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THERAPEUTIC POTENTIAL OF THE PARTITION OF THE AQUEOUS EXTRACT OF THE LEAVES OF *TERMINALIA LEIOCARPA* (DC.) BAILL. (COMBRETACEAE) AGAINST MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* INFECTIONS

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ARTICLE HISTORY	ABSTRACT
Received on: 14-07-2026 Revised on: 24-08-2026 Accepted on: 19-09-2026	The Ivorian pharmacopoeia offers diverse phytotherapeutic alternatives for common ailments. <i>Terminalia leiocarpa</i> (DC.) Baill. (Combretaceae) is an important medicinal plant traditionally used for its antibacterial properties. This study evaluated the antibacterial activity of chloroform, ethyl acetate, n-butanol, and residual aqueous fractions against seven <i>Pseudomonas aeruginosa</i> strains, including six clinical isolates (four resistant and two wild-type strains) and one reference strain. Agar well diffusion and broth macrodilution methods were employed to assess antibacterial activity. The n-butanol fraction exhibited the highest activity, producing an inhibition zone of 25 ± 1.3 mm against strain 885C at 50 mg/mL. All fractions showed antibacterial activity, with an identical minimum inhibitory concentration (MIC) of 0.019 mg/mL. Although minimum bactericidal concentration (MBC) values varied slightly, the differences were not statistically significant, suggesting comparable antibacterial efficacy among the fractions. Thin-layer chromatography (TLC) revealed various secondary metabolites, including terpenes, saponins, polyphenols, flavonoids, xanthenes, naphthoquinones, alkaloids, tannins, coumarins, and anthrones. These compounds may contribute to the observed antibacterial effects. Overall, the findings support the traditional use of <i>T. leiocarpa</i> in Ivorian medicine and highlight its potential as a source of antibacterial agents against <i>P. aeruginosa</i> .
Keywords: Antibacterial activity; <i>Terminalia leiocarpa</i> ; phytochemical.	
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INTRODUCTION

Humans have long utilized plants initially as a food source and subsequently as medicine to meet their healthcare needs. For millions of people, particularly those in rural areas, plant-based medicines constitute the primary source of healthcare, whether due to geographical isolation or the extreme poverty affecting the majority of rural populations [1]. Today, plant-based treatments are regaining prominence as modern medicine faces a critical shortage of new therapies and the efficacy of drugs such as antibiotics once viewed as the near-universal solution for severe infections declines. Bacteria have gradually adapted to medications, leading to increased antibiotic resistance [2]. The art of plant-based healing has long been known and practiced in Africa, drawing on knowledge transmitted orally across generations to specific groups of initiates, such as traditional health practitioners and herbalists. Many of these plants are commonly used to treat various infectious diseases. Among them, *Terminalia leiocarpa* (DC.) Baill. (Combretaceae) stands out due to its heavy use in both therapeutic and commercial contexts. This plant is

employed to treat numerous conditions, including those caused by *Pseudomonas aeruginosa* a major opportunistic bacterium classified as one of the greatest threats to human health regarding antibiotic resistance [3]. Indeed, recent scientific research has revealed the emergence of highly resistant strains of this bacterium particularly against carbapenems-prompting the World Health Organization to designate the pathogen as a critical priority threatening human health. This study was initiated with the aim of contributing to a reliable and sustainable solution to this issue by leveraging plants from the Ivorian pharmacopoeia. This study is part of efforts to valorize the African pharmacopoeia and aims to evaluate, *in vitro*, the anti-*Pseudomonas* potential of the organic and residual aqueous fractions obtained from the liquid-liquid separation of the aqueous extract of *T. leiocarpa* leaves.

Furthermore, a phytochemical screening of the chemical constituents of the various fractions was conducted using thin-layer chromatography (TLC).

MATERIALS AND METHODS

Material

Bacterial strains

The bacterial material was obtained from the bacterial strain bank of the Department of Bacteriology and Virology at the Institut Pasteur de Côte d'Ivoire and the Cocody University Hospital (CHU). It consists of seven (7) clinical strains, including two (2) wild-type strains, four (4) resistant strains, and a *Pseudomonas aeruginosa* reference strain (ATCC 27853) used for quality control (Table 01).

