTRACT OF *TERMINALIA MACROPTERA* (COMBRETACEAE) BARK AGAINST RESISTANT CLINICAL STRAINS OF *ESCHERICHIA COLI* FROM THE INSTITUT PASTEUR OF CÔTE D'IVOIRE, AND ANTIOXIDANT POTENTIAL

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DOI: https://doi.org/10.5281/zenodo.23803847

How to cite this Article: Traore Youssouf Zanga[1*], Ouattara Mohamed Baguy[2], Yao N'guessan Alain Roland[1], Kouadio Guesssend Nathalie[2], Ouattara Abou[3] (2026). Antibacterial Activity Of The 70% Hydro-Ethanolic Extract Of *Terminalia Macroptera* (Combretaceae) Bark Against Resistant Clinical Strains Of *Escherichia Coli* From The Institut Pasteur Of Côte D'ivoire, And Antioxidant Potential. European Journal of Biomedical and Pharmaceutical Sciences, 13(11), 42–48.
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Article Received on 27/09/2026 **Article Revised on 17/10/2026** **Article Published on 01/11/2026**

ABSTRACT

Background: This study is part of the ongoing effort to combat antibiotic resistance and diseases associated with oxidative stress. The aim of this work is to determine the antibacterial activity and antioxidant capacity of the hydroethanolic extract of *Terminalia macroptera* against clinical strains of *Escherichia coli*. **Methods:** The hydroethanolic extract was prepared using the technique described by Zihiri (2003). Tube-based staining techniques were used to identify phytochemical constituents, while the method of Blois (1958) involving the dissolution of the DPPH radical in absolute ethanol to achieve a concentration of 0.3 mg/mL was used to determine the antioxidant capacity of the 70% hydroethanolic extract**. Results:** The hydroethanolic extract yielded an acceptable 11.75 mg/mL. Several phytochemicals were identified, including polyphenols, saponins, tannins, flavonoids, steroids, and terpenoids. Inhibition zone diameters ranged from 19 to 25 mm, indicating good extract activity, while an MBC/MIC ratio of less than 2 demonstrated the extract's bactericidal effect against the clinical *Escherichia coli* strains tested. Antioxidant activity was assessed via DPPH radical inhibition, showing 80.19% inhibition for the extract compared to 71.53% for the reference compound, vitamin C. The concentration required for 50% inhibition (IC50) confirmed the extract's antioxidant potency, with an IC50 of 0.0022 mg/mL lower than that of vitamin C (0.0035 mg/mL). **Conclusion:** This study demonstrates that the 70% hydroethanolic extract possesses antibacterial properties against clinical strains of *Escherichia coli* and strong antioxidant activity for combating oxidative stress-related diseases.

KEYWORDS: Phytochemical screening, 70% hydro-ethanolic extract, *Terminalia macroptera* bark, clinical strains, *Escherichia coli*

INTRODUCTION

Uropathogenic *Escherichia coli* is a primary driver of antimicrobial resistance (AMR)[1] Globally, more than 405 million people are infected annually, resulting in approximately 230,000 deaths.[2] In Africa, the prevalence of resistance in *Escherichia coli* varies significantly by country. In Mali, rates of 18.5% and 13.62% were recorded in Bamako and Sikasso, respectively, in 2009.[3] In Guinea specifically in Conakry the prevalence is 62.2%[4], whereas in Côte d'Ivoire, it stands at 18%[5], particularly among newborns in Abidjan. However, it stands at 16.8% in

Daloa.[6] The prevalence of resistance in *Escherichia coli* (*E. coli*) is an alarming reality, as even newborns are not spared from this phenomenon. *Escherichia coli* is both a commensal of the digestive tract and a major pathogen. Regarding uropathogenic infections, *E. coli* is responsible for over 80% of cases. Between 20% and 40% of urinary tract infections are associated with *E. coli*.[7] Indeed, *E. coli* disrupts the intestinal system thanks to its genomic plasticity, which enables it to adapt effectively to both intestinal and extra-intestinal environments. Furthermore, the emergence and spread of multiple acquired resistance mechanisms have led to the appearance of multidrug-resistant strains. This phenomenon is observed in both environmental and hospital settings, sometimes leaving clinicians with limited therapeutic options.[8] Moreover, alongside antimicrobial resistance, other widespread diseases threaten public health in both urban and rural areas specifically, cancer. In Côte d'Ivoire, this is a genuine public health issue, as no social group or gender is spared; according[9], the prevalence rate is estimated at 35% across the various organs affected by this silent killer. While treatments do exist, they are very limited and prohibitively expensive for middle-income individuals. Given the damage caused by these scourges, alternative solutions are essential. It is in this context that we focused our interest on medicinal plants specifically *Terminalia macroptera*, which is highly effective in the traditional treatment of infectious diseases and certain types of cancer. The aim of this study is to determine, using a bio-guided approach, the effect of 70% hydro-ethanolic extracts of *Terminalia macroptera* on resistant clinical strains of *E. coli*, as well as the plant's antioxidant capacity.

