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Evaluation of the Antibacterial Activity of Extracts of Alchornea Cordifolia (Schum Et Thonn Mull) Leaves on the in Vitro Growth of Ureolytic Germs Isolated from Urethral Samples At the Korhogo Rural Hospital Centre, North Côte d'Ivoire

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Abstract The germs involved in urinary tract infections are a major concern for clinicians. The increasing multi-resistance of these bacteria to conventional antibiotics is a real public health problem. The aim of this study was to contribute to the treatment of urinary tract infections using the leaves of *Alchornea cordifolia*. The leaves were subjected to two extraction methods (infusion and hydro-ethanolic maceration). A tri-phytochemical analysis using colorimetry and tube precipitation revealed the polyphenol composition of the extractsThe sensitivity of the extracts was assessed by agar well diffusion and the antibacterial parameters determined by diffusion in liquid medium followed by spreading on Mueller Hinton agar. The results showed that the extracts were rich in sterols, polyterpenes, polyphenols, flavonoids, tannins, alkaloids and saponins, and possessed interesting antibacterial activity. All strains were sensitive to extremely sensitive to *A. cordifolia* extracts at 50 mg/ mL. Low MIC values ranging from 0.78 to 12.5 mg/mL were recorded. All the extracts had a bactericidal effect on all the strains tested. This plant has good antibacterial activity and is therefore a good candidate for the treatment of urinary tract infections.

Keywords: *urinary diseases, Alchornea cordifolia, antibacterial, phytochemistry*

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1. Introduction

Urinary diseases are shameful diseases. Clinical presentation is delayed, leading to delays in medical management. With few symptoms, these diseases are associated with complications of several chronic diseases with a poor vital prognosis, notably pyelonephritis, bacteraemia and prostatitis [1]. The literature reports that chronic urinary tract infections are regularly associated with chronic interstitial nephropathy [2,3] revealed urinary tract infections in 54% of cases of chronic renal failure at Treichville University Hospital between January and December 1990.Another study by Kamara and al, (2017) [4] reported a prevalence of 4% in sickle cell patients at Treichville University Hospital. In paediatrics [5], neonatology [6,7] diabetology [8] and infectious diseases (tetanus) [9], cases of urinary tract infection were frequently cited as a complicating factor. Urinary tract infections are therefore a cause for concern and deserve special attention. These mainly infectious diseases are characterised by an abnormal proliferation of micro-organisms in the urinary tract.The microorganisms responsible for urinary tract infections include *Escherichia coli* (70-95%) [10], Klebsiella, Enterobacter, Proteus and Serratia (10-15%), *Pseudomonas aeroginosa* (10-15%) and the staphylococcus genus (5%) [11]. Antibiotic

therapy is the indicated method of treatment. However, these microorganisms are formidable and resistant despite advances in antibiotic therapy [1]. The search for solutions using medicinal plants offers an alternative.To this end, *A. cordifolia* is a good candidate, given its numerous prescriptions in traditional environments. Previous studies have reported that this plant is regularly cited in recipes for the treatment of various diseases, including malaria, haemorrhoids [12], painful periods, dysentery, high blood pressure, diabetes, vaginal hygiene [13] and diseases linked to oxidative stress in the town of Nkongsamba [14]. However, scientific research remains insufficiently documented as to the antibacterial activity of this plant on the growth of ureolytic germs. This is why this study was initiated. It will help to update the data on this plant and could justify its use in traditional settings for the treatment of urinary tract infections in Côte d'Ivoire.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

The plant material consisted of *Alchornea cordifolia* leaves. The leaves were collected in August 2023 in Korhogo in the Poro region and then identified at the Centre National of Floristique (CNF) of Université Félix Houphouët-Boigny, Côte d'Ivoire.