Table 01: List of different bacterial strains

Coded species	Organic product	Resistance phenotype
<i>Pseudomonas aeruginosa</i> 891C	Pus	IPM ^R
<i>Pseudomonas aeruginosa</i> 489C	Wound	IPM ^R
<i>Pseudomonas aeruginosa</i> 261C	Urine	IPM ^R
<i>Pseudomonas aeruginosa</i> 1076C	Urine	IPM ^R
<i>Pseudomonas aeruginosa</i> 885C	Pus	Savage
<i>Pseudomonas aeruginosa</i> 793C	Urine	Savage
<i>Pseudomonas aeruginosa</i> (ATCC 27853)		

IPM^R: Imipenem resistance; ATCC: American Type Collection Culture. Methods

Laboratory equipment

The laboratory equipment used to obtain the crude extract consisted of a rotary evaporator, a freeze-dryer, a vortex mixer, a magnetic stirrer, a Densimat, an oven, a fume hood, a refrigerator, automatic micropipettes (P200 and P1000), and a balance for weighing samples. A UV lamp, a chromatography tank, a digital camera, a hot plate, and various reagents were used to perform phytochemical screening. The glassware and accessories used for antibacterial assays included a 50 mL separatory funnel, beakers (100 mL and 250 mL), test tubes, graduated cylinders, funnels, sterile Petri dishes (90 mm diameter), jars, Pasteur pipettes, hemolysis tubes, a retort 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 µL and 1000 µL pipette tips, silica gel F254 plates, microcapillary tubes, and pipette bulbs. Sterile distilled water was the solvent used to obtain the crude extract. Hexane (Prolabo®), chloroform (Prolabo®), ethyl acetate (Prolabo®), and n-butanol (Prolabo®) were the solvents used to obtain the various extract fractions. The reagents consist of 10% Folin-Ciocalteu reagent, 2% aluminum trichloride (AlCl₃) (2 g of AlCl₃ in 100 mL of methanol), sodium carbonate (Na₂CO₃) (17%, w/v), Godin's reagent, Dragendorff's reagent, 5% basic lead acetate, 10% FeCl₃ solution, and 5% ethanolic KOH solution.

Methods

Preparation of crude extracts

The leaves were harvested during the dry season in the savanna region of Côte d'Ivoire, specifically in the Karakoro department. Harvesting took place at dusk, a time when the

plants had had sufficient opportunity to secrete maximum levels of active compounds to cope with the day's stressors. The samples were dried and then ground into a plant powder. Aqueous extraction was performed using a method from [4], adapted for this study. In this procedure, 50 g of plant powder was macerated in 500 mL of distilled water for 24 hours under magnetic stirring (Stuart Scientific model). This process was repeated twice. The mixture was filtered twice under vacuum using a Büchner funnel and Whatman® 3 mm filter paper. The filtrate was freeze-dried at -70°C (Christ® Alpha 1-2 freeze-dryer) to obtain the total aqueous extract as a powder. It was stored in a freezer at 4°C until use for liquid-liquid separation.

This separation method was adapted from that of [5]. It involves successive maceration using four organic solvents of increasing polarity (hexane, chloroform, ethyl acetate, and n-butanol). Specifically, 10 g of aqueous extract was dissolved in 100 mL of sterile distilled water. Subsequently, 100 mL of hexane was added. The mixture was blended (Philips, Boroglass®) and transferred to a separatory funnel. This process was repeated until the organic phase became clear. Following separation, the aqueous residue underwent further separation under the same conditions using chloroform (100 mL x 4), followed by ethyl acetate (100 mL x 4) and n-butanol (100 mL x 4). The resulting organic phases were concentrated at 40°C using a rotary evaporator (Büchi type). This partitioning series yielded five fractions: the hexane fraction, the chloroform fraction, the ethyl acetate fraction, the n-butanol fraction, and the residual aqueous fraction. The latter was evaporated in an oven at 50°C. Due to the small quantity obtained, the hexane fraction could not be used for antibacterial testing.