MATERIALS AND METHODS

Materials

Plant material

Terminalia macroptera was collected in the Korhogo department, specifically in the village of "Tawara." Following collection, the plant was transported to the National Floristic Center (CNF) at Félix Houphouët-Boigny University to confirm its identification.

Microbial Material

The material consists of six clinical strains of *Escherichia coli* (*E. coli*). These clinical strains are: *E. coli* 505C/26, *E. coli* 944C/26, and *E. coli* 1011C/26, isolated from urine; *E. coli* 1015C/26 and *E. coli* 1374C/26, isolated from human blood; and finally, *E. coli* 1412C/26, isolated from a vaginal swab. All these strains originated from the Pediatrics and Neonatology Department of the Cocody University Hospital (CHU) and are stored in the biobank of the Institute Pasteur de Côte d'Ivoire.

Methods

Drying and pulverization of the leaves

After harvesting, the bark was dried for two to three weeks at laboratory temperature. Once dried, the bark was ground using a blender (Silver Crest, Germany) into a fine powder to increase the contact surface area between the solvent and the plant material. The resulting powder was packaged in kraft paper envelopes.

Extraction of bioactive compounds

The extraction of bioactive compounds is based on the affinity of these compounds for the solvent used. This extraction was performed according to the method described by.[10]

70% hydro-ethanolic extraction by maceration

A mixture of 100 g of plant powder and 1000 ml of 70% ethanol was placed in a blender for 5 minutes. The resulting mixture underwent several filtration steps to remove coarse particles. The obtained filtrate was evaporated in an oven at 40°C for 48 hours. Finally, the resulting extract was weighed to calculate the yield.

Yield calculation

Extraction yield is defined as the ratio of the mass of the obtained dry extract to the mass of the plant powder used. This yield (R) was calculated using the formula by.[11]

R (%) = (m/M) × 100 (1)

R = extraction yield as a percentage

m = mass of the dry extract

M = mass of the plant powder

Qualitative analysis of phytochemical compounds

Qualitative phytochemical analysis is performed using characteristic color tests to identify the major groups of chemical compounds present in the plant extract. Test-tube phytochemical assays are conducted according to the protocols described by.[12] Results are recorded as (+) if the target chemical compound is present and (-) if it is absent. Each phytochemical test was repeated twice under the same conditions.

Detection of polyphenols

Polyphenols are detected using the ferric chloride ($FeCl_3$) reaction. Three drops of a 2% alcoholic ferric chloride solution are added to 2 ml of the plant extract solution. The appearance of a blue-black or green coloration varying in intensity indicates the presence of polyphenols.

Detection of flavonoids

Flavonoids are detected using the sodium hydroxide reaction. Two to three drops of a basic sodium hydroxide solution (10% NaOH) are added to a test tube containing 2 ml of the extract solution. The appearance of a red to yellow-orange coloration indicates the presence of flavonoids.

Detection of anthocyanins

Anthocyanins are identified through a reaction with sulfuric acid. We added 2 ml of 2N sulfuric acid (H2SO4) to 2 ml of each solution. The appearance of a pink-red coloration, which turns bluish-violet upon the addition of ammonia, indicates the presence of anthocyanins.

Alkaloid detection

Two drops of Dragendorff's reagent (potassium iodobismuthate reagent) are added to a test tube containing 2 ml of plant extract. The appearance of a precipitate or an orange coloration indicates the presence of alkaloids. In the second test, two drops of Bouchardat's reagent (iodine-iodide reagent) are also added. The appearance of a reddish-brown coloration indicates a positive reaction.