2.1.2. Bacterial Strains

Three codified clinical strains: *E. coli* (Dam), E. coli (8039), *S. aureus* (9044) and two reference strains: E. coli ATCC 25922, and *S. aureus* ATCC 19213, were the subject of this study. The clinical strains were supplied by the analysis Laboratory of the Centre Hospitalier Régional (CHR) of Korhogo and were derived from pathological products of urinary tract infection.

2.1.3. Reagents and Chemicals

Several products and reagents were used in this study. Muller-Hinton agar, nutrient agar, nutrient broth, distilled water, physiological water (Nacl 0.9%), TBX agar and CHAPMAN agar were used for the antibacterial tests. Folin-ciocalteu reagent, 2% iron III chloride (FeCl3), 1% aluminium chloride (AlCl3) and sulphuric vanillin were used. Gentamicin (CN-30 mcg) and Cefotaxime (CTX-5 µg) are used as standard.

2.2. Methods

2.2.1. Verification of Germ Purity

The purity of the target strains is checked using selective isolation media. Strains are isolated on appropriate selective agar and purified on nutrient agar, before being transferred to Mueller Hinton agar. The colonies obtained after 18 hours incubation are used to prepare the bacterial suspension.

2.2.2. Preparation of Total Extracts

Extractions were carried out using the methods described by [15].

2.2.3. Phytochemical Screening

Secondary metabolites were revealed by means of staining and precipitation tests in test tubes following the methods described by [16,17]. Total polyphenols; Total flavonoids; Tannins; Total alkaloids, Saponins Quinones; Sterols and terpenes.

2.2.4. Preparation of the Extract Concentration Range

The concentration range for the extracts was prepared by double dilution. A series of plant extract concentrations, ranging from 200 mg/mL to 1.56 mg/mL, were prepared in test tubes marked T1 to T8. The resulting concentration range was autoclaved at 121°C for 15 minutes.

2.2.5. Preparation of Inoculum

Using a Pasteur pipette, two 18-hour-old bacterial colonies were picked and placed in a test tube containing 10 mL of sterile Mueller-Hinton broth. The mixture was incubated at 37°C for 3 hours. After opalescence, 0.1 mL of this preculture was removed and diluted in 2 mL of physiological water (0.85% NaCl). This suspension was homogenized using a vortex and calibrated to the 0.5 Mac Farland scale [18,19].

2.2.6. Agar Well Diffusion Method

Agar well-diffusion method was followed to determine the antimicrobial activity. Mueller Hinton plates were swabbed (sterile cotton swabs) with 18 hours old - broth culture, wells approximately 6 mm in diameter were made in each of these plates using a sterile cookie cutter. Each well received 80 µL of the test substance at concentrations of 100 and 50 mg/mL. Control experiments comprising inoculums without plant extract were set up. After 15 min of diffusion at laboratory temperature, The Plates were incubated at 37°C for 18-24 h. [18,19]. The diameter of the inhibition zone (mm) was measured.

2.2.7. Determination of Antibacterial Parameters of Extracts

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are determined according to [18,19]. and interpreted according to [20]. Thus, a bacterial strain is said to be non-sensitive or resistant when the diameter of the zone of inhibition is < 8 mm. It is said to be sensitive when the diameter is between 9 and 14 mm. It is said to be highly sensitive when the diameter is between 15 and 19 mm. It is said to be extremely sensitive when the diameter > 20 mm.

2.2.8. Antibiotic Susceptibility Testing

All isolates clinical germs studied (E. coli 8039, E. coli DAM and S. aureus 9044). were tested for susceptibility to a panel of 4 families (Aminosides, Penicillins, Cephalosporins and Beta-lactams) using the disk diffusion method in accordance to guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM) [2023]. Each antibiotic family contains two antibiotics tested, except for the Beta-lactam family, which contains a single antibiotic (Ceftazidine: CAZ-30µg). The flooding technique was used to inoculate

Mueller-Hinton agar in Petri dishes. Excess inoculum was removed with a swab. The Petri dish is dried at 37°C for 15 mn. Antibiotic-impregnated discs are applied to the agar surface at 2 cm intervals. The plates were incubated at 37°C for 24 h. The diameters of the zones of inhibition are measured in mm using a ruler and interpreted according to CA-SFM (2023) as Sensitive (S), Intermediate (I) or Resistant (R).

