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Characterization and antibacterial potential of aqueous and ethanolic extracts of *Combretum collinum* Fresen (Combretaceae) trunk bark against extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae strains

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ABSTRACT

The rise of bacterial resistance is shaking the foundations of conventional antibiotics, prompting medical research to explore new therapeutic avenues. It is within this context that this study was conducted, dedicated to exploring the antibacterial potential of aqueous and ethanolic stem extracts of Combretum collinum Fresen (Combretaceae). The evaluation targeted a reference strain and 10 clinical isolates of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, using a combination of well diffusion on agar and macrodilution in liquid medium. Furthermore, thin-layer chromatography (TLC) analysis was performed to map the active secondary metabolites. The results reveal widespread susceptibility of the strains, although the intensity varied depending on the specific extract. At a concentration of 50 mg/ml, the ethanolic fraction proved particularly effective against the opportunistic pathogen Shigella sp. 55C, inducing a zone of inhibition of 22 ± 4.1 mm. Quantitatively, this ethanolic extract exhibited a purely bactericidal profile across the entire panel (MICs ranging from 0.78 to 6.24 mg/ml), while the aqueous extract eradicated 66% of the tested organisms. Phytochemical screening revealed a high content of tannins, flavonoids, polyterpenes, anthocyanins, and polyphenols. These bioactive molecules support the observed efficacy against enterobacteria and provide a solid scientific basis for the empirical use of Combretum collinum in traditional medicine.

Keywords: *Antibacterial activity, multidrug resistance, Combretum collinum, phytochemical screening, Côte d'Ivoire.*

Introduction

Infections caused by microorganisms have always been the primary global health threat. Indeed, one-third of global mortality and 43% of deaths in developing countries are due to infectious diseases [1]. Overall, bacterial infections alone account for 70% of deaths caused by microorganisms [2]. They therefore pose a significant global public health problem [3] [4].

In Côte d'Ivoire, infectious diseases represent a major health challenge, accounting for 50 to 60% of overall mortality according to [5]. At the heart of this situation, bacterial infections are particularly devastating for the most vulnerable members of the population. Bacterial infections are devastating, claiming the lives of 5% of newborns and up to 15 to 20% of children under five, while also being associated with 16% of all deaths recorded nationwide.

To combat bacteria, modern medicine has developed effective antibiotic therapies that have significantly reduced the spread of these microorganisms. However, the continuous, excessive, and even uncontrolled use of these antibiotics has led to the emergence of multidrug resistance in hospitals [6].

Among these multidrug-resistant bacteria are extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae. In Côte d'Ivoire, bacterial resistance to antibiotics reached 24.6% in 2012 [7]. This rapid increase in the prevalence of ESBL-producing Enterobacteriaceae poses a serious public health problem, especially since these germs are responsible for several pathologies, including diarrhea, urinary tract infections, suppurative infections, chronic wounds, and more. Faced with this threat, the search for new bioactive molecules has become urgent, as medicinal plants remain the primary resource, used by over 80% of the population in Côte d'Ivoire to meet their healthcare needs, according to [8]. Among these plants is *Combretum collinum* Fresen (Combretaceae), widely used in African traditional medicine to treat various illnesses, including those caused by enterobacteria.

This study is situated within the context of scientific validation of the traditional use of this plant species. Its objective is to evaluate the antibacterial activity of aqueous and ethanolic extracts of the trunk bark of *Combretum collinum* Fresen (Combretaceae) on the *in vitro* growth of extended-spectrum beta-lactamase-producing Enterobacteriaceae strains.

Furthermore, a phytochemical screening was carried out in order to identify the major groups of chemical compounds responsible for the therapeutic interest of this plant.

Materials and Methods

Materials

Bacterial strains

The bacterial material comes from the bacterial strain bank of the Department of Bacteriology and Virology of the Pasteur Institute of Côte d'Ivoire and the Cocody University Hospital Center (Abidjan). It consists of ten (10) clinical strains, including three (3) wild-type strains, seven (7) extended-spectrum beta-lactamase-producing Enterobacteriaceae, and one reference strain, *Escherichia coli* ATCC 25922, used for quality control (Table 1).