Detection of sterols and polyterpenes

The Liebermann reaction is used to detect sterols and polyterpenes. To perform this test, 5 ml of plant extract was evaporated to dryness in a dish on a sand bath. The residue was dissolved while hot in 1 ml of acetic anhydride in a dish. Then, 0.5 ml of concentrated sulfuric acid (H2SO4) was added to the solution. The appearance of a purple-violet ring at the interface shifting to blue and then green indicates a positive reaction.

Detection of tannins

¬ Catechic tannins (condensed tannins)

Catechic tannins are detected using the Stiasny reaction. We added 2 mL of Stiasny reagent (30% formalin / concentrated HCl) to 2 mL of each plant extract. The mixture was then heated in a water bath at 80°C for 30 minutes and subsequently cooled. The appearance of large, light-brown flakes indicates the presence of catechic tannins.

¬ Gallic tannins (hydrolyzable tannins)

To detect gallic tannins, the solution previously used to test catechic tannins is filtered to remove the large flakes. The filtrate is then saturated with sodium acetate (CH3COONa), and a drop of 2% alcoholic ferric chloride solution is added. A positive reaction is indicated by the appearance of an intense blue-black coloration, signifying the presence of gallic tannins.

Detection of coumarins

Coumarins were detected using a 10% NaOH reaction. Two milliliters of each extract were distributed into two test tubes; 1.5 ml of 10% NaOH was added to one of the tubes, and both were then heated in a water bath until boiling. After cooling, 4 ml of distilled water was added to each test tube. The reaction is considered positive if the tube containing the alkaline solution appears clear or more transparent than the control tube (which lacks the alkaline solution). To confirm the presence of coumarins, extract solutions were prepared by dissolving 10 mg of extract in 4 ml of hot distilled water. The resulting solution was transferred into two test tubes. The first served as a control, while 0.5 ml of 10% ammonia solution was added to the second tube. The tubes were then observed under an ultraviolet (UV) lamp at 365 nm. The appearance of blue or green fluorescence indicates the presence of coumarins.

Detection of saponins

To detect saponins, 5 ml of the extract was placed in a test tube. The tube was then shaken vigorously for 15 seconds and left to stand for 15 minutes. The presence of saponins was indicated by a persistent foam layer exceeding 1 cm in height.

Detection of quinones

To detect quinones, 0.5 g of the dry extract was dissolved in 3 ml of hydrochloric acid (5% HCl), and the mixture was heated in a water bath until boiling. After cooling, 4 ml of chloroform was added, resulting in the formation of two phases. The supernatant was collected in a separate test tube. The appearance of a violet color upon the addition of 0.5 ml of ammonia indicated the presence of quinones.

Evaluation of the extract's antibacterial activity

Extract sterility test

This test was conducted to verify that the extract was not contaminated with bacteria. To perform the test, 0.1 g of the extract was added to 10 ml of thioglycollate broth and incubated at 37°C for 24 hours. Following this period, the broth was inoculated onto a Petri dish containing nutrient agar, and the dish was then incubated for 24 hours.

Preparation of extract solutions

The methods described by[13] were used to prepare the extract solutions. Specifically, 2 g of extract was added to 10 ml of distilled water to obtain a stock solution with a concentration of 200 mg/ml. This solution was then subjected to a series of twofold dilutions.

Preparation of culture medium

Mueller-Hinton medium was used for the antibacterial tests. The medium melted in an autoclave at 121°C for 15 minutes and kept in a supercooled state. It was then poured into appropriately labeled sterile Petri dishes and left to cool on the benchtop prior to inoculation with the bacterial strains.

Preparation of bacterial suspensions

Bacterial suspensions were prepared using the liquid medium method described by.[14] This method involves adding a well-isolated colony to a test tube containing 10 ml of sterile Mueller-Hinton (MH) broth. The resulting mixture is incubated at 37°C for 3 hours. A 0.1 ml aliquot of this solution (containing Enterobacteriaceae, such as *E. coli*) is then withdrawn and homogenized with 10 ml of sterile Mueller-Hinton broth using a vortex mixer. The bacterial concentration of the inoculum is estimated at 10^6 bacteria/ml.