Performance of antibacterial tests

Preparation of the inoculum for tests on solid media

24-hour-old cultures were emulsified in 2 mL of 0.85% NaCl suspension, and the optical density was adjusted to a 0.5 McFarland standard using a densitometer. A 1 mL volume was diluted in 10 mL of physiological saline (0.9% NaCl), thereby preparing the bacterial inoculum, estimated at 10⁶ bacteria/mL.

Tests of microbial sensitivity to extracts

Prior to evaluating their biological activity, the microbiological purity of the fractions was verified via a sterility test to rule out any pre-existing bacterial or fungal contamination. Antibacterial properties were then determined using two complementary approaches: the agar diffusion method (well or cup method) and the liquid macro-dilution method [6]. For the diffusion test, Petri dishes containing Mueller-Hinton agar (MHA) were inoculated by swabbing with a standardized bacterial inoculum. Wells were created in the agar and loaded with 50 µL of the fraction solution, adjusted to a concentration of 50 mg/mL [7]. Following a 24-hour incubation period at 37°C, the diameter of the inhibition zone (DI) surrounding each well was precisely measured using a caliper (Figure 1). The activity level of the fractions was standardized and interpreted according to the scale established by [8], structured as follows: DI < 8 mm : ineffective substance; 9 mm ≤ DI ≤ 14 mm: effective substance ; 15 mm ≤ DI ≤ 19 mm: highly effective substance; DI ≥ 20 mm: extremely effective substance.

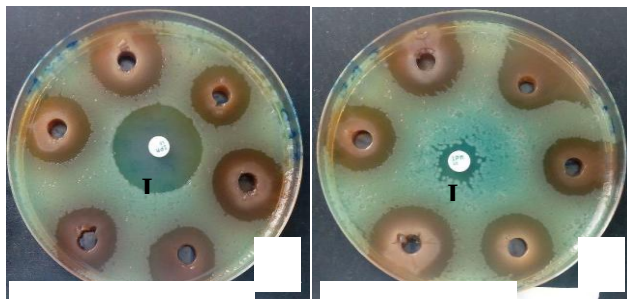


Figure 01: Antibacterial activity of fractions at 50 mg/mL and an antibiotic (Imipenem) against *Pseudomonas aeruginosa* cultures: 885C (A) and *Pseudomonas aeruginosa* 261C (B).

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

Preparation of the concentration range

The concentration range was obtained using the twofold serial dilution method. To this end, a solution of the extracts at a concentration of 50 mg/mL was prepared in sterile distilled water. A series of twofold dilutions was performed starting from this solution to obtain a concentration range of 25 to 0.19 mg/mL.

Determination of antibacterial parameters

Antibacterial parameters were determined using the liquid medium dilution method described by [9]. Specifically, 0.2 mL of each extract concentration was mixed with 1.8 mL of bacterial inoculum in 10 experimental test tubes. The growth control tube received 0.2 mL of sterile distilled water in addition to the inoculum, while the sterility control received only 2 mL of sterile Mueller-Hinton broth. This setup yielded a concentration range of 5 to 0.019 mg/mL (a 10-fold dilution series).

The tubes were incubated for 24 hours at 37°C. Following incubation, the lowest concentration showing no visible bacterial growth was identified as the Minimum Inhibitory Concentration (MIC). The Minimum Bactericidal Concentration (MBC) is defined as the concentration resulting in 0.01% viable bacteria after 24 hours of incubation at 37°C. Determination of the MBC began with a bacterial count. This involved diluting the initial inoculum (from 10^{-1} to 10^{-4}) and streaking these dilutions onto Mueller-Hinton agar (MHA) plates using a calibrated 2 μ L loop to create 5 cm long streaks followed by 24 hours of incubation; these Petri dishes were labeled "A." After reading the MIC results, the contents of the tubes showing no visible growth were used to inoculate MHA plates, again using 5 cm streaks. This series of Petri dishes is designated as B. The MBC was determined by comparing bacterial growth in dishes A and B; thus, the lowest concentration showing less than 0.01% viable bacteria relative to the initial inoculum was identified as the MBC.

