

Antibacterial Profile of the Fractionated 80% Methanolic Extract of *Mallotus oppositifolius* (Geisel.) Müll. -Arg. (Euphorbiaceae) Against the Challenges of Multidrug Resistance in *Pseudomonas aeruginosa*

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Abstract

Original Research Article

Infectious diseases pose a significant challenge in Côte d'Ivoire. While treatments for these conditions exist, repeated therapeutic failures due to pathogen resistance have called into question the curative efficacy of current anti-infective agents. This study aims to provide a scientific basis for the traditional therapeutic use of the medicinal plant *Mallotus oppositifolius* (Geisel.) Müll.-Arg (Euphorbiaceae). It involved investigating the antibacterial activity of hexane, chloroform, ethyl acetate, and butanol fractions derived from an 80% methanolic extract of *Mallotus oppositifolius* leaves against seven strains of *Pseudomonas aeruginosa* (comprising six clinical isolates with resistant phenotypes and one reference strain). An agar well diffusion method was used to assess strain susceptibility. A screening test identified the two most active fractions for the determination of antibacterial parameters via the broth macro-dilution method. Biological analyses demonstrated the superior activity of the ethyl acetate fraction; at a concentration of 50 mg/mL, it produced the largest zone of inhibition (18.5 ± 0.6 mm) against *P. aeruginosa* strain 1780C/14. With the exception of the chloroform fraction, the other fractions generally exhibited remarkable activity, characterized by zones of inhibition exceeding 10 mm. Minimum Inhibitory Concentrations (MICs) ranged from 0.19 to 0.78 mg/mL for the ethyl acetate fraction and from 0.39 to 0.78 mg/mL for the butanol fraction. The latter demonstrated bactericidal activity against all tested bacterial strains. Furthermore, TLC analysis of the fractions revealed a profile rich in secondary metabolites, including sesquiterpene lactones, saponins, flavonoids, anthrones, anthraquinones, alkaloids, tannins, and coumarins. The combined presence of these bioactive compounds supports the therapeutic potential of *Mallotus oppositifolius*. These results validate the therapeutic use of this plant in African pharmacopoeia.

Keywords: Antimicrobial activity, *Mallotus oppositifolius*, phytocompounds, Côte d'Ivoire.

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INTRODUCTION

The use of plants for therapeutic purposes is an ancient practice (Lebreton, 2014). It has enabled peoples around the world to treat themselves and overcome various ailments. Healthcare systems based on this practice were established and demonstrated lasting efficacy and safety for both human and animal health (Matzigkeit, 1993). Plants thus represent a source for developing affordable, improved traditional medicines, as well as a promising avenue for discovering new antimicrobial molecules to combat the growing phenomenon of bacterial resistance to antibiotics (Sanogo, 2006). In Côte d'Ivoire, populations still rely heavily on traditional medicine to meet their healthcare needs, despite the availability of conventional medicine

(Koné & Kamanzi, 2006). Ethnobotanical surveys have shown that the Ivorian flora includes numerous medicinal plants used to treat various human and/or animal diseases (Zirihi *et al.*, 2010). Several pharmacological and phytochemical studies have confirmed that Ivorian plants possess biological activities and contain chemical compounds that could be beneficial in managing various diseases and in the search for new therapeutic molecules (Kouakou *et al.*, 2015). This is particularly significant given that, globally, nearly 25% of prescriptions are plant-based and 60–70% of antibacterial drugs are substances of natural origin (Diallo, 2005). A case in point is Echinaforce®, an antiviral, antibacterial, and immunomodulatory agent derived from *Echinacea purpurea* Moench (Sharma *et al.*, 2010). Given the threat of microorganism resistance

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to anti-infectives, the question arises as to whether medicinal plants from Côte d'Ivoire specifically those containing antibacterial compounds—offer a viable alternative for managing infections caused by multidrug-resistant bacteria.

The aim of this study is to evaluate the antibacterial activity of hexane, chloroform, ethyl acetate, and butanol fractions derived from an 80% methanolic extract of *Mallotus oppositifolius* leaves against seven strains of *Pseudomonas aeruginosa*, comprising six clinical isolates with a resistant phenotype and one reference strain. Additionally, phytochemical screening was conducted to identify the major classes of chemical compounds present in the plant.