2.2.9. Statistical Analysis

Statistical analyses of the results were carried out using Statistica software version 7.1 and. Fisher's minimum significant difference (LSD) test was used to determine significant differences between several means. For all statistical analyses, the differences were considered significant at the 5% significance level.

3. Results and Discussion

3.1. Phytochemical Investigations of Leaf Extracts

Phytochemical investigations showed the presence of sterols, polyterpenes, polyphenols, flavonoids, tannins, alkaloids and saponins in the three extracts. The difference in phytochemical compounds between the extracts was observed in tannins and quinones. The 70% ethanol extract did not contain any tannins or quinones (Table 1).

3.2. Abacterial Activity of Extracts

3.2.1. Sensitivity of Germs to Extracts

Bacterial sensitivity tests to the various extracts gave variable inhibition diameters, as shown in Table 2 and Table 3. At a concentration of 100 mg/mL, *E. coli* strains were highly sensitive to *A. cordifolia* extracts, with inhibition diameters of between 15 and 18 mm. *S. aureus* strains were extremely sensitive, with diameters in excess of 25 mm. This high sensitivity was observed in all extracts. At a concentration of 50 mg/mL, all the *E. coli* strains were sensitive to the extracts, with diameters varying between 11 and 14 mm, while the *S. aureus* strains were extremely sensitive, with inhibition diameters greater than 21 mm. The *S. aureus* 9044 strain was the most sensitive to *A. cordifolia* extracts and the *E. coli* 8039 strain the least sensitive. These results are comparable to those obtained with standard antibiotics. Gentamicin obtained similar sensitivities in all the strains tested, while *E. coli* 8039 was resistant to cefotaxime but sensitive to *A. cordifolia* extracts. *S. aureus* ATCC 19213 and *E. coli* DAM clinical strain showed statistically identical sensitivity with the 5% and 10% infusions.

Table 1. Photochemical screening

Extraits	Sterols et Polyterpenes	Polyphenols	Flavonoïdes	Tannins		Quinones	Alcaloïdes		Saponines
				Cat	Gal		B	D	
A.C 5 %	+ +	+ + +	+ +	+	-	+ +	+	+ +	+ + +
A.C 10 %	+ +	+ + +	+ +	+ +	+ +	+	+	+ +	+ + +
A.C 70 %	+ + +	+ + +	+ +	-	-	-	+	+ +	+

(+): Presence ;(-): Absence; (Cat): Catéchistes ; (Gal): Galliques ; B: Bouchardât ; D: Dragendorff

Table 2. Antibacterial activity (zone of inhibition, mm) of various extract of *A. cordifolia* at 100mg/mL

sensitivity test at 100 mg/mL						
Origins	Germs	A.C. 5%	A.C 10%	A.C 70%	Genta	Cefo
Reference strains	*E. coli* ATCC 25922	13.00±1.00^{b}	14.67±0.57^{a}	16.67±0.57^{ab}	S	R
	S. aureus ATCC 19213	15.00±1.00^{a}	15.67±0.57^{a}	25.33±1.15^{d}	S	R
Clinical strains	*E. coli* DAM	15.33±1.15^{a}	15.67±0.57^{a}	15.67±0.57^{a}	S	S
	E. coli 8039	18.67±0.57^{c}	15.33±1.52^{a}	18.33±0.57^{b}	R	R
	S. aureus 9044	25.67±0.57^{d}	25.00±1.00^{b}	21.33±1.52^{c}	R	R

A.C: Alchornea cordifolia ; Genta: Gentamicine ; Cefo: Cefotaxime ; a,b,c,d: indicate the different statistical classes of germ sensitivity to extracts