The MBC/MIC ratio made it possible to characterize the mode of action of the extracts [10]. According to [11], an extract is bactericidal when its MBC equals its MIC or when the MBC/MIC ratio is less than or equal to 4. It is considered bacteriostatic when the MBC/MIC ratio exceeds 4. When this ratio equals 32, the strain is classified as tolerant.

Phytochemical screening

The detection of various chemical compounds in the fractions was carried out using thin-layer chromatography (TLC) according to the method described by [9]. This method allows for the identification of several groups of secondary metabolites through specific color reactions, observed either in the visible range or at a specific wavelength [12]. A 10 mg mass of each fraction was dissolved in 1 mL of absolute methanol to obtain a 10 mg/mL solution. Ten microliters (10 μ L) equivalent to 100 μ g of this solution were spotted onto a silica gel F254 plate (stationary phase) using a microcapillary tube. The chromatograms were developed in tanks pre-saturated with the eluent (mobile phase): CHCl_3 -MeOH- H_2O (65:35:5 v/v/v), and then dried. The plates were examined before and after visualization, either under visible light or under a UV lamp at 254 and 366 nm. The retardation factors (Rf) of the observed spots were calculated as the ratio of the distance traveled by the compound to the distance traveled by the solvent front.

Detection of terpenoids and saponins

These compounds were detected using Godin's reagent. After spraying the plate with Godin's reagent and heating it at 100°C for 10 minutes, various colors appeared. Under visible light, violet and red spots indicated the presence of monoterpenes, while blue spots indicated saponins [13].

Detection of alkaloids

After spraying with Dragendorff's reagent and heating the chromatogram at 100°C for 10 minutes, alkaloids appeared as orange spots under visible light.

Detection of polyphenols

After spraying the chromatogram with 10% Folin-Ciocalteu reagent and heating at 100°C for 10 minutes, blue spots observed under visible light confirmed the presence of polyphenols [14].

Detection of flavonoids and sesquiterpene lactones

After spraying the chromatogram with 5% (w/v) aluminum chloride (AlCl_3) and heating, the presence of flavonoids was indicated by yellow spots visible under either visible light or UV light at 366 nm. Sesquiterpene lactones were indicated by fluorescence of various colors at 366 nm [13].

Detection of tannins

The appearance of spots of various colors (blue, green, black), visible under visible light after spraying the chromatogram with a 10% FeCl_3 solution, indicated the presence of tannins.

Detection of anthraquinones and anthrones

A 5% ethanolic KOH solution was sprayed onto the chromatogram. Red spots, visible under both visible light and at 366 nm, confirm the presence of anthraquinones. Anthrones, on the other hand, appear as yellow spots at 366 nm [15].

Statistical analysis of results

One-way ANOVA followed by Tukey's test was used to compare MIC values among themselves and MBC values among themselves, and to determine whether the activity of the fractions was statistically influenced by bacterial phenotypes. Results are expressed as means $\pm$ standard deviations. Probability values of $P < 0.05$ were considered statistically significant. R software (version 4.4.3) was used to perform these statistical tests.

RESULTS

Antibacterial activity results

Bacterial susceptibility

The fractions derived from the aqueous extract of *T. leiocarpa* showed varying degrees of activity against all the bacteria tested. This is evidenced by the mean inhibition diameters obtained, ranging from 25 ± 1.3 to 14 ± 1.6 mm for the organic fractions (chloroform, ethyl acetate, and n-butanol fractions) and from 22 ± 0.5 to 17 ± 0.9 mm for the residual aqueous fraction (Table 2). This inhibitory activity was particularly notable regarding the *in vitro* growth of *Pseudomonas aeruginosa* 885C; the largest mean inhibition diameters were observed with this strain specifically 25 ± 1.3 mm for the n-butanol fraction and 22 ± 1.4 mm for the residual aqueous fraction. In other words, this wild-type strain, isolated from the pus of a patient with a *Pseudomonas* infection, proved to be the most susceptible to both categories of fractions. Overall, the organic fractions yielded mean inhibition diameters comparable to those of the residual aqueous fraction, and the various values obtained indicate that susceptibility varied according to the polarity of the extraction solvents.

