

Phytochemical screening and evaluation of the antibacterial activity of *Atractogyne bracteata* (Rubiaceae) bark extracts

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Abstract

Medicinal plants are a valuable source of bioactive molecules, which are generally secondary metabolites. This study is part of the search for new natural molecules from a plant in the Tonkpi region. The plant in question is *Atractogyne bracteata*, belonging to the Rubiaceae family, and used in traditional medicine. Phytochemical screening revealed the presence of bioactive substances, namely polyphenols, flavonoids, saponins, and tannins. Antibacterial activity was assessed through a qualitative study (disc diffusion and wells), and the results were confirmed by a quantitative study that determined the MICs and MBCs of the studied extracts against six bacterial strains (ATCC *Escherichia coli*, ESBL *Escherichia coli*, methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* and susceptible *Klebsiella*). The aqueous extract showed significant antibacterial activity against the tested strains, particularly *P. aeruginosa*, *Staphylococcus aureus* Meti-R and *E. coli* ESBL (Extended-Spectrum Beta-Lactamase), with inhibition zone diameters of 20 and 14 mm, respectively, at concentrations of 200 mg/ml. Minimum inhibitory concentration (MIC) values showed a wide range, from 3.12 mg/ml to 20 mm. This study supports the antibacterial properties attributed to this plant.

Keywords: *Atractogyne Bracteata*; Secondary Metabolite; Antibacterial Activity; MIC; MBC.

1. Introduction

The proliferation of multidrug-resistant pathogenic bacteria has been recognized by the World Organisation for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO), and the World Health Organization (WHO) as a serious global human and animal health problem. The development of bacterial resistance to antimicrobials is neither a random nor a new phenomenon [1]. However, concerns are growing due to the frequency with which new, emerging resistant phenotypes appear among pathogenic bacteria or in commensal microorganisms, such as resistance to carbapenems, colistin, linezolid, macrolides, etc. Experts estimate that between 2015 and 2050, antibiotic-resistant infectious diseases will be the leading cause of death from disease [2, 3]. One of the major causes of this mortality is the exponential emergence of multidrug-resistant bacteria. Those involved in enteric infections are primarily Enterobacteriaceae and Enterococci, as well as certain germs such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Clostridium difficile* [4]. Furthermore, many infections could be successfully treated based on the clinician's prior clinical experience or because the susceptibility of the germs could be reliably predicted (thus allowing for empirical treatment) [1]. This threat of multidrug-resistant bacteria is particularly worrying in developing countries where bacterial infections are endemic. In this context, studying the active ingredients and antibacterial activities of local

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medicinal plants could be an alternative. Plants used in traditional medicine should normally undergo botanical, phytochemical, and pharmacological studies to ensure their efficacy and justify their use. Today, many laboratories are turning to plants to search for active compounds [5]. It is estimated that approximately 25% of medical prescriptions are derived directly or indirectly from plants [6].

Atractogyne bracteata is a plant used in traditional medicine by the population of western Ivory Coast for its purgative, laxative, and anti-diarrheal and anti-typhoid fever properties. It is therefore useful to evaluate the antibacterial activities of this plant to improve traditional healers' recipes and lead to the production of improved, standardized traditional medicines at affordable prices for the population. This study aims to contribute to the valorization of medicinal plants. It seeks to evaluate the antibacterial activity of *Atractogyne bracteata* extracts against *Escherichia coli* ATCC, *Escherichia coli* ESBL, *Staphylococcus aureus* Meti-R, *Pseudomonas aeruginosa*, *Salmonella* and susceptible *Klebsiella* germs frequently implicated in illnesses treated with traditional medicine and to identify the phytochemical compounds responsible for this activity.

2. Materials and Methods

2.1. Plant Material

The plant material consisted of *Atractogyne bracteata* bark collected in June 2025 at the Polytechnic University of Man, in the Tonkpi region of western Ivory Coast. The plant was authenticated at the Swiss Center for Scientific Research (CSRS) in Abidjan (Ivory Coast) where a specimen is deposited (CSRS 005440). These barks were dried in the laboratory (approximately 25°C) for four weeks.