Table 3. Bacterial sensitivity test at 50 mg/mL

sensitivity test at 50 mg/Ml						
Origins	Germs	A.C. 5%	A.C 10%	A.C 70%	Genta	Cefo
Reference strains	*E. coli* ATCC 25922	11.00±1.00^{a}	12.67±0.57^{a}	13.67±0.57^{a}	S	R
	S. aureus ATCC 19213	12.00±1.00^{ab}	12.67±0.57^{a}	21.33±1.15^{c}	S	R
Clinical strains	*E. coli* DAM	12.67±0.57^{b}	11.67±0.57^{a}	12.67±0.57^{a}	S	S
	E. coli 8039	14.67±0.57^{c}	12.33±1.52^{a}	13.67±0.57^{a}	R	R
	S. aureus 9044	21.67±0.57^{d}	21.33±1.52^{b}	16.00±1.00^{b}	R	R

A.C: Alchornea cordifolia, Genta: Gentamicine ; Cefo: Cefotaxime

3.2.2. Antibacterial Parameters of Extracts

The antibacterial parameters were determined and reported in Table 4. The inhibitory concentrations obtained varied from 0.78 to 12.5 mg/mL. The CMB/CMI activity ratios varied from 1 to 8. The highest activity ratio (8) was observed for the extract infused 5% with *E. coli* strain 8039. The other extracts obtained low activity ratios ranging from 1 to 4. The 5% infused extract exerted a bacteriostatic effect on *E. coli* 8039 but a bactericidal effect on all the other strains studied. The 10% infused and 70% ethanol extracts had a bactericidal effect on all the strains tested.

3.3. Antibiogram of Bacteria Tested

The antibiotics tested were grouped into 4 families (Aminosides, Penicillins, Cephalosporins and Beta-lactams). The results are shown in Table 5. According to the CASFM guide (2023) *E. coli* 8039 was sensitive to Amikacin (AK-30 µg) from the Aminoside family but resistant to cephalosporins, penicillins and Beta-lactam antibiotics (Ceftazidine, CAZ-30µg). As for *E. coli* DAM, it was sensitive to two Aminoside antibiotics (Gentamicin: CN-30 µg and Amikacin: AK-30 µg), to Cephalosporins (Ceftriaxone: CRO-30 µg) and resistant to the penicillins, cephalosporins and Beta-lactamines tested. With regard to *S. aureus* 9044, this strain was resistant to the two antibiotics tested Gentamicin: CN-30 mcg (Aminosides) and Cefotaxime: CTX-5 µg (Cephalosporins). These strains were resistant to at least one antibiotic from two different families. They are multi-resistant germs.

4. Discussion

The emergence and spread of bacteria that are multi-resistant to conventional drugs is the result of the therapeutic impasse observed in the treatment of several chronic infectious diseases in human populations. This phenomenon has become a major public health problem. It is a genuine concern that has prompted the ongoing search for new, more effective and less costly molecules. The antibacterial tests carried out as part of the present study demonstrated the inhibitory activity of *Alchornea cordifolia* leaf extracts on the in vitro growth of *E. coli* 8039, *E. coli* DAM and *S. aureus* 9044, three multi-resistant clinical strains. The sensitivity of the strains to the extracts varied from one bacterial strain to another. All strains were sensitive to the extracts studied at a concentration of 50 mg/ mL. The most sensitive strain was *S. aureus* 9044 and the least sensitive was *E. coli* 8039 to the 5% infusion. This difference in sensitivity between the strains can be explained by the difference in structure, chemical composition and membrane permeability of the bacteria. [21]. Several studies have reported that bacterial resistance to biomolecules is linked to bacterial structure and the nature of the extract [22,23]. According to these authors, the resistance of Gram-negative bacteria is linked to the presence of the peptidoglycan layer, which acts as an effective barrier to biomolecules. The results of our study corroborated those of Guedé-Guina et al (1995). These authors observed *in vitro* a sensitivity of *E. coli* and *S. aureus* in the presence of the total extract of a leaf mixture of *A. cordifolia*, *Cassia.alata* and *Staurospermum verticillatum*. Determination of the antibacterial parameters showed that the *A. cordifolia* extracts had a bactericidal effect on all the strains tested. A bacteriostatic effect was observed with the 5% infusion on *E. coli* 8039. The antibacterial activity of the extracts may be due to the presence of flavonoids, sterols, polyterpenes, polyphenols, alkaloids and saponins observed in the infused and ethanolic extracts of *A. cordifolia*. These phytochemical compounds act either by modifying the bacterial cell membrane, by acting as an antimetabolite, or by inhibiting the synthesis of nucleic acid, proteins and/or the cell wall [24]. The richness of *A. cordifolia* leaves in phytochemical compounds could explain the biological activities (antimicrobial, antioxidant, anti-inflammatory and antiparasitic activity) inherent in this plant and thus justify its involvement in the treatment of several pathologies in traditional medicine.