This plant was selected based on an ethnobotanical survey conducted among traditional medicine practitioners in the Lakota department.

According to the literature, it is widely used in African pharmacopoeia to treat various conditions, including bacterial infections such as diarrhea, urinary tract infections, chronic wounds, and dental caries (Uchendu *et al.*, 2008).

MATERIALS AND METHODS

1- Materials

1-1- Plant material

The plant material consisted of an extract obtained from the powdered leaves of *Mallotus oppositifolius* (Geisel.) Müll.Arg. (Euphorbiaceae).

1-2- Bacterial strains

The fractions were tested against seven strains of *Pseudomonas aeruginosa*, comprising six carbapenem-resistant clinical isolates (PARC) and one reference strain (Table I).

Table I: List of bacterial strains studied

Bacteria	Codes	Origins	Resistance phenotypes
Clinical strains			
<i>Pseudomonas aeruginosa</i>	891C/11	suppuration	PARC due to porin D2 deficiency.
<i>Pseudomonas aeruginosa</i>	489C/11	Wound	PARC due to porin D2 deficiency.
<i>Pseudomonas aeruginosa</i>	1060C/11	Probe tip	PARC due to porin D2 deficiency.
<i>Pseudomonas aeruginosa</i>	1810C/11	Catheter aspiration	PARC due to porin D2 deficiency.
<i>Pseudomonas aeruginosa</i>	1076C/11	Urine	PARC due to porin D2 deficiency.
<i>Pseudomonas aeruginosa</i>	1780C/14	Urine	PARC due to porin D2 deficiency.
Reference strains			
<i>Pseudomonas aeruginosa</i>	ATCC 27853		

PARC: Carbapenem-resistant *Pseudomonas aeruginosa*

1-3- Technical equipment, solvents, and reagents

Conducting the antibacterial study required the following solvents: methanol (Prolabo®), distilled water, hexane (Prolabo®), chloroform (Prolabo®), ethyl acetate (Prolabo®), and n-butanol (Prolabo®); an antibiotic: imipenem (IPM) (Biorad®); reagents: Godin's reagent, Dragendorff's reagent, Folin-Ciocalteu reagent (10%), aluminum chloride, basic lead acetate (5%), FeCl₃ solution (10%), and ethanolic KOH solution (5%); technical equipment: UV lamp, chromatography tank, digital camera, hot plate, and oven; as well as glassware and small laboratory tools.

2- Methods

2-1- Preparation of the crude extract

Mallotus oppositifolius leaves were harvested in August in southwestern Côte d'Ivoire. Harvesting took place at dusk. According to Sofowora (1996), active principles are more concentrated in plants between sunset and midnight. The harvested plant parts were air-dried in an air-conditioned room and pulverized; the resulting powders were stored in paper envelopes to prevent mold growth. Extraction was performed by successively exhausting the plant powder via maceration using solvents of increasing polarity (Kouadio *et al.*, 2015). 125 g of plant powder was placed in a glass jar

with 1 L of hexane and left to macerate for 24 hours under mechanical agitation to remove fats. The residual marc was dried at room temperature on blotting paper and then macerated in 80% (v/v) methanol for 24 hours. The mixture was filtered under the same conditions as before, and the resulting filtrate was concentrated using a rotary evaporator at 40°C to remove the alcohol. The aqueous residue was evaporated in an oven at 50°C to obtain the 80% methanolic extract.

2-2- Fractionation of crude extracts

The various fractions were obtained using a liquid-liquid partitioning technique, based on the method described by N'guessan *et al.* (2007). To do this, 10 g of the 80% methanolic extract from *Mallotus oppositifolius* leaves were dissolved in 100 mL of sterile distilled water. Once fully dissolved, the aqueous solution was transferred to a separatory funnel, and 100 mL of the chosen solvent was added. The funnel containing the mixture was shaken and then allowed to settle completely. After the two phases (organic and aqueous) had fully separated, the organic phase was collected. This process was repeated until the organic phase became clear. The procedure was then continued with the next solvent, following an order of increasing polarity. The solvents used in this sequence were hexane, chloroform,

ethyl acetate, and butanol. These fractions were evaporated at 40°C to yield the hexane, chloroform, ethyl acetate, and butanol fractions. Finally, the residual aqueous phase was also evaporated in an oven at 50°C.