Table 1. List of selected Enterobacteriaceae

Codes	Species	Organic products	Phenotypes
13C	*Escherichia coli*	Urine	ESBL, RCFQ, KTG
60Y	*Escherichia coli*	Urine	Savage
63P	*Escherichia coli*	Urine	ESBL ; RAc, AMC, C, CEF
11C	*Escherichia coli*	wastewater	ESBL
31C	*Shigella* sp.	Feces	ESBL ; RA
55C	*Shigella* sp.	Feces	Savage
92P	*Shigella flexneri*	Feces	ESBL ; RATC
09C	*Klebsiella pneumoniae*	Blood	ESBL ; RCFQ
50C	*Salmonella* sp.	Pus	Savage
90Y	*Salmonella* sp.	Blood	ESBL ; AQ, P
Reference strain			
ATCC25922 *Escherichia coli*			

ESBL: *Extended-spectrum beta-lactamases; KTG: Kanamycin-tobramycin-gentamicin; AQ: Aminoglycoside, quinolones; P: Penicillinase; RATC: Resistance to amoxicillin, ticarcillin, cefotaxime; RAC: Resistance to ampicillin; AMC: Amoxicillin + clavulanic acid; C: Cefoxitin; CEF: Ceftazidime; ATCC: American Type Culture Collection.*

Laboratory equipment

The laboratory equipment used to obtain the crude extracts consisted of a rotary evaporator, a freeze dryer, a Voltex shaker, a magnetic stirrer, a Densimat, an oven, a fume hood, a refrigerator, automatic micropipettes (P200 and P1000), and a balance for determining the collected masses. A UV lamp, a chromatography tank, a digital camera, a hot plate, and reagents were used for phytochemical sorting. As for the glassware used to carry out the antibacterial activities, it consists of beakers (100 mL and 250 mL), test tubes, graduated cylinders, funnels, sterile Petri dishes of 90 mm diameter, jars, Pasteur pipettes, hemolysis tubes, a stand, syringes (5 mL and 10 mL), sterile glass pipettes (10 mL), microfilters (0.22 µm and 0.45 µm), swabs, Whatman® 3 mm filter paper, aluminum foil, spatulas, latex gloves, masking tape, absorbent cotton, a timer, 100 and 1000 µL tips, F254 silica gel plates, micropillar tubes and bulbs. Sterile distilled water, hexane, and ethanol were the solvents used to prepare the extracts.

Methods

Preparation of crude extracts

The plant material, consisting of trunk bark, was collected in the savannah region of northern Côte d'Ivoire during the dry season. To maximize the concentration of secondary metabolites accumulated by the plant in response to diurnal physiological stresses, sampling was targeted for late afternoon, just before dusk. After thorough drying in the dark, the samples were ground into a fine powder. The extraction protocol implemented was based on the methodology described by [9], with adjustments specific to our objectives.

To obtain the organic fraction, a preliminary defatting was performed by macerating 25 g of powder under magnetic stirring in 250 mL of hexane for 24 hours. The residue, collected and dried on filter paper, was then immersed in 250 mL of ethanol for another 24-hour maceration. The resulting filtrate was concentrated at 40 °C under reduced pressure using a rotary evaporator, then completely dehydrated under a fume hood to yield the ethanolic extract. In parallel, the aqueous extract was prepared by macerating 50 g of powder in 500 mL of sterile distilled water for 24 hours, followed by freeze-drying the filtrate. Both final extracts were packaged in sterile glass vials and stored in a refrigerator.

Performing antibacterial tests

Preparing the inoculum for solid-state tests

Young 24-hour strains were emulsified in 2 mL of 85% NaCl suspension, and the optical density was adjusted to 0.5 MaC Farland using a densimat. A 1 mL volume was diluted in 10 mL of physiological saline (0.9% NaCl), thus constituting the bacterial inoculum estimated at 10^6 bacteria/mL.