Table 4. CMI and CMB performance of different extracts of *A. cordifolia*

		Alchornea. cordifolia					
		Infusé 5 %		Infusé 10 %		Infusé 70 %	
Origins	Bacteria	CMI (mg/mL)	CMB / CMI	CMI (mg/mL)	CMB / CMI	CMI (mg/mL)	CMB / CMI
Reference strains	*E. coli* ATCC 25922	12.5	2	12.5	2	12.5	2
	S. aureus ATCC 19213	3.12	1	3.12	1	0.78	4
Clinical strains	*E. coli* DAM	6.25	2	6.25	2	3.12	4
	E. coli 8039	3.12	8	6.25	4	6.25	2
	S. aureus 9044	6.25	1	3.12	2	3.12	2

Cmi: concentration minimale inhibitrice; cmb: concentration minimale bactericide

Table 5. Resistance partterns of strains tested

Antibiotics / Germs	Aminosides		Pénicillines		Céphalosporines		Beta-lactamines
	CN-30mcg	AK-30µg	AX-30mcg	AUG-30µg	CRO-30µ	CTX-5µg	CAZ-30µg
E. coli 8039	R	S	R	R	R	R	R
E. coli DAM	S	S	R	R	S	R	R
S. aureus 9044	R	-	-	-	-	R	-

CN-30mcg: Gentamicine ; AK-30µg: Amikacine ; AX-30mcg: Amoxicilline ; AUG-30µg: Amoxicilline acide clavulanique ; CRO-30µ: Ceftriaxone ; CTX-5µg: Cefotaxime ; CAZ-30µg: Ceftazidine ; S: Sensible ; R: Résistant ; (-): Non determine

5. Conclusion

This study updated scientific data and justified the traditional use of *Alchornea cordifolia* leaves in the treatment of urinary tract infections in northern Côte d'Ivoire. This objective was achieved by carrying out a phytochemical screening and assessing the antibacterial activity of extracts from the leaves of this plant. Evaluation of sensitivity to the extracts showed that all the strains tested were sensitive to extremely sensitive to *A. cordifolia* extracts. Determination of antibacterial parameters, activity complementary to germ sensitivity, showed that the extracts exerted a bactericidal effect on all the strains tested except the 5% infused extract, which exerted a bacteriostatic effect on the in vitro growth of *E. coli* 8039. The 10% infused and 70% macerated extracts were the most effective. *S. aureus* 9044 was the most sensitive strain and *E. coli* 8039 the least sensitive. The biological activities of the extracts of this plant are due to their phytochemical compounds. *A. cordifolia* therefore has antibacterial potential. These extracts are good candidates in the fight against urinary tract infections. This work justifies the use of this plant in traditional medicine, particularly in the treatment of pathologies associated with urinary tract infections.

Statement of Competing Interests

The authors have no competing interests

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