Detection of coumarone

Table 02: Activity (mean $\pm$ SD) of fractions derived from the aqueous extract of *T. leiocarpa* leaves at 50 mg/mL against *Pseudomonas aeruginosa* strains.

Coded species	Inhibition diameters (mm)			
	Chloroform fraction	Ethyl acetate fraction	n-Butanol fraction	Residual aqueous fraction
<i>Pseudomonas aeruginosa</i> 891C	$16 \pm 0,4$	$20 \pm 0,3$	$20 \pm 0,2$	$19 \pm 0,4$
<i>Pseudomonas aeruginosa</i> 489C	$15 \pm 1,2$	$19 \pm 1,4$	$21 \pm 2,4$	$19 \pm 2,2$
<i>Pseudomonas aeruginosa</i> 261C	$16 \pm 1,6$	$18 \pm 3,4$	$19 \pm 1,4$	$17 \pm 0,9$
<i>Pseudomonas aeruginosa</i> 1076C	$19 \pm 2,4$	$21 \pm 0,4$	$20 \pm 0,4$	$20 \pm 0,7$
<i>Pseudomonas aeruginosa</i> 885C	$17 \pm 4,4$	$19 \pm 1,5$	$25 \pm 1,3$	$22 \pm 1,4$
<i>Pseudomonas aeruginosa</i> 792C	$14 \pm 1,6$	$18 \pm 0,9$	$24 \pm 0,6$	$19 \pm 0,4$
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	$19 \pm 0,4$	$20 \pm 0,4$	$21 \pm 0,5$	$22 \pm 0,5$

5% (w/v) basic lead acetate ((CH₃COO)₂Pb) was used to spray the chromatogram. Green and blue fluorescent spots under UV light at 366 nm indicated the presence of coumarins

Antibacterial parameters

Both types of fractions yielded identical MICs, inhibiting all bacterial growth at 0.019 mg/mL; these values were thus obtained independently of the bacterial phenotypes. Regarding minimum bactericidal concentrations (MBCs), values ranged from 0.019 to 0.156 mg/mL for the organic fractions and from 0.019 to 0.078 mg/mL for the residual aqueous fraction (Table 3). Individually, the chloroform, n-butanol, and residual aqueous fractions exhibited a bactericidal effect on all strains, whereas the ethyl acetate fraction was bactericidal against 57.14% of them.

Table 03: Antibacterial parameters of fractions derived from the aqueous extract of *T. leiocarpa* leaves at 50 mg/mL against *Pseudomonas aeruginosa* strains.

		<i>Pseudomonas aeruginosa</i> strains						
		891C	489C	261C	1076C	885C	793C	(ATCC 27853)
Chloroform fraction	MIC	0,019	0,019	0,019	0,019	0,019	0,019	0,019
	MBC	0,078	0,039	0,019	0,078	0,039	0,019	0,078
	MBC/MIC	4	2	1	4	2	1	4
	Power	Bcide	Bcide	Bcide	Bcide	Bcide	Bcide	Bcide
Ethyl acetate fraction	MIC	0,019	0,019	0,019	0,019	0,019	0,019	0,019
	MBC	0,019	0,156	0,156	0,078	0,039	0,039	0,156
	MBC/MIC	1	8	8	4	2	2	8
	Power	Bcide	Btique	Btique	Bcide	Bcide	Bcide	Btique
Fraction of n-butanol	MIC	0,019	0,019	0,019	0,019	0,019	0,019	0,019
	MBC	0,019	0,039	0,078	0,039	0,039	0,039	0,078
	MBC/MIC	1	2	4	2	2	2	4
	Power	Bcide	Bcide	Bcide	Bcide	Bcide	Bcide	Bcide
Residual aqueous fraction	MIC	0,019	0,019	0,019	0,019	0,019	0,019	0,019
	MBC	0,019	0,078	0,078	0,019	0,039	0,019	0,078
	MBC/MIC	1	4	4	1	2	1	4
	Power	Bcide	Bcide	Bcide	Bcide	Bcide	Bcide	Btique

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; Bcidal: bactericidal; Bstatic: bacteriostatic.