2-3- Antibacterial testing

2-3-1- Preparation of the inoculum for solid-medium tests

Using a Pasteur pipette fitted with a bulb, two isolated colonies from a 24-hour Petri dish culture on selective medium were collected to prepare a suspension in NaCl with an optical density equivalent to a 0.5 McFarland standard. The suspension was then diluted by adding 10 µL of the *P. aeruginosa* culture to 10 mL of physiological saline (Bassaibis *et al.*, 2009). This resulting bacterial solution served as the final inoculum, with an estimated concentration of 10^6 CFU/mL. Microbial sensitivity tests to the partitions

Before evaluating the antibacterial activity of the various partitions, a sterility test was performed to ensure they were free of microorganisms (Guessennd, 2005). Subsequently, Mueller-Hinton (MH) agar plates were inoculated using the flood inoculation method. The plates were allowed to dry at room temperature for 15 minutes. Four wells, each 6 mm in diameter, were cut into the agar. Each well was filled with 50 µL of a specific partition. The control well received only 50 µL of sterile distilled water. The inoculated plates were left at room temperature (26°C) for 30 minutes to allow for the pre-diffusion of the partitions. They were then incubated at 37°C for 24 hours. The diameter of the zones of inhibition (ZI) around the wells was measured using a caliper (Figure 1). Bacterial sensitivity was assessed according to the scale established by Poncé *et al.* (2003), structured as follows: ZI < 8 mm: ineffective substance; 9 mm ≤ ZI ≤ 14 mm: effective substance; 15 mm ≤ ZI ≤ 19 mm: highly effective substance; ZI ≥ 20 mm: extremely effective substance.

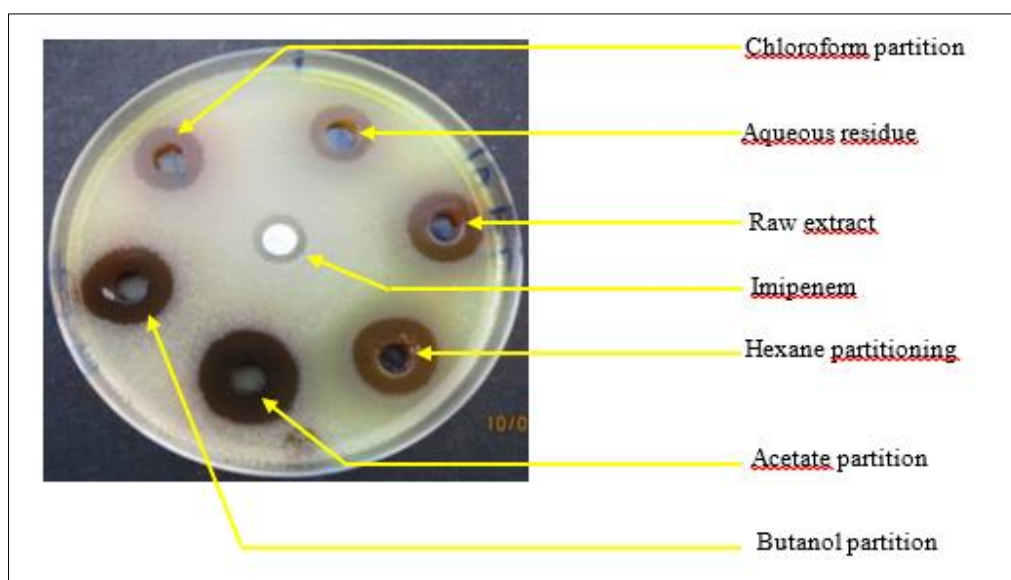


Figure 1: Illustration of the activity of *Mallotus oppositifolius* fractions against an imipenem-resistant *P. aeruginosa* strain

2-3-2-Preparation of the inoculum for liquid medium tests

The bacterial inoculum was prepared using young colonies and Mueller-Hinton broth (MHB). Using a Pasteur pipette fitted with a bulb, two isolated colonies were picked from a 24-hour bacterial culture. These colonies were homogenized in 10 mL of MHB. The resulting solution was incubated for 3 hours at 37°C to obtain a bacterial preculture. Next, 0.3 mL of the preculture was added to 10 mL of BMH. The resulting bacterial suspension was estimated to contain approximately 10^6 cells/mL and served as the pure inoculum referred to as the "100" inoculum.