Determination of inhibition zone diameters

Inhibition zone diameters were determined using the Chabbert and Kirby-Bauer methods via diffusion on Mueller-Hinton agar. Inoculated Mueller-Hinton agar plates were first dried in an incubator for 15 minutes. Subsequently, a 50 µl volume was deposited into wells previously created using Pasteur pipettes. The Petri dishes were then closed and left to allow diffusion at room temperature for 30 minutes before being incubated at 37°C for 24 hours. Readings were taken by measuring the diameters of the inhibition zones around each well using a caliper. Each result was expressed as the diameter of the inhibition zone for the strains against the extract.[15] The procedure was repeated three times for each diameter to determine the mean value. A strain is classified as resistant if the inhibition zone diameter is less than 8 mm, and susceptible if it falls between 9 and 14 mm. A diameter between 15 and 19 mm indicates high susceptibility, and diameters of 20 mm or greater indicate high susceptibility; in both these cases, the extract is considered effective.[16]

Determination of the Minimum Inhibitory Concentration (MIC)

The various concentration ranges of the plant extract to be tested were added to a set of seven experimental tubes, following the methods of.[17,18] Specifically, 1 mL of the extract at the highest concentration was transferred to tube T1, the extract at the next concentration to tube T2, and so on, down to the lowest concentration in tube T7. By the end of this process, the concentrations across tubes T1 through T7 had been serially diluted by half. Additionally, two control tubes were used: TC (growth control) and TS (sterility control). Tube TC received 1 mL of sterile distilled water to serve as growth control, while tube TS received 2 mL of Mueller-Hinton broth to serve as sterility control. All tubes were incubated at 37°C for 24 hours. Subsequently, the results were observed and recorded; the MIC was determined to be the concentration in the first tube showing no growth visible to the naked eye.

Determination of the Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration is the lowest concentration of a substance that results in a microbial survival rate of no more than 0.01%. Using a calibrated 2 µL loop, the tubes showing no visible growth were streaked onto agar Petri dishes in parallel lines 5 cm long, starting with the MIC tube. After 24 hours of incubation at 37°C, the number of colonies on the streaks of plate B was compared with that of plate A (prepared during the bacterial inoculum count). This procedure was repeated at least three times.

Evaluation of the antioxidant activity of the extracts studied

This test aims to demonstrate the **in vitro** antioxidant effects of the hydro-ethanolic extract of **Terminalia macroptera** by determining its anti-radical properties in the presence of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antioxidant potential of the extract was evaluated using the method described by[19] Blois (1958). DPPH was dissolved in absolute ethanol to obtain a solution with a concentration of 0.3 mg/mL. A concentration series (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, and 0.0625 mg/mL) of the hydro-ethanolic extract from the selected plant species was prepared in absolute ethanol. 2.5 mL of the extract and 1 mL of the ethanolic DPPH solution were placed into dry, sterile tubes. After mixing, the tubes were incubated in the dark for 30 minutes. The absorbance of the mixture was then measured at 517 nm against a blank consisting of 2.5 mL of pure ethanol and 1 mL of DPPH solution. Ascorbic acid (vitamin C) served as the positive reference control (at concentrations of 2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, and 0.0625 mg/mL).

The percentage of DPPH inhibition was calculated using the following formula.

$$\% \text{ DPPH inhibition} = 1 - [(100) \times \frac{\text{Extract absorbance}}{\text{Control absorbance}}] \quad (2)$$

The 50% inhibitory concentrations (IC50) of the aqueous extracts and vitamin C were determined using the graphical method. This experiment was repeated three times.

The antioxidant capacity of the extracts, expressed in mg of Trolox equivalents per gram of dry matter (mg TE·g^{-1} DM), was determined using formula (3) proposed by.[20]

DPPH value (mg TE·g^{-1} DM) = [(Extract absorbance– Control absorbance) / slope) (V/v) / (m) (1000)] (3)

Where.

Slope = slope of the line representing absorbance values as a function of Trolox concentrations;

V = total volume of extract prepared to obtain the stock solution;

V = volume of extract used for the assay;

m = mass of sample taken to prepare the stock solution;

r = yield and ms = dry matter

TE = Trolox equivalent

Statistical analysis of results

GraphPad Prism 8.0.1 software was used to perform the statistical analysis of experimental data. Raw data regarding inhibition zone diameters were processed using Excel 2016 to create a database and generate the various graphs. Values are presented as the mean ± standard deviation (or standard error). The data were evaluated using one-way ANOVA to assess differences. Where a difference was observed, Tukey's multiple comparison test was applied at the 5% significance level to evaluate significant differences between group means. The difference was considered significant if $P < 0.05$ and not significant if $P > 0.05$.