Statistical analysis results

The statistical analysis performed in this study enabled a comparison of the mean values $\pm$ standard deviations obtained for wild-type bacterial strains (*Pseudomonas aeruginosa* 792C; 885C) and those of other *Pseudomonas* strains with a resistant phenotype. Analysis of variance revealed that the differences observed in MIC and MBC values were not significant at the 0.05 level (Tables 4 and 5 ; $P > 0.05$).

Table 04: Comparison of mean values (mean $\pm$ SD of three trials) of MIC and MBC for alcoholic fractions according to bacterial strain phenotypes.

Antibacterial parameters	Wild-type bacterial strains	Resistant bacterial strains	p-value
MIC (mg/ml)	0,021 $\pm$ 0,4	0,033 $\pm$ 1,2	P > 0,05
MBC (mg/ml)	0,043 $\pm$ 1,2	0,11 $\pm$ 0,4	P > 0,05

Table 05: Comparison of mean values (mean $\pm$ SD of three trials) of MIC and MBC for the residual aqueous fraction according to bacterial strain phenotypes.

Antibacterial parameters	Wild-type bacterial strains	Resistant bacterial strains	p-value
MIC (mg/ml)	0,025 $\pm$ 0,2	0,374 $\pm$ 1,2	P > 0,05
MBC (mg/ml)	0,041 $\pm$ 1,0	0,517 $\pm$ 0,5	P > 0,05

Phytochemical screening results

Phytochemical screening revealed the presence of ten groups of phytoconstituents in the various fractions. These include terpenes, saponins, polyphenols, flavonoids, xanthenes, naphthoquinones, alkaloids, tannins, coumarins, and anthrones (Table 6).

Table 06: Compounds identified by TLC in *T. leiocarpa* leaves

Developing solvent : Chloroform/methanol/water (65:35:5)									
Leaf extracts	Before revelation		Color after development						Possible types of compounds
	Rf	Color	Godin		Folin-Ciocalteu		AlCl ₃		
			Rf	Color	Rf	Color	Rf	Color	
Hexane fraction	0,87	Blue	0,87	Purple	0,87	Blue	0,87	Yellow	Terpenes (G), saponins,
			0,67	Yellow	0,67	Blue	0,67	Yellow	
			0,25	Purple	0,25	Blue	0,25	Yellow	
Chloroform fraction	0,87	Purple	0,88	Purple	0,88	Blue	0,87	Blue	Terpenes (G), polyphenols (G, F), Flavonoids (G, A) or xanthones (G) or naphthoquinones (G)
			0,67	Yellow	0,67	Blue	0,69	Yellow	
			0,28	Purple	0,28	Blue	0,28	Yellow	
							0,22	Yellow	
							0,15	Yellow	
Ethyl acetate fraction	0,87	Blue	0,87	Purple			0,87	Blue	Terpenes (G), saponins (G), polyphenols (F), flavonoids (A)
					0,43	Blue	0,67	Yellow	
			0,52	Purple			0,52	Yellow	
					0,28	Blue	0,28	Yellow	
n-Butanol fraction	0,88	Blue	0,88	Purple			0,88	Blue	Saponins (G), terpenes (G), polyphenols (F), flavonoids (A)
					0,67	Blue	0,67	Yellow	
			0,25	Purple			0,25	Yellow	
Residual aqueous fraction			0,88	Purple			0,88	Blue	Terpenes (G), polyphenols (F), flavonoids (A)
					0,67	Blue	0,67	Yellow	
							0,25	Yellow	