2-3-3- Preparation of the concentration range

The concentration range was prepared using the twofold serial dilution method. To do this, a solution of

the fractions 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.

2-3-4- Determination of antibacterial parameters

Anti-*Pseudomonas* parameters were determined using the broth macro-dilution method, adapted from the protocol described by Kouadio *et al.* (2015). In a series of 10 hemolysis tubes, 0.2 mL of each fraction concentration was mixed with 1.8 mL of bacterial inoculum. This procedure resulted in a 1:10 dilution of the initial solutions, yielding a final concentration range of 5 to 0.019 mg/mL. A growth control was prepared by substituting the fraction with 0.2 mL of sterile distilled water, while a sterility control contained only 2 mL of sterile Mueller-Hinton broth.

After incubation at 37°C for 24 h, the Minimum Inhibitory Concentration (MIC) was defined as the lowest extract concentration at which no bacterial growth was visible to the naked eye. The Minimum Bactericidal Concentration (MBC) defined as the concentration resulting in a viable bacterial count less than or equal to 0.01% of the initial inoculum was determined by colony counting. Initially, serial dilutions (from 10^{-1} to 10^{-4}) of the starting inoculum were streaked onto Mueller-Hinton agar (MHA) in 5 cm-long lines using a calibrated 2 μ L loop (set of plates A) and then incubated at 37°C for 24 hours. After reading the MIC, the contents of all tubes showing no visible turbidity were plated using the same procedure (set of plates B) and incubated under identical conditions. The MBC was determined by comparing colony counts between plates A and B, thereby identifying the lowest partition concentration that reduced the initial bacterial load by 99.99%.

The MBC/MIC ratio was used to characterize the mode of action of the extracts (Fauchere, 2002). According to this author, if the MBC/MIC ratio is less than or equal to 2, the substance is considered bactericidal; conversely, if it is greater than 2, the substance is considered bacteriostatic.

2-4- Phytochemical screening

The detection of various chemical compounds in the partitions was performed using thin-layer chromatography (TLC) following the method described by Kouadio *et al.* (2015). This method allows for the identification of several groups of secondary metabolites through specific staining, observed either in the visible

range or at a specific wavelength (Sanogo *et al.*, 2016). A 10 mg mass of each partition 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): CHCl₃-MeOH-H₂O. (65:35:5 v/v/v), then dried. These 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 various observed spots were calculated; these correspond to the ratio of the distance traveled by the compound to the distance traveled by the solvent.

RESULTS AND DISCUSSION

3-1- Results

3-1-1- Bacterial sensitivity to partitions

The fractions obtained from the crude 80% methanolic extract of *Mallotus oppositifolius* leaves all showed activity against *P. aeruginosa* strains, with the exception of the aqueous residue. The acetate fraction demonstrated the most notable activity, with inhibition zone diameters ranging from 16.5 ± 0.6 to 18.5 ± 0.6 mm (Figure 2). The largest value was observed against *P. aeruginosa* 1780C/14, a carbapenem-resistant strain isolated from the urine of a patient with a urinary tract superinfection. Inhibition diameters ranged from 10.67 ± 0.6 to 12.5 ± 1.5 mm for the hexane fraction, from 8 ± 1.2 to 9 ± 0.6 mm for the chloroform fraction, and from 10.67 ± 1.0 to 16.5 ± 1.0 mm for the butanol fraction.