Germ susceptibility testing of extracts

Prior to biological testing, the absence of any microbial contamination in the extracts was validated by a rigorous sterility test. The evaluation of antibacterial potential then relied on the complementarity of two methodological approaches: well diffusion (wells) on agar and macrodilution in liquid medium, in accordance with the protocols described by [10] [11]. Specifically, Petri dishes filled with Mueller-Hinton (MH) agar were uniformly inoculated by swabbing. Wells were prepared in the dishes to hold a 50 µL volume of each extract, adjusted to a concentration of 50 mg/mL [12]. After a 24-hour incubation period at 37°C, the inhibition zones surrounding the wells were precisely measured using calipers (Figure 1). The antibacterial activity of the extracts was categorized according to the evaluation grid of [13]. According to this scale, the activity of a substance is defined as: Insufficient (ineffective), for an inhibition diameter (ID) of less than 8 mm; Moderate (effective), when the ID ranges between 9 and 14 mm; Strong (very effective), for an ID extending from 15 to 19 mm; Optimal (extremely effective), when the ID exceeds 20 mm.

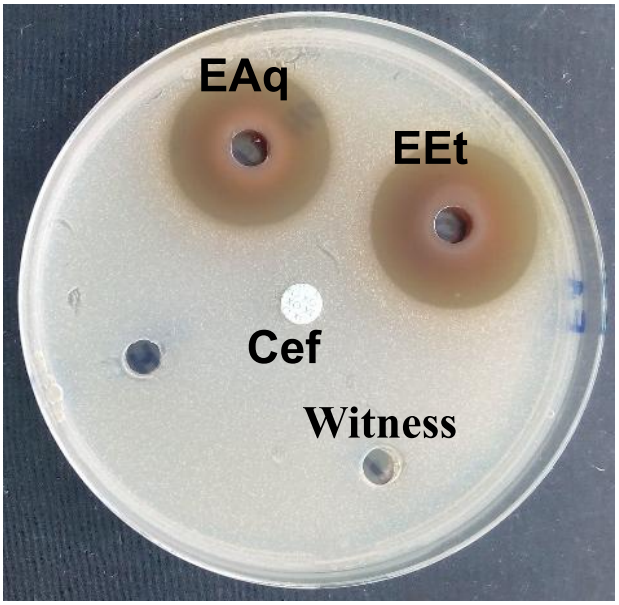

Fig. 1: Antibacterial activity of aqueous (EAq) and ethanolic (EEt) extracts of Combretum collinum Fresen trunk bark at 50 mg/ml and of antibiotic (Cef: ceftazidime) on Shigella sp. 55C culture.

Preparation of the inoculum for liquid-based tests
Young colonies of 24 hours were collected and emulsified in a test tube containing 10 mL of sterile Muller-Hinton broth. The mixture was incubated at 37°C for 3 hours. After this incubation, a 0.3 mL suspension of this preculture was diluted in 10 mL of sterile Muller-Hinton broth and then homogenized.

Preparation of the concentration range
The concentration range was obtained by the double dilution method. To do this, a 50 mg/mL solution of the extracts was prepared in sterile distilled water. A series of 2:1 dilutions were performed on this solution to obtain a concentration range from 25 to 0.19 mg/mL.

Determination of antibacterial parameters
The antibacterial profile of the extracts was refined by macrodilution in liquid medium according to [14]. In a series of 10 tubes, 0.2 mL of each extract concentration was inoculated with 1.8 mL of bacterial suspension, resulting in a 1/10 dilution for a final range of 5 to 0.019 mg/mL. A growth control (inoculum + distilled water) and a sterility control (broth alone) completed the series. The MIC corresponds to the lowest extract concentration free of macroscopic turbidity after 24 hours of incubation at 37 °C. The MBC, reflecting the destruction of 99.99% of the initial inoculum, was determined by comparative enumeration. On the one hand, the original inoculum was diluted from 10^{-1} to 10^{-4} and streaked in 5 cm bands onto MH agar using a 2 µL calibrated loop (Plates A). On the other hand, the contents of the clear tubes were subculturated identically (Plates B). After 24 hours at 37 °C, the MBC was extracted from the lowest concentration showing a bacterial load less than or equal to 0.01% compared to control A. Pharmacodynamic guidance was dictated by the MBC/MIC ratio [15]. According to [16] an extract is bactericidal when its MBC is equal to its MIC or if the MBC/MIC ratio is less than or equal to 4. It is said to be bacteriostatic when the MBC/MIC ratio is greater than 4. When this ratio is equal to 32, the strain is said to be tolerant.

