

Phytochemical screening and antibacterial activity of *Cassia sieberiana* DC. (Fabaceae) and *Zanthoxylum zanthoxyloides* (Lam.) Zepernick et Timler (Rutaceae) leaves extracts on *Escherichia coli* involved in urinary tract infections.

Abstract

Aims: *Cassia sieberiana* and *Zanthoxylum zanthoxyloides* are plants identified during an ethnobotanical survey, to treat urinary tract infections. The aim of this study was to investigate the antibacterial activity of aqueous and hydroalcoholic extracts obtained from leaves of *C. sieberiana* and *Z. zanthoxyloides*.

Methodology: Phytochemical compounds were identified using colorimetric and precipitation methods. The sensitivity of isolates to plant extracts and their level of resistance to conventional antibiotics were assessed by agar diffusion on *Escherichia coli* isolates from patients with urinary tract infections. Antibacterial parameters were determined by culture in liquid medium coupled with spreading on Mueller Hinton agar.

Results: The investigations revealed a predominance of tannins, flavonoids, polyphenols, saponins, terpenes and sterols, but an absence of alkaloids and phenolic acids in the plants extracts studied. The bacteria selected for the study showed high levels of resistance to cephalosporins, quinolones, penicillins and cyclins, but high sensitivity to aminoglycosides. These isolates were found to be resistant to beta-lactam antibiotics, with an ESBL phenotype. *C. sieberiana* extracts achieved inhibition diameters ranging from 09.5 ± 0.5 to 22.33 ± 1.78 mm. All strains were sensitive to these extracts. *E. coli* strain (Ech Dam) was the most sensitive to the decoctate of the aqueous extract, with an inhibition diameter of 22.33 ± 0.89 mm. All strains studied were equally sensitive to aqueous extract and *C. sieberiana* decoctate, and to gentamicin as a reference molecule. No significant

difference was observed between the inhibition diameters of gentamicin and those of crude *C. sieberiana* extracts.

Conclusion: Antibacterial activities of the extracts would justify the use of *C. sieberiana* leaves in traditional environments. However, *Z. zanthoxyloides* leaves extracts showed no activity on all the strains tested. Their use would therefore be unrelated to the roots and not the leaves.

Key words: bacterial sensitivity, phytochemical screening, resistance profile, *Escherichia coli*, urinary infection, *Cassia sieberiana*, *Zanthoxylum zanthoxyloides*

INTRODUCTION

Urinary tract infections are a health safety issue in low-and middle-income countries [1]. In Côte d'Ivoire, a prospective cross-sectional study involving 202 children aged from 6 to 24 months showed a prevalence of urinary tract infection around 10.89 %, with a predominance among girls and children aged from 6 to 12 months [2]. Numerous micro-organisms can infect the urinary tract, including *Klebsiella*, *Enterobacter*, *Proteus* and *Serratia* (10-15%), *Pseudomonas aeruginosa* is responsible for 10-15 % of cases, while *Staphylococcus* is responsible for 5 % [3]. But the most frequent agent is *Escherichia coli*. It accounts for the majority (70-95 %) and more than half of all urinary tract infections. The treatment of bacterial urinary tract infections is generally based on antibiotics use. However, their misuse has led to bacterial resistance to many antibiotics. This complicates the therapeutic management of these pathologies and justifies the search for new antimicrobials. For this, researchers have undertaken research into the pharmacological actions of medicinal plants, which constitute a valuable source of natural products for the maintenance of human health. According to the World Health Organization, medicinal plants are the best source for obtaining drugs with high pharmacological potential [4]. Some 80 % of the world's population, mainly in low-and middle-income countries, rely on traditional medicine derived from medicinal plants. Consequently, these plants need to be studied in order to better understand their properties, safety and efficacy [5]. For this reason, we focused on *Cassia sieberiana* and *Zanthoxylum zanthoxyloides*, two plants used in northern Côte d'Ivoire for the traditional treatment of urinary tract infections.

C. sieberiana is a tree in the Fabaceae family. Its fruits are used to treat fever, jaundice, stomach ache, gonorrhoea, hemorrhoids and ulcers. It is also used as a vermifuge, laxative and wound dressing [6]. According to the same author, in Côte d'Ivoire, the decoction is taken to treat intestinal worms, while the infusion of root bark is used against venereal diseases, sterility and dysmenorrhoea. After soaking the roots in water, the liquid is used for baths against fatigue and to massage the body. *Z. zanthoxyloides* is a Rutaceae with several therapeutic virtues. Root bark is used to treat high blood pressure, parasites [7], sickle-cell anemia [8], diarrhoea, dysentery, cholera, abdominal pain and stomach ulcers [9]. The plant has anti-free radical activity [10] and a vasodilatory effect [11]. The aim of the present study is to evaluate the antibacterial activity of *C. sieberiana* and *Z. zanthoxyloides* leaves extracts on *Escherichia coli* isolates isolated from patients suffering from urinary tract infections.

2. MATERIALS AND METHODS

2.1-Materials

2.1.1. Plant material

The biological material consisted of the leaves of *C. sieberiana* and *Z. zanthoxyloides*. These plants were selected following an ethnobotanical survey conducted by our research team, focusing on the traditional management of urinary tract infections. Plant identification was confirmed by Dr Soro Dramane, ethnobotanist at Péléforo GON COULIBALY University. The plants were harvested in February 2023 in Korhogo, in the Poro region of northern Côte d'Ivoire.

2.1.2 Bacterial isolates

The bacterial strains consisted of 4 clinical isolates and an *E. coli* ATCC 25922 reference strain (Table 1). These isolates were supplied by the medical analysis laboratory at the Korhogo Regional Hospital Centre (CHR)

Table 1: Origin of the tested strains

Souche	Code	Origin
<i>E. coli</i>	Ech Dam	Urine

<i>E. coli</i>	Ech 1	Urine
<i>E. coli</i>	Ech 6577	Urine
<i>E. coli</i>	Ech 528	Urine
<i>E. coli</i>	ATCC 25922	Reference

2.2-Methods

2.2.1 Pre-treatment of leaves

The leaves were washed and left to dry in the shade at room temperature in an airy place for 10 days. The dry leaves were crushed using an electric grinder, and the crushed material was stored in jars in a dry place protected from light and moisture until use.

2.2.2. Preparation of aqueous and hydroalcoholic extracts

The aqueous extract was obtained by decoction according to [12]. A 100 g test sample of plant powder was placed in a flask containing 1000 mL of distilled water. The mixture was boiled for 20 minutes. After cooling and filtration, the different filtrates were combined and placed in an oven at 50 °C for 3 days. To obtain the hydroalcoholic extract (30 :70 V/V), 100 g of plant powder was placed in a blender containing 1000 mL of ethanol. The whole was kept under agitation for 5 min ,3 times successively. After filtration, the filtrate was then placed in an oven