2.2. Microorganisms tested

The study was conducted on six bacterial strains (*Salmonella*, ATCC *Escherichia coli*, *Escherichia coli* ESBL, *Staphylococcus aureus* Meti-R, *Pseudomonas aeruginosa* and susceptible *Klebsiella*). These bacterial strains were provided by the Supply and Management Unit (SMU) of the Pasteur Institute of Ivory Coast.

2.3. Preparation of plant extracts

2.3.1. Preparation of aqueous extract

100 g powder of the root of *Atractogyne bracteata* were macerated for 24 hours in 1L of distilled water [7]. The macerate has been wrung into a square of sterile tissue, filtered successively on cotton wool and one-fold on filter paper (Whatman paper® 2 mm). The filtrate was dried slowly in the stove at 50° C. The powder obtained was stored in a hermetically sealed jar and refrigerated at 4 °C [8].

2.3.2. Preparation of ethanolic 70% extract

It was carried out using modified [9] method. A mass of 100 g of plant powder was added in 1L of ethanol 70% and subjected to maceration for 72 hours. The macerate was treated according to the same procedure like the aqueous extract.

2.4. Phytochemical screening

2.4.1. Test for sterols and polyterpenes (reaction LIEBERMANN)

After evaporation to dryness 5mL of each solution in a capsule on a sand bath without charring, the residue was dissolved in hot acetic anhydride and 1 mL in a test tube, we poured cautiously with 0.5 mL of concentrated sulfuric acid along the tube wall to the solution. The applications to the interphase of a purple or purple ring, turning blue to green, indicate a positive reaction [10].

2.4.2. Test for alkaloids (reactions Dragendorff and Bouchardat)

Six milliliters of plant extract were evaporated. The residue was taken up in six milliliters of alcohol at 60 ° and the alcoholic solution thus obtained was divided into two test tubes.

In the first tube was added two drops of Dragendorff reagent. The appearance of a precipitate or an orange color indicated the presence of alkaloids.

In the second tube was added two drops of reagent Bouchardat. The appearance of a reddish-brown color indicated a positive reaction to the presence of alkaloids [11].

2.4.3. *Test for polyphenols*

Two milliliter of extract was added a drop of alcoholic solution of ferric chloride at 2%. The appearance of a dark green or lighter or darker blue color indicated the presence of polyphenolic derivatives [12].

2.4.4. *Test for flavonoids*

For this research, two milliliter of the extract was evaporated to dryness in a porcelain dish on a sand bath. The residue was taken after cooling in five milliliter hydrochloric alcohol half. The successive addition of three magnesium shavings and three drops of isoamyl alcohol showed an intense pink or violet in the presence of flavonoids [9].

2.4.5. *Test for saponosides*

A volume of two milliliters of each extract was evaporated and taken up in five milliliters of water. After vigorous stirring, the foaming of more than one centimeter, stable and persistent high for 30 minutes indicated the presence of saponins [11].

2.4.6. *Test for catechol or condensed tannins (reaction Stiasny)*

A volume of five milliliter of each extract was evaporated and an amount of 10 ml of a reagent solution Stiasny was added to the residue. This mixture was placed in a water bath at 80 ° C for 30 minutes and was cooled to room temperature. Positive feedback had resulted in the formation of large flakes brown clear or dirty precipitates [10].

2.4.7. *Quinonic substances research*

For this research, 2 mL of each extract solution is first evaporated to dryness in a sand-bath capsule without charring, then the residue is triturated in 5 mL of 1: 5 hydrochloric acid. Then the solution obtained is brought to the boiling water bath for half an hour. Finally, after cooling on a current of cold water, the hydrolyzate is extracted with 20 ml of chloroform and the chloroform phase is collected in another test tube supplemented with 0.5 ml of ammonia diluted by half. The appearance of a color ranging from red to purple indicates the presence of quinones [10].

2.4.8. *Research anthocyanins*

The presence of anthocyanins in an extract solution is indicated by a red color which increases with the addition of dilute HCl and turns purplish-blue-green by the addition of ammonia [10].