RESULTS

Yield

The hydro-ethanolic extract produced a yield (R) of 11.75%.

Qualitative phytochemical analysis of the 70% hydro-ethanolic extract.

The analysis revealed the presence of several phytomolecules: flavonoids, steroids, terpenoids, gallic tannins, saponins, and cardiotonic glycosides (Table 1).

Table 1: Phytomolecules of the 70% hydroethanolic extract.

Phytomolecules								
Poly	**Flav**	**Anth**	**Cat**	**Gal**	**Sap**	**Quin**	**Alc**	**Ste & Pol**
+	+	–	–	+	+	–	–	+

+ = Presence of the compound

– = Absence of the compound

Poly: polyphenol, Flav: flavonoid, Ste & Pol: sterol and polyterpenes Anth: anthocyanin, Sap: saponoside, Cat: catechic, Gal: Gallic, Alc: Alkaloid, Quin: quinone.

Antibacterial activity of the 70% hydroethanolic extract

The inhibition zone diameters determined for the 70% hydroethanolic extract of **Terminalia macroptera** ranged from 19 ± 0.17 mm to 25 ± 0.32 mm. The reference compounds ceftazidime (CAZ) and ceftriaxone (CRO) yielded different inhibition zone diameters. Specifically, diameters for ceftazidime ranged from 8 ± 0.12 mm to 16 ± 0.10 mm, while those for cefoxitin ranged from 8 ± 0.88 mm to 14 ± 0.31 mm. All inhibition zone measurements exceeded 9 mm, indicating significant activity for the hydroethanolic extract. Furthermore, when compared to the reference compounds, the extract consistently produced larger inhibition zone diameters, suggesting that the extract possesses superior activity compared to the reference compounds (Table 2).

Table 2: Measurement of the various inhibition diameters for the extract and reference molecules.

	Measurements of inhibition diameters					
	***E.coli* 505C/26**	***E.coli* 944C/26**	***E.coli* 1011C/26**	***E.coli* 1015C/26**	***E.coli* 1374C/26**	***E.coli* 1374C/26**
Extract	22±0,11	25±0,17	19±0,32	24±0,22	20±0,43	24±0,27
CRO	10,5±1,20	14±0,31	13,5±1,12	10±1,10	8± 0,88	13,5±0,36
CAZ	8±0,12	16 ±0,10	13±0,20	14±0,03	13,5±0,31	12±1,20

CRO= Ceftriaxone ; **CAZ**= Ceftazime ; **Extract**= 70% hydroethanolic extract

Antibacterial effect of the 70% hydro-ethanolic extract

The antibacterial effect of the 70% hydro-ethanolic extract was determined using the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) parameters. The determined MIC values ranged from 6.25 ± 0.6 to 25 ± 0.16, while MBC values ranged from 12.5 ± 0.32 to 50 ± 0.13; the MIC/MBC efficacy ratios ranged between 1 and 2. Analysis of the efficacy ratios—all of which were below 4—indicates the bactericidal effect of the 70% hydro-ethanolic extract (Table 3).

Table 3: Antibacterial parameters of the 70% hydro-ethanolic extract.

Antibacterial parameters: MIC and MBC				
Bacterial strains	**CMI**	**CMB**	**CMB/CMI**	**Interpretation**
***E. coli* 505C/26**	25±0,02	50±0,13	2	Bactericidal
***E. coli* 944C/26**	12,5±0,06	25±0,09	2	Bactericidal
***E. coli* 1011C/26**	6,25±0,22	12,5±0,17	2	Bactericidal
***E. coli* 1015C/26**	25±0,13	25±0,03	1	Bactericidal
***E. coli* 1374C/26**	25±0,16	25±0,14	1	Bactericidal
***E. coli* 1412C/26**	6,25±0,61	12,5±0,32	2	Bactericidal

Antioxidant activity of the 70% hydro-ethanolic extract

The antioxidant activity of the 70% hydro-ethanolic extract was determined by measuring the percentage of DPPH radical inhibition. The higher the percentage, the stronger the antioxidant capacity. The IC50 represents the concentration required to inhibit 50% of DPPH radicals; a lower IC50 value indicates more potent antioxidant activity. R^2 represents the regression correlation coefficient. Analysis of the hydro-ethanolic extract's antioxidant activity, compared to the reference compound (vitamin C), shows a DPPH radical inhibition rate of 80.19% for the extract versus 71.53% for vitamin C. Additionally, the IC50 was 0.0022 mg/mL for the extract compared to 0.0035 mg/mL for vitamin C. These values indicate that the hydro-ethanolic extract possesses

strong antioxidant capacity, superior to that of the reference compound, vitamin C (Table 4).