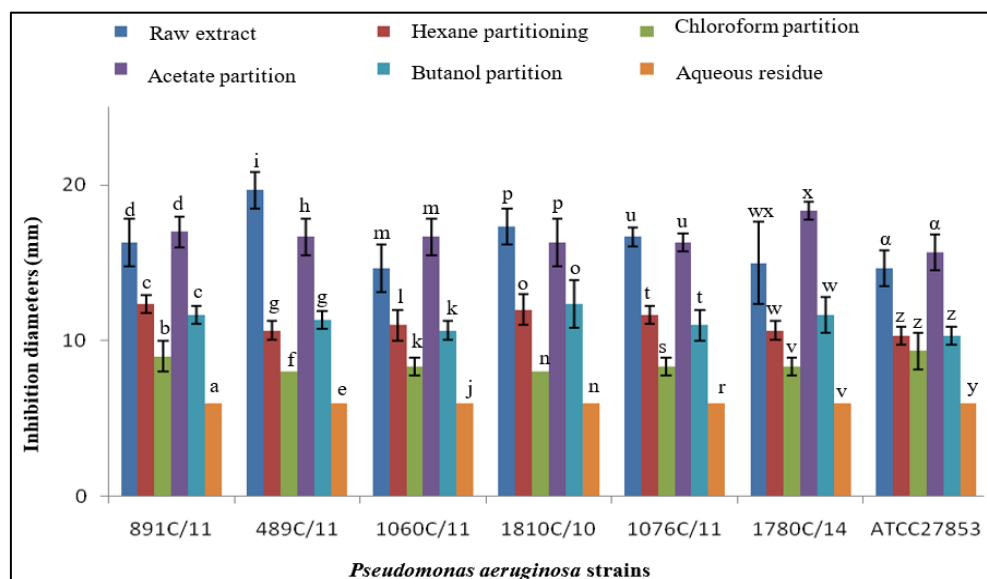


Figure 2: Activity of *Mallotus oppositifolius* fractions (mean $\pm$ SD) against *P. aeruginosa* strains

Bars sharing the same letters are not significantly different at the $\alpha = 5\%$ level.

A study was conducted to select the most active fractions (Table II). The results indicate that the acetate

and butanol fractions were the most active, exhibiting the largest inhibition diameters 18.1 ± 5.5 mm and 16.9 ± 5.7 mm, respectively. Consequently, these fractions were selected for the determination of MIC and MBC values.

Table II : Analysis of variance of the inhibition zone diameters

Excerpt	Mean ± Standard deviation		
Hexane partitioning	14.89	±	4.79 ^b
Chloroform partition	13.95	±	3.73 ^b
Acetate partition	18.08	±	5.48 ^a
Butanol partition	16.90	±	5.70 ^a
Aqueous residue	14.00	±	3.64 ^b
ANOVA statistical parameters			
dl	4		
F	9.061		
P	< 0.00		

Bars sharing the same letters are not significantly different at the $\alpha = 5\%$ level.

3-1-2- Antibacterial parameters of the fractions

The ethyl acetate fraction derived from the 80% methanolic crude extract of *Mallotus oppositifolius* leaves was bactericidal against all strains except *P. aeruginosa* 891C/14 (Table III). The lowest MIC value for this fraction (0.19 mg/mL) was observed against the

P. aeruginosa strains 891C/14 and 1076C/14. MBC values ranged from 0.39 to 1.56 mg/mL.

The n-butanol fraction was bactericidal against all strains tested in this study, with MICs ranging from 0.39 to 0.78 mg/mL; this activity was most notable against *P. aeruginosa* strains 489C/11 and 1076C/11. MBC values ranged from 0.78 to 1.56 mg/mL.

Table III : MIC and MBC of fractions from the 80% methanolic extract of *Mallotus oppositifolius*

Bacterial strains	Ethyl acetate partition				n-Butanol partition			
	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	Power	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Power
<i>P. aeruginosa</i> 891C/11	0.19	1.56	6	Bctique	0.78	1.56	2	Bcide
<i>P. aeruginosa</i> 489C/11	0.78	1.56	2	Bcide	0.39	0.78	2	Bcide
<i>P. aeruginosa</i> 1060C/11	0.39	0.78	2	Bcide	0.78	1.56	2	Bcide
<i>P. aeruginosa</i> 1810C/10	0.39	0.78	2	Bcide	0.78	1.56	2	Bcide
<i>P. aeruginosa</i> 1076C/11	0.19	0.39	2	Bcide	0.39	0.78	2	Bcide
<i>P. aeruginosa</i> 1780C/14	0.39	0.78	2	Bcide	0.78	1.56	2	Bcide
<i>P. aeruginosa</i> ATCC 27853	0.19	0.39	2	Bcide	0.39	0.78	2	Bcide