Phytochemical Screening
The phytochemical profile of the extracts was determined by thin-layer chromatography (TLC) using an adaptation of the methodology described by [14]. This qualitative analysis technique relies on the appearance of specific colors under the effect of appropriate reagents, observed in daylight or at targeted wavelengths [17], thus allowing the isolation of major families of secondary metabolites. Experimentally, a 10 mg/mL stock solution was prepared by dissolving 10 mg of each extract in 1 mL of absolute ethanol. Spots of 10 µL (equivalent to a 100 µg load) were applied using microcapillaries onto F^{254} silica gel plates, which served as the stationary phase. The migration took place in chromatographic tanks previously saturated with the CHCl3-MeOH-H2O elution system (65:35:5 v/v/v). After development and drying of the chromatograms, spot detection was performed comparatively, before and after chemical detection, under white light as well as under ultraviolet illumination at 254 nm and 366 nm.

Detection of Terpenoids and Saponins
Chemical profiling of the eluates was performed using Godin's reagent. After spraying, the support was incubated at 100 °C for 10 minutes to reveal the banding patterns. In daylight, the diagnosis was based on the selectivity of the induced colors: the emergence of violet and red spots characterizes the monoterpene fraction, while the detection of a blue color indicates the presence of saponinic compounds [18].

Detection of Alkaloids
The characterization of the alkaloid fraction was carried out by applying Dragendorff's reagent. Following nebulization of this developer and a control oven temperature of 100 °C for 10 minutes, the presence of these metabolites was indicated by the emergence of orange spots that were perfectly distinguishable under white light.

Detection of Polyphenols
The profiling of phenolic nuclei was completed via a redox reaction induced by Folin-Ciocalteu reagent (10%). The analytical process required nebulization of the support followed by a controlled heat shock in an oven at 100 °C for 10 minutes. The presence of these molecules of interest was then indicated by the macroscopic appearance of blue chromatic foci under white light, thus materializing the chemical signature described by [19].

Detection of Flavonoids and Sesquiterpene Lactones
The profiling of flavonoids was based on their complexation capacity with 5% (w/v) aluminum chloride (AlCl3). Following nebulization of the metallic salt and thermal activation, the formation of these chelates was evidenced by the emergence of yellow spots, highly detectable under conventional lighting or by fluorescence under ultraviolet radiation at 366 nm. This same level of light excitation (366 nm) revealed, through the appearance of polychromatic emission foci, the concomitant presence of sesquiterpene lactones, perfectly consistent with the interpretation framework of [18].

Detection of Coumarins
The diagnosis of coumarin presence was based on the detection of specific optical signals under UV illumination at 366 nm. This protocol required prior spraying of the TLC plates with a 5% (w/v) basic lead acetate solution. The subsequent emergence of green and blue fluorescent spots thus validated the physicochemical signature of these compounds of interest.

Detection of Tannins
The targeting of tannic compounds was achieved by exploiting their affinity for 10% FeCl3 chloride. After nebulization of this ferric salt onto the chromatographic support, the formation of metallo-polyphenolic chelates resulted in the macroscopic appearance of chromatic sedimentation foci in the visible spectrum. The emergence of these polychromic spots, oscillating between blue, green, and black, unequivocally confirmed the presence of these polyphenolic macromolecules within the plant matrix.

Identification of Anthraquinones and Anthrones
The characterization of the quinone derivatives was carried out by topical alkalinization using an ethanolic solution of potassium hydroxide (KOH 5%). The basic reaction generated highly differentiated color profiles under light exposure. On the one hand, the emergence of red spots, discernible head-on in white light and under ultraviolet radiation at 366 nm, attested to the presence of anthraquinones.