G: Compounds revealed with Godin's reagent; F: Compounds revealed with Folin-Ciocalteu reagent; A: Compounds revealed with AlCl₃

Table 06 (continued): Compounds identified by TLC in *T. leiocarpa* leaves

Developing solvent : Chloroform/methanol/water (65:35:5)									
Leaf extracts	Before revelation		Color after development						Possible types of compounds
	Rf	Color	FeCl ₃		Dragendorff		KOH 5%		
			Rf	Color	Rf	Color	Rf	Color	
Hexane fraction	0,87	Blue							alkaloids (D), anthrones (K), coumarins (K)
			0,81	Black			0,81	Yellow	
			0,12	Black	0,12	Orange	0,12	Yellow	
Chloroform fraction	0,87	Purple	0,87	Black					Tanins (F), alkaloids (D), coumarins (K)
			0,81	Black			0,81	Yellow	
			0,12	Black	0,12	Orange			
Ethyl acetate fraction	0,87	Blue					0,87	Yellow	Tannins (F), alkaloids (D), coumarins (K)
							0,81		
			0,12	Black	0,12	Orange			
n-Butanol fraction	0,87	Blue					0,87	Yellow	Tannins (F), alkaloids (D), coumarins (K)
			0,12	Black	0,12	Orange			
Residual aqueous fraction							0,87	Yellow	Tannins (F), alkaloids (D), coumarins (k), anthrones (K)
			0,12	Black	0,12	Orange	0,12	Yellow	

D : Compounds revealed with Dragendorff's reagent; F : Compounds revealed with FeCl₃ ; K: Compounds revealed with 5% KOH

DISCUSSION

The anti-*Pseudomonas* activity of fractions obtained from the liquid-liquid separation of the aqueous extract of *T. leiocarpa* leaves was evaluated against the *in vitro* growth of six clinical strains of this bacterium: two wild-type strains, four strains exhibiting resistance to carbapenems (specifically imipenem), and one reference strain. The tests demonstrated that the plant species *T. leiocarpa* possesses remarkable antibacterial activity, as all fractions showed varying degrees of activity against the tested bacterial strains. This is evident from the observed inhibition zone diameters, which ranged from 14 ± 1.6 mm to 25 ± 1.3 mm. Indeed, using the solid-medium diffusion method, an extract is considered active if it produces an inhibition zone of 10 mm or greater [16] a criterion met by the extracts used in this study. The n-butanol fraction was the most notable, as it inhibited the *in vitro* growth of *Pseudomonas aeruginosa* 885C, yielding the largest inhibition zone diameter (25 ± 1.3 mm) at a concentration of 50 mg/mL.

This bacterium is responsible for iatrogenic local infections following instrumental procedures such as urinary tract infections after catheterization, bronchopulmonary infections in patients on ventilators, and contact lens-related ocular infections as well as septicemia in burn victims and granulopenic patients. It also causes bronchial superinfection in cystic fibrosis patients, facilitated by the production of elastase [17]. In light of the foregoing, the observed activity could justify the traditional use of *T. leiocarpa* in treating these conditions [18]. The aqueous fraction showed similar activity against the same strain, yielding an inhibition zone diameter of 22 ± 1.4 mm at a concentration of 50 mg/mL. The results obtained with both types of fractions were nearly identical, suggesting that the active compounds responsible for this antibacterial activity are soluble in both types of polar solvents. This significant antibacterial activity may be attributed to the fact that the Combretaceae family is rich in various constituents notably flavonoids, tannins, polyterpenes, polyphenols, and saponins which confer this property [19].

Regarding antibacterial parameters (MIC and MBC), the lowest value at which the inhibitory action of the fractions began to manifest was 0.019 mg/mL. This value was observed across all selected strains. It can therefore be stated that the fractions were effective against the entire set of strains involved in this study. These results appear similar to those reported in [20]. Indeed, that author demonstrated that leaf and root extracts of *Anogeissus leiocarpus* (DC) Guill. & Perr. possess bactericidal and bacteriostatic activity against a wide range of multidrug-resistant bacteria, including **Pseudomonas aeruginosa**, a pathogen responsible for major opportunistic infections.