Bcide: bactericidal; Bctique: bacteriostatic

Phytochemical screening results

TLC-based phytochemical screening of the fractions derived from the 80% methanolic crude extract of *Mallotus oppositifolius* leaves identified eight compounds, including flavonoids, polyphenols, saponins, alkaloids, tannins, coumarins, sesquiterpene

lactones, and anthrones. Alkaloids, tannins, and sesquiterpene lactones were detected in all fractions. Coumarins were observed in the hexane, ethyl acetate, and n-butanol fractions, whereas saponins were found only in the n-butanol fraction and the aqueous residue (Table IV).

Table IV: Phytochemicals identified in the fractions of the 80% methanolic extract of *Mallotus oppositifolius* leaves and trunk bark

Chemical compounds	Hexane partitioning	Acetate partition	Butanol partition	Aqueous residue
Saponins	--	--	+	+
Tannins	+	+	+	+
Coumarins	+	+	--	--
Alkaloids	+	+	+	+
Flavonoids	--	+	+	--
Anthrones	+	+	+	--
Sesquiterpene lactones	+	+	+	+
Anthraquinones	--	--	+	--

DISCUSSION

The anti-*Pseudomonas* activity of fractions obtained from the liquid-liquid separation of an 80% (v/v) methanolic extract of *Mallotus oppositifolius* leaves

was evaluated *in vitro* against seven strains of *Pseudomonas aeruginosa*. This panel included one reference strain and six clinical isolates resistant to carbapenems, particularly imipenem. Biological assays

revealed that *M. oppositifolius* possesses remarkable antibacterial activity. With the exception of the chloroform fraction, all fractions inhibited bacterial growth to varying degrees. The measured diameters of the zones of inhibition ranged from 10.67 ± 0.6 mm to 18.5 ± 0.6 mm. According to the criteria established by Biyiti (2004), an extract is considered active in the agar diffusion method if it produces a zone of inhibition of 10 mm or greater a threshold met by all the active fractions in this study. The ethyl acetate fraction proved to be the most active, displaying the largest zone of inhibition (18.5 ± 0.6 mm) at a concentration of 50 mg/mL against the multidrug-resistant strain *P. aeruginosa* 1780C/14. This clinical isolate was obtained from the urine of a female patient suffering from a urinary tract infection resulting from a superinfection. It is worth noting that this bacterial species is also responsible for severe bronchial superinfections in patients with cystic fibrosis, notably through the production of elastase (Pierre and Marie, 2003).

The observed antibacterial activity provides scientific justification for the traditional use of *M. oppositifolius* in treating these conditions (Kouadio *et al.*, 2015). This efficacy can be attributed to the polarity of the extraction solvents, which influences fractionation and selectively concentrates secondary metabolites possessing biological properties. Furthermore, plants of the Euphorbiaceae family are known to be rich in bioactive compounds specifically flavonoids, tannins, polyterpenes, and saponins—chemical classes widely documented for their antimicrobial properties (Koné, 2005).

Regarding antibacterial parameters (MIC and MBC), the lowest concentration at which inhibitory activity was observed in the fractions was 0.19 mg/mL for the ethyl acetate fraction and 0.39 mg/mL for the butanol fraction. The former value was obtained with *P. aeruginosa* strains 891C/11 and 1076C/11, while the latter was recorded for strains 489C/11 and 1076C/11. These results indicate that the inhibitory activity of these fractions was particularly effective against these specific strains. These findings align with those of Kouadio *et al.* (2015), who demonstrated that purified extracts from *M. oppositifolius* leaves possess bactericidal activity against a wide range of multidrug-resistant bacteria, including *P. aeruginosa*, a pathogen responsible for major opportunistic infections.