On the other hand, the anthrone fraction was selectively revealed in the UV spectrum at 366 nm in the form of yellow emission foci, thus corroborating the identification criteria validated by [20].

Statistical analysis of the results

Analysis of variance (one-way ANOVA) followed by Tukey's test was used to compare the variations in MICs and MBCs, and to determine whether the activity of *Combretum collinum* stem bark extracts was statistically influenced by bacterial phenotypes. Results are expressed as means ± standard deviations. A p-value < 0.05 was considered statistically significant. The R software [21] was used to perform these statistical tests.

Results

Antibacterial activity

Extracts from the trunk bark of *Combretum collinum* were active to varying degrees against all the bacteria studied. This is evidenced by the mean inhibition diameters obtained, ranging from 22 ± 4.1 to 13 ± 1.3 mm for the ethanolic extract and from 20 ± 4.1 mm to 12 ± 4.0 mm for the aqueous extract (Fig. 2). This inhibitory activity was most pronounced in preventing the growth of *Shigella* sp. 55C, as evidenced by the highest inhibition diameter values obtained against the growth of this strain: 22 ± 4.1 mm with the ethanolic extract and 20 ± 4.1 mm with the aqueous extract. In other words, this wild-type strain, obtained from the stool of an infected patient, was the most sensitive to both types of extracts. In general, the different extracts were active against all bacteria.

Regarding antibacterial parameters, the two types of extracts produced MICs ranging from 0.78 to 12.48 mg/ml. The lowest value (0.78 mg/ml), obtained with the alcoholic extract, inhibited the bacterial growth of *Shigella* sp. 55C. This activity not only inhibited bacterial growth but also neutralized it, judging by its bactericidal effect. As for the minimum bactericidal concentrations (MBCs), these also varied from 1.56 to 12.48 mg/ml for the ethanolic extract and from 3.12 to 24.96 mg/ml for the aqueous extract (Table 2). Furthermore, the ethanolic extract showed a bactericidal effect on all bacterial strains. Whereas, the aqueous extract was bactericidal on 66% of them.

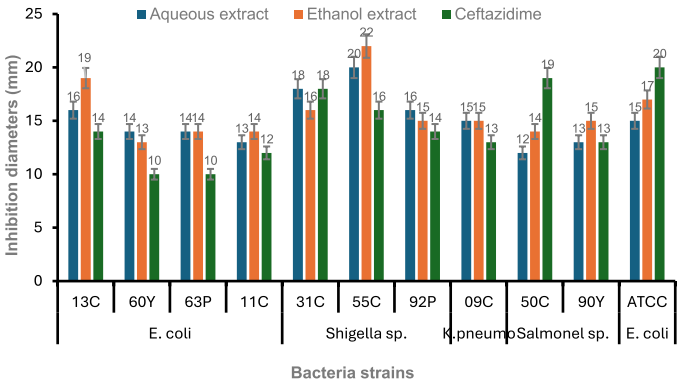

Fig. 2: Activité (moyenne ± SD) des extraits de l'écorce de tronc de de Combretum collinum à 50 mg/ml sur les souches d'entérobactéries.

Table 2: Mean values (mean ± SD of three trials) of the antibacterial parameters of extracts of the trunk bark of Combretum collinum on enterobacteria strains.

	Ethanol extract (mg/ml)				Aqueous extract (mg/ml)			
Enterobacteriaceae	MIC	MBC	$\frac{MBC}{MIC}$	power	MIC	MBC	$\frac{MBC}{MIC}$	Power
E. coli 13C	6,24	12,48	2	Bactericide	3,12	6,24	2	Bactericide
E. coli 60Y	6,24	12,48	2	Bactericide	3,12	24,96	8	Bacteriostatic
E. coli 63P	6,24	12,48	2	Bactericide	3,12	24,96	8	Bacteriostatic
E. coli 11C	6,24	6,24	1	Bactericide	12,48	12,48	1	Bactericide
Shigella sp.31C	1,56	3,12	2	Bactericide	3,12	24,96	8	Bacteriostatic
Shigella sp.55C	0,78	1,56	2	Bactericide	3,12	18,72	6	Bacteriostatic
Shigella flexneri 92P	1,56	1,56	1	Bactericide	1,56	3,12	2	Bactericide
K. pneumoniae 09C	6,24	12,48	2	Bactericide	3,12	6,24	2	Bactericide
Salmonella sp. 50C	6,24	12,48	2	Bactericide	3,12	18,72	6	Bacteriostatic
Salmonella sp. 90Y	6,25	12,48	2	Bactericide	3,12	24,96	8	Bacteriostatic
E. coli ATCC25922	3,12	3,12	1	Bactericide	3,12	3,12	1	Bactericide