Table 4: Antioxidant activity parameters of the 70% hydro-ethanolic extract.

Antioxidant parameters			
	% Inhibition of DPPH radical	**CI50 (mg/mL)**	**R2**
70% hydro-ethanolic extract	80,19	0,0022	1
Vitamin C	71,53	0 ,0035	0,98

DISCUSSION

The 70% hydro-ethanolic extract of **Terminalia macroptera** used in this study was obtained via cold maceration. This gentle method preserves heat-sensitive compounds while ensuring the efficient extraction of secondary metabolites.[21, 22] The 70% hydro-ethanolic mixture is more effective for a wider range of compounds (polar to semi-polar). This observation is supported by several previous studies, notably[23], which demonstrated that extraction yield is optimal with solvents of intermediate polarity capable of solubilizing various classes of compounds (flavonoids, saponins, alkaloids). Extraction from the powdered bark of **Terminalia macroptera** yielded 11.75 mg/mL. This yield is lower than the approximately 16 mg/mL obtained by[22] using the same method. This difference could be attributed to the soil texture at the plant's origin. Phytochemical analysis of the extract revealed the presence of several phytomolecules, notably polyphenols, flavonoids, gallic tannins, steroids, terpenoids, and saponins. These compounds are likely responsible for the observed biological activities. Indeed, flavonoids can inhibit bacterial proteases and boost immune defenses[24], while tannins possess recognized astringent and antimicrobial properties.[25] Antibacterial activity was demonstrated by measuring inhibition zone diameters for various extract concentrations and by calculating the MBC/MIC ratio. The 70% hydroethanolic extract exhibited strong antibacterial activity, as the measured diameters exceeded 19 mm and the MBC/MIC ratio was less than 4. This ratio reflects the bactericidal effect of the extract on the bacterial strains studied, a property that may be attributed to the presence of flavonoids and saponins, compounds known for their antibacterial activity.[26] The antioxidant capacity of the extract was evaluated by determining the DPPH radical inhibition rate and the IC50 value, comparing the extract's performance with that of vitamin C, the reference molecule. The extract achieved a DPPH inhibition rate of 80.19%, compared to 71.53% for vitamin C, indicating superior performance. Furthermore, the extract's IC50 value was 0.0022 mg/mL, which is lower than that of vitamin C (0.0035 mg/mL). These results suggest that the 70% hydroethanolic extract possesses strong antioxidant properties relative to vitamin C, the study's reference molecule. This potent antioxidant activity is likely attributable to the presence of tannins. Indeed, tannins are known for their interaction with proteases, which influences vascular permeability, protein digestion, and blood coagulation.[27]

CONCLUSION

This study has highlighted the presence of several compounds including phenolics and flavonoids, which exhibit antibacterial activity, and tannins, known for their antioxidant properties. These findings provide a rationale for the plant's use in traditional medicine to treat conditions associated with oxidative stress and gastroenteritis. Furthermore, this work could serve as a focal point for leveraging traditional knowledge in collaboration with local communities, herbalists, and rural development stakeholders. Additionally, a bio-guided study using solvents other than ethanol could be considered to maximize the extraction of active principles.

ACKNOWLEDGMENTS

We extend our thanks to the Faculty of Biological Sciences and the Department of Biochemistry and Genetics at Peleforo Gon Coulibaly University in Korhogo, and particularly to the institution's Microbiology Unit. We also offer our sincere thanks to the Institute Pasteur in Côte d'Ivoire for providing laboratory equipment and biological resources.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

Author Contributions

Traore designed the study, contributed to data analysis alongside Ouattara, and drafted the manuscript. Yao Nguessan Alain collected the plant material and participated in oil extraction in collaboration with Ouattara Abou. Kouadio Guessend Nathalie contributed to the interpretation of the results. All authors read and approved the manuscript.

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