Statistical analysis enabled the selection of the most active fractions by comparing the mean inhibition diameters ($\pm$ standard deviations) across all tested bacterial strains. The results show that the ethyl acetate and butanol fractions produced the largest inhibition diameters, measuring 18.1 ± 5.5 mm and 16.9 ± 5.7 mm, respectively. These values are statistically similar, suggesting, on the one hand, that the active secondary metabolites are highly soluble in these two polar solvents. On the other hand, although the diameters differ

numerically, their statistical similarity indicates that they exert comparable effects on the microorganisms. This phenomenon highlights a likely synergy or complementarity of action among the various phytocompounds responsible for the observed biological activity.

Furthermore, the ethyl acetate fraction demonstrated bactericidal activity against 83.33% of the microorganisms included in the study, whereas the butanol fraction neutralized all the bacteria (with all MBC/MIC ratios being less than or equal to 2). This bactericidal potency of *M. oppositifolius* confirms the remarkable anti-*Pseudomonas* activity observed previously. It fully justifies the plant's traditional use in African pharmacopoeia for treating chronic wounds (Kouadio *et al.*, 2015) and various other conditions caused by bacteria of the genus *Pseudomonas* (Pierre and Marie, 2003).

A medicinal plant identified for treating a specific microbial infection is often prescribed for other common ailments. These multiple uses can be attributed to the presence of various groups of biologically active secondary metabolites, as revealed by phytochemical screening (Tra Bi, 2008). Indeed, phytochemical investigations have revealed the presence of polyphenols, flavonoids, saponins, alkaloids, tannins, coumarins, sesquiterpene lactones, and anthrones. These phytoconstituents are responsible for the observed pharmacological effects and determine the plant's therapeutic value. Several of these chemical groups are known for their antibacterial properties. In particular, the flavonoids identified in this species possess significant antibacterial potency. Due to their abundance of phenolic groups, these flavonoid compounds can bind to specific proteins and enzymes, thereby altering bacterial enzymatic balances (Sanogo, 2018). These constituents play a major role in the observed antibacterial activity. They provide a scientific basis for the traditional use of *M. oppositifolius* both internally and externally to treat dental caries and Buruli ulcer, two conditions in which *P. aeruginosa* frequently acts as a secondary infection agent (Koné, 2001).

Saponosides, including saponins, are also recognized for their anti-inflammatory, anti-edematous, analgesic, and antibacterial properties (Hoffman, 2003). Their presence likely explains the plant's role in alleviating conditions such as eye ailments and sepsis in burn victims (Pierre & Marie, 2003), which cause excruciating pain. Tannins are known for their antiseptic, bactericidal, and astringent properties (Sepulveda *et al.*, 2011). The use of *M. oppositifolius* to treat skin wounds may be attributed to its tannins specifically both catechin and gallic types.

The results of this study indicate that *M. oppositifolius* could represent a promising alternative in the fight against antibiotic resistance. Indeed, the

combination of a drying up of new antibiotic discoveries and the rise of multidrug-resistant bacteria poses a major threat of therapeutic dead-ends in the coming years. Faced with this public health challenge, the World Health Organization (WHO) actively advocates and encourages the development and dissemination of simple treatments derived from traditional medicine, provided that their authenticity, safety, and efficacy are scientifically guaranteed (Die-Kacou *et al.*, 2009 ; Soro *et al.*, 2010).

CONCLUSION

This study confirms the remarkable anti-*Pseudomonas* activity of *M. oppositifolius* against various multidrug-resistant bacterial strains. Determination of MICs and MBCs revealed that the ethyl acetate and butanol fractions were the most effective, exerting a bactericidal effect on the vast majority of the tested strains. These results demonstrate the good solubility of the active principles in these polar solvents, in which the phytochemicals responsible for this efficacy are likely concentrated. These promising observations offer encouraging prospects for addressing the public health challenge posed by antibiotic resistance. They strongly support further investigation, particularly through deeper ethnopharmacological study, *in vivo* trials, and a rigorous assessment of the plant's safety. Finally, given the demonstrated therapeutic potential, strengthening conservation measures for this species is essential to preserve this valuable part of the 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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