MIC: *minimum inhibitory concentration ; MBC : minimum bactericidal concentration.*

Statistical Analysis

This analysis compared the mean ± standard deviation values obtained for wild-type bacterial strains (*E. coli* 60, *Shigella* sp. 55C, and *Salmonella* sp. 50C) with those of other resistant bacteria. Analysis of variance revealed that the observed differences in MICs and MBCs were not significant at the 5% level (Tables 3 and 4 ; P > 0.05).

Table 3: Comparison of mean values (mean ± SD of three trials) of MIC and MBC of the ethanolic extract according to the phenotypes of the bacterial strains.

Antibacterial parameters	Wild bacterial strains	Bacterial strains ESBL	P-value
MIC (mg/ml)	0,855 ± 1,3	1,135 ± 3,0	**P** > 0,05
MBC (mg/ml)	1,732 ± 1,1	1,140 ± 2,0	**P** > 0,05

MIC: *minimum inhibitory concentration ; MBC : minimum bactericidal concentration.*

Table 4: Comparison of mean values (mean ± SD of three trials) of MIC and MBC of the aqueous extract according to the phenotypes of the bacterial strains

Antibacterial parameters	Wild bacterial strains	Bacterial strains ESBL	P-value
MIC (mg/ml)	1,465 ± 2,2	1,161 ± 2,5	**P** > 0,05
MBC (mg/ml)	2,139 ± 3,3	1,446 ± 3,4	**P** > 0,05

MIC: *minimum inhibitory concentration ; MBC : minimum bactericidal concentration.*

Phytochemical Screening

Qualitative phytochemical analyses revealed the presence of seven groups of phytoconstituents in the extracts. These are polyterpenes, polyphenols, catechin and gallic tannins, anthraquinone glycosides, anthocyanins, and flavonoids (Table 5).

Table 5: Chemical compounds identified in the different plant extracts

Plant extracts	Chemical compounds detected
Aqueous extract	Catetechic and gallic tannins, polyphenols, flavonoids,
Ethanolic extract	Catechetic and gallic tannins, anthraquinone heterosides, polyphenols, polyterpenes, anthocyanins, flavonoids.

DISCUSSION

The antibacterial activity of extracts from the trunk bark of *Combretum collinum* Fresen. (Combretaceae) was evaluated on the in vitro growth of ten clinical Enterobacteriaceae strains, including three wild-type strains, seven extended-spectrum beta-lactamase producing strains, and one reference strain. The tests showed that the ethanolic and aqueous extracts were active to varying degrees against all bacterial strains. This is reflected in the differences observed in the inhibition zone diameters, which ranged from 22 ± 4.1 mm to 12 ± 4.0 mm. Accordingly, using the solid-state diffusion method, an extract is considered active when it induces an inhibition zone greater than or equal to 10 mm [22]. This is the case for the extracts used in this study. The ethanolic extract produced the largest diameter (22 ± 4.1 mm) that inhibited the growth of *Shigella* sp. 55C at a concentration of 50 mg/ml. Bacteria of this genus induce pathologies such as gastroenteritis, colic, diarrhea, bacillary dysentery, and fevers [23]. In light of the above, the observed activity could therefore justify the traditional use of *Combretum collinum* in the treatment of these conditions [24]. The aqueous extract was equally active against the same strain, with an inhibition diameter of 20 ± 3.0 mm at 50 mg/ml. The results obtained with the two types of extracts are almost identical. This suggests that the active compounds responsible for this antibacterial activity are soluble in both types of polar solvents. This significant antibacterial activity could be explained by the richness of the Combretaeae family in various constituents, notably flavonoids, tannins, polyterpenes, and polyphenols, which confer this property [25].