Statistical analysis allowed for the assessment and comparison of mean values for the different fractions across all selected bacterial strains. The analysis indicated that the differences observed in MIC and MBC values between the two categories of strains were not statistically significant. This suggests, on the one hand, that the activity of the fractions was not statistically influenced by bacterial phenotypes. On the other hand, while the values differ, they produce the same effects on the bacteria, suggesting a probable synergy or complementarity of action among the

various chemical constituents responsible for the observed activity. For future, more in-depth studies, it would therefore be more advantageous to use the plant in the form of a crude extract. This approach aligns closely with African pharmacopoeia practices, particularly regarding the choice of water as the primary solvent.

Furthermore, with the exception of the ethyl acetate fraction, all fractions exhibited bactericidal activity against all strains tested in this study, as the MBC/MIC ratios were all less than or equal to 4 [11]. This bactericidal activity of *T. leiocarpa* leaves could fully justify its widespread use in African pharmacopoeia against various conditions, including those caused by *Pseudomonas*. A plant identified as a remedy for a microbial infection is often prescribed to treat various other common conditions. It thus has multiple uses, which can be attributed to the presence of numerous groups of biologically active chemical compounds revealed by chemical screening of its plant parts [21]. Indeed, phytochemical investigations have identified ten (10) groups of chemical compounds, including terpenes, saponins, polyphenols, flavonoids, xanthenes, naphthoquinones, alkaloids, tannins, coumarins, and anthrones. These phytoconstituents are responsible for the pharmacological effects observed with the plant's use and, consequently, determine its therapeutic value. Many of these compounds are known for their antibacterial effects; the flavonoids present in this plant species are secondary metabolites with significant antibacterial potency. Due to their abundance of phenolic groups, these flavonoid compounds can bind to specific proteins and enzymes, thereby altering enzymatic balances [18]. These constituents likely contribute to the observed antibacterial activity and provide a rationale for the traditional use of *T. leiocarpa* internally to treat bacterial urinary tract and bronchopulmonary infections, and externally to treat eye ailments and sepsis in burn victims [17]. Saponosides, including saponins, are also recognized for their anti-inflammatory, anti-edema, analgesic, and antibacterial properties [22]. Their presence likely explains the plant's role in alleviating conditions such as eye and ear infections, as well as urinary tract infections that cause excruciating pain. Tannins are known for their antiseptic, bactericidal, and astringent properties [23]. The use of *T. leiocarpa* to treat pimples, boils, and skin wounds may be attributed to its tannins-specifically both catechin and gallic types *T. leiocarpa* is well known among local populations for its efficacy in traditional medicine. It is also utilized in handicrafts, dyeing, construction, food production, and livestock farming [18]. For these reasons, it is being overexploited and has begun to become scarce in certain areas. Protecting this plant must be a priority in the effort to safeguard biodiversity in Côte d'Ivoire.

Given that *Pseudomonas aeruginosa* is currently classified as a critical pathogen threatening human health, it would be worthwhile to explore the potential of medicinal plants an inexhaustible source of plant secondary metabolites.

CONCLUSION

This study confirms the anti-*Pseudomonas* activity of *Terminalia leiocarpa* leaves against strains representing various bacterial phenotypes. Measurements of MIC and MBC values demonstrate the bactericidal effect of most

fractions, confirming that the active principles dissolve optimally in water and alcohol ; indeed, the chemical compound classes responsible for this efficacy are highly concentrated in these solvents. These promising results warrant further research, specifically to delve deeper into the ethnopharmacological aspects, conduct *in vivo* tests, and assess the plant's safety. *T. leiocarpa* thus offers encouraging prospects for addressing the public health challenge posed by microbial diseases. Finally, strengthening the protection of this species is essential to safeguard this valuable component of Ivorian flora.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all the authors mentioned. They participated in all stages of the manuscript's development, up to its final version. They have therefore read and approved the final manuscript.

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