2.4.9. *Test for Gallic tannins*

The above solution was saturated and one or two drops of alcoholic solution of iron chloride to 2% have been added. The positive response has led to the appearance of blue-black coloration characteristic intense tannins Gallic [10].

2.5. Preparation of bacterial inoculum

Two isolated colonies from each bacterial culture for 18 hours were homogenized in 10 mL of Muller-Hinton broth and incubated for 3 hours at 37° C for preculture. A levy of 0.1 mL of the preculture broth was diluted in a tube containing 10 mL of Mueller-Hinton (MH). This bacterial suspension was made consisting of 10⁰ dilution of bacterial inoculum so as to obtain a bacterial load estimated to 10⁶ Unit Format colonies per milliliter (CFU / mL).

2.5.1. *Preparation of extracts concentration ranges*

A range of concentration of each extract was prepared with a series of ten vice tubes through the method of double dilution an in medium liquid. This range of concentration is 200 mg/mL to 0.39 mg/mL numbered T1 to T10. For this, 10 mL of a mixture solution of DMSO / sterile distilled water (V / V) were placed in the tubes T1 and 5mL in all the other tubes. Two grams (2g) of each extract were dissolved in the tubes T1 to obtain a concentration of 200 mg/mL. A 5 mL volume of the tubes T1 was transferred into the tubes T2 and then homogenized. This operation was repeated until T10 tubes where 5 mL of T10 tubes are rejected. All tubes are kept refrigerated at 4 °C [12].

2.5.2. *Determination of growth inhibition zones*

The method of holes punch in the MH agar described by [13] has been accepted. Each pit or holes of 6 mm diameter was filled with 80 µL of extract concentrations of 200 and 100 mg / mL, taking care to separate two holes of at least 20 mm.

A negative control wells was performed for each bacterial strain with 80 μ L of the mixture of DMSO / sterile distilled water solution (V/V). After a pre-release of 45 minutes at laboratory temperature to 16° C, all the Petri dishes were incubated in an incubator at 37° C for 18-24h. Meanwhile, Ceftriaxone (CRO 30 μ g) for Enterobacteriaceae and oxacillin (OX 5 μ g) for staphylococci were used as positive controls. After incubation, the activities of the extracts were assessed by measurement of a growth inhibition area around the wells using a caliper. According to [14], a strain is called insensitive or resistant, sensitive and very sensitive if the diameters of inhibition are respectively less than 8 mm, between 9 and 14 mm and between 15 and 19 mm.

2.5.3. Determination of Minimum Inhibitory Concentration (MIC)

The macro dilution method in liquid medium described by [15] was used to determine these antimicrobials parameters. Thus, in a series of 10 hemolysis tubes numbered C1 to C10 for each extract was introduced 1 mL of the bacterial inoculum. Then 1 mL of each extract concentration well known by the range of prepared concentration was added in the same tubes. This distribution of plant extract is made so that 1 ml of plant extract of 200 mg/mL was transferred in the tube C1, that of 100 mg/mL in the tube so C2 to C9 tube receive 1mL plant extract of 0.78 mg/mL. C10 has been tube, received instead of plant extract, 1 mL of DMSO / Sterile distilled water (V/V), was used as a control. This distribution of plant extract concentration is well known in each tube already containing 1 mL of inoculum reduced the concentration of plant extract in medium at its half. Tube and the concentration of C1 increased from 200 mg/mL to 100 mg/mL, 100 mg/mL to 50 mg/mL for C2 so on until a concentration of 0.39 mg/mL for T9. This experiment was performed identically for each sample tested. The first nine (9) tubes (C1 to C9) are called "experimental tubes" and the last tube (C10) is rated "growth control tube or TC." The loaded tubes were incubated at 37° C for 24 h. The MIC is the concentration of the first tube where it finds no trouble visible to the naked eye.

2.5.4. Determination of Minimum Bactericidal Concentration (MBC)

From the MIC, the lowest concentration that leaves no more than 0.01% survival of bacteria suspended starting 24 hours corresponds to the CMB. It is determined by plating by a streak on Mueller-Hinton agar by streaking 5 cm using a loop, beginning with the first and incubated undisturbed at 37° C for 24 h tube.

2.5.5. Antibacterial activity of the extracts tested

The antibacterial effect of different extracts tested was considered bactericidal or bacteriostatic depending on the MBC / MIC ratio. According [16] when this ratio is greater than 4, the extract has bacteriostatic and bactericidal, if the ratio is less than or equal to 4.

3. Results

3.1. Yields

Yield values were calculated in relation to the initial mass of *Atractogyne bracteata* powder for one trial. Plant extraction yields are 14.33% for the ethanolic extract (EEth) and 17% for the aqueous extract (EAq). Both extracts have a powdery appearance. In terms of color, the extracts have the same coloration (Table I).

Table 1 Yield of aqueous and ethanolic extracts of *Atractogyne bracteata* bark

Extracts	Weight (g)	Yield (%)	Color	Aspect
EAq	25.5	17	Maroon	Powder
EEth	21.5	14.33	Maroon	Powder

EAq : Aqueous extract ; EEth : Ethanolic extract

3.2. Phytochemistry screening

Phytochemical screening revealed the presence of secondary metabolites in the plant tissues of this plant. These include polyphenols, tannins, flavonoids, saponins, anthocyanins, alkaloids, sterols, and polyterpenes in the plant bark. The intensity of the color observed during qualitative tests indicates that these secondary metabolites are more abundant in the aqueous extract than in the ethanolic extract. However, anthocyanins and quinones were completely absent from the aqueous extract, and gallic tannins were absent from both extracts (aqueous and ethanolic).

Table 2 Phytochemical analysis of ethanolic and aqueous extracts of *Atractogyne bracteata* bark

Extracts		EEth	EAq
alkaloids	B	-	-
	D	+	+
Saponosides		+	+
anthocyanins		-	++
Tannins	Cat	+	+
	Gal	-	-
flavonoids		++	+++
Polyphenols		+	++
Quinones		-	+
Sterols and polyterpenes		+	+

-: Absence; +: Presence; ++: Strong presence; +++: Very strong presence; EAq: Aqueous extract; EEth: Ethanolic extract; Gal: Gallic; Cat: Catéchiques; B: Bouchardaf; D: Dragendoff

3.3. Antimicrobial activity

The disk diffusion method was used to determine the action of plant extracts dissolved in DMSO on different strains. This action is characterized by the appearance of an inhibition zone around the paper disk previously impregnated with the extract, indicating the absence of bacterial growth in this zone. The diameter of the inhibition zone differs from one bacterium to another and from one extract to another.

Table 3 Diameters of inhibition zones (mm) of ethanolic and aqueous extracts of *Atractogyne bracteata* and Ceftriaxone on tested strains

Extracts	Strains tested	Concentrations of extracts (mg/mL)				Ceftriaxone (CRO)
		200	100	50	25	
EEth	<i>K. sensible</i>	08	08	06	06	08
	<i>E. coli ATCC</i>	12	10	08	06	12
	<i>E. coli ESBL</i>	08	06	06	06	06
	<i>Salmonella</i>	10	08	06	06	06
	<i>S. aureus Méti-R</i>	06	06	06	06	08
	<i>P. aeruginosa</i>	10	08	06	06	06
EAq	<i>K. sensible</i>	12	10	06	06	10
	<i>E. coli ATCC</i>	12	10	06	06	12
	<i>E. coli ESBL</i>	14	10	06	06	12
	<i>Salmonella</i>	10	08	06	06	10
	<i>S. aureus Méti-R</i>	14	10	06	06	14
	<i>P. aeruginosa</i>	20	16	12	10	18

Where: Ts = T = 0: Sterility control including well diameter (6 mm) with DMSO/Water (0.5: 0.5; V/V); CRO = Ceftriaxone, EEth: Ethanolic extract; EAq: Aqueous extract.

The variation in the antibacterial activity of the extracts explains the variations in their chemical compositions. The inhibition zone diameters range from 6 to 20 mm for both extracts. The results of the evaluation of the antibacterial capacity of the aqueous extract of *A. bracteata* indicate that it possesses significant antibacterial activity against

susceptible *P. aureus* (20 mm), followed by *Staphylococcus aureus* Meti-R and *E. coli* (14 mm), and the ATCC *E. coli* and *K. sensitive* strains (12 mm) at concentrations of 200 mg/mL and above (Table 3). Furthermore, no sensitivity was observed in *E. coli* ESBL and *S. aureus* Meti-R strains with the EEth extract.

3.4. Antibacterial Parameters

After 24 hours of incubation at 37°C in liquid medium (BMH), the study of the effect of *A. bracteata* extracts on the in vitro growth of the strains yielded the results shown in Table 4. Based on Minimum Inhibitory Concentrations (MIC), the antibacterial parameters of the aqueous extract (EAq) showed sensitivity against the tested strains. The measured MIC and MBC values for these pathogenic bacteria show that the lowest MIC (3.12 mg/mL) was recorded with *P. aeruginosa*. Thus, the calculated MBC/MIC ratios, less than 4 (Table 4), demonstrate that the aqueous extract has bactericidal activity against these bacteria. Regarding the ethanolic extract, it showed no sensitivity against producing *E. coli* ESBL and *Staphylococcus aureus* Meti-R strains. Furthermore, the calculated MBC/MIC ratios, greater than 4 (Table 4), suggest that the crude ethanolic extract has a bacteriostatic effect on these bacterial strains. However, the antibacterial parameters of the ethanolic extract (EEth) are not bactericidal against all the tested strains.

Table 4 Antibacterial Parameters of Ethanol and Aqueous Extract Fractions of *A. bracteata* bark on *in Vitro* Growth of Test Organisms

Bacterial strains							
Extract	Antibacterial parameters (mg/mL)	<i>Salmonella</i>	<i>E. coli</i> ESBL	<i>E. coli</i> ATCC	<i>Klebsiella</i> sensible	<i>S. aureus</i> Méti-R	<i>Pseudomonas aeruginosa</i>
EEth 70	MIC	100	>100	25	25	>100	50
	MBC	100	>100	50	100	>100	100
	MBC/MIC	1	Nd	2	4	Nd	2
	Effect	Bacteridal	-	Bacteridal	Bacteridal	-	Bacteridal
EAq	MIC	100	25	50	50	12.5	3.12
	MBC	100	50	100	100	25	3.12
	MBC/MIC	1	2	2	2	2	1
	Effect	Bacteridal	Bacteridal	Bacteridal	Bacteridal	Bacteridal	Bacteridal

4. Discussion

The choice and yield of solvents can be important criteria. Based on the results obtained, it appears that extraction yields vary considerably from one extract to another. Calculating yields allows for a quantitative assessment of the extracts that can be obtained from each species. From a phytochemical perspective, the results show that both the aqueous (EAq) and ethanolic (EEth) extracts contain flavonoids, tannins, phenols, alkaloids, saponins, sterols, and polyterpenes. The presence of several of these chemical groups is consistent with the work carried out by other authors, specifically [17], who revealed the presence of flavonoids, tannins, phenols, alkaloids, saponins, and anthraquinones in extracts from certain plants (*Picralima nitida*, *Nauclea pobeguini*).

Water, being a highly polar compound, allows the extraction of several metabolites simultaneously, particularly those with ketone and enol groups in their formulas [18]. The high activity observed with the extracts can be explained by the fact that these extracts contain several compounds with synergistic activities. This synergy resulted in a much more significant inhibitory capacity for the extracts, unlike the fractions obtained from a solvent system or solvent mixture with increasing polarity.

Regarding antibacterial activity, it can depend on the composition of the culture medium [19]. Organic matter in the culture medium can reduce the effectiveness of an antibacterial agent by combining with it to form inactive compounds, by adsorbing it and decreasing its concentration, or by precipitating and completely eliminating it. Other factors influence the activity such as the harvest period, the climate, the extraction method, the rest time [20, 21].

The absence of inhibition zones in certain strains may indicate variations in the chemical composition of each extract, as well as variations in bacterial type and their sensitivity to the extracts. Resistance of strains to natural substance extracts may be due to the difference in cell wall structure between Gram positive and Gram negative bacteria. Indeed, Gram negative bacteria are resistant because they possess an outer membrane composed of phospholipids, proteins, and lipopolysaccharides, making it impermeable to the active ingredients of the extracts studied. In contrast, Gram positive bacteria are the most sensitive [22]. According to [14], an extract is considered active if it induces an inhibition zone greater than or equal to 10 mm. The minimum inhibitory concentration is defined as the lowest concentration reported to give complete inhibition of the tested bacteria after 48 hours of incubation [23, 24].

In general, the extraction of plant material in various solvents revealed that, for the same plant, the antimicrobial potential of the extract differed depending on the solvent used. The diversity of the phytochemical content of the different extracts from the same plant could explain the diversity in the results of the antimicrobial tests obtained. The different solvents and solvent systems would have solubilized the biologically active compounds in different proportions depending on their polarity; these compounds would therefore have affected the cellular metabolism of microorganisms differently, given their different chemical nature [25].

Furthermore, the results of the phytochemical screening of extracts from this plant showed that it contained alkaloids, saponins, tannins, and terpenoids. The presence of these secondary metabolites could explain the observed antimicrobial activities.

Indeed, the work of [26], on the phytochemistry of the species *Sapium ellypticum*, revealed that the bark of this plant contains triterpenes, notably lupeol and lupeol acetate, as well as stigmasterol (a phytosterol), which have demonstrated antibacterial and antifungal properties according to the work carried out by. Furthermore, work by [30], showed that the antimicrobial activity of plant extracts does not necessarily depend on the number of active compounds present in these extracts, but rather on the efficacy of each individual compound. To our knowledge, no studies on the antibacterial potential of this plant have yet been reported. These results are therefore reported here for the first time. Furthermore, the ethanolic fraction of the bark had a very weak effect on most strains. Thus, this result partially confirms observations made among the population of the Tonkpi region, who use bark decoctions to treat diarrhea and typhoid fever.

Furthermore, the MBC/MIC ratio allowed us to determine the bactericidal and bacteriostatic activity of the plant extracts. According to [31], when this ratio is greater than 4, the extract is considered bacteriostatic, and bactericidal when it is less than or equal to 4. These results therefore allow us to conclude that each extract exhibited bactericidal activity against *S. aureus* ATCC, *S. aureus* Meti-R, *E. coli* ATCC and *E. coli* ESBL, as the MBC/MIC ratios were less than 4. Considering the IC₅₀ values presented in Table 4, we observe that against *P. aeruginosa*, the EAq extract has the lowest IC₅₀ value (3.12 mg/ml). This indicates that this extract is the most active. The high activity of this extract could be due to a difference in concentration among the various chemical groups present in the extract.

Furthermore, the bactericidal effects (MBC/MIC ≤ 4) could be explained by the fact that the bioactive substances in the plant extracts caused the destruction of these germs by acting on one or more metabolic steps essential to their survival [32]. Thus, the results of this work would justify the traditional use of *Atractogyne bracteata* bark for the treatment of certain infectious diseases.

5. Conclusion

This study evaluated the antibacterial activity of aqueous and ethanolic extracts (EAq and EEth) of *A. bracteata* barks against *E. coli* ATCC, *E. coli* ESBL, *K. sensitive*, *S. aureus* Meti-R, *Salmonella* and *P. aeruginosa*. The results show that the tested extracts inhibited the growth of the studied organisms to varying degrees. The crude aqueous extract was the most active. Phytochemical screening of these tested extracts identified flavonoids, polyphenols, saponins, and tannins, which are thought to be responsible for the observed activities. These results partially validate the use of the plant in treating infections caused by the tested microorganisms and contribute to the scientific justification for the various uses of this plant in the traditional pharmacopoeia of the Tonkpi region.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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