Regarding antibacterial parameters, the lowest minimum inhibitory concentration (MIC) of the ethanolic extract was 0.78 mg/ml, obtained against *Shigella* sp. 55C. This extract thus proves particularly effective against this strain, frequently implicated in various infectious diseases [23]. The results are similar to those of [24] concerning the alcoholic extract. Indeed, these authors obtained a similar MIC with the hydro-ethanolic extract of *Combretum collinum* leaves against a wide range of multidrug-resistant bacteria, including enterobacteria, responsible for opportunistic infections. The statistical analysis indicated that the observed differences in MIC and MBC values between the two strain categories were not significant. In other words, the extracts showed no selectivity towards the bacteria. This reveals, firstly, that the activity of the extracts was not statistically influenced by the bacterial phenotype. Secondly, although these values are numerically different, they reflect similar biological efficacy. This homogeneity of action could be explained by a synergy or complementarity of the various phytoconstituents responsible for the observed activity.

Furthermore, the ethanolic extract was bactericidal against all bacterial strains tested in this study, as the MBC/MIC ratios were all less than 4 [16]. The bactericidal properties of *C. collinum* trunk bark could fully justify its common use in African traditional medicine against several pathologies, including those caused by enterobacteria [24].

The therapeutic range of a medicinal plant frequently goes beyond the scope of a single infectious pathology. This empirical versatility finds its scientific basis in the diversity of secondary metabolites synthesized by its organs [26].

In the case of *Combretum collinum*, phytochemical investigations revealed a remarkable molecular complexity, characterized by the presence of seven large groups of phytoconstituents: polyterpenes, polyphenols, anthocyanins, anthraquinone glycosides, flavonoids, as well as tannins in their catechic and gallic forms. This chemical composition dictates the pharmacodynamic properties of the species and forges its interest in traditional medicine. Within this matrix, flavonoids are distinguished by a major antibacterial potential linked to their polyphenolic structure. By interacting with membrane proteins and denaturing vital enzyme systems of pathogens, these compounds disrupt bacterial homeostasis. This targeted action legitimizes the internal use of *C. collinum* in the treatment of dysentery, gonorrhea, as well as urinary or ear infections [27]. Furthermore, the high prevalence of tannins known for their astringent, antiseptic and bactericidal properties [28] provides a rational justification for the topical application of the plant. The joint presence of catechic and gallic fractions supports the healing processes and the eradication of cutaneous suppurations or chronic wounds.

C. collinum is one of the many plant species commonly used by local populations for its numerous therapeutic benefits. It deserves to be included among the cultivated and protected plants in Côte d'Ivoire.

Furthermore, the identification of its active principles constitutes an important contribution to the development of beta-lactamase inhibitors. Given that there is currently no inhibitor capable of inhibiting all classes of beta-lactamases, it would be worthwhile to explore the potential of medicinal plants, an inexhaustible source of plant secondary metabolites.

Conclusion

The work carried out has confirmed the antibacterial activity of *C. collinum* trunk bark against various strains of Enterobacteriaceae with different phenotypes. The determination of MICs and MBCs revealed a bactericidal effect of the ethanolic extract on all bacterial strains. Furthermore, the major groups of chemical compounds likely responsible for this activity were identified and found to be more concentrated in both solvents. It is therefore conceivable to conduct further ethnopharmacological studies, confirm the in vitro results with in vivo tests, and perform toxicity tests on *C. collinum*, which could offer hope for alleviating microbial infections, a significant public health threat. Moreover, the scope of measures to strengthen the protection of endangered species must be broadened to guarantee the preservation and safeguarding of biodiversity, which constitutes a true treasure of Ivorian flora.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTES OF THE AUTHORS

This work was carried out jointly by all the co-authors. The final version of the manuscript was read and approved by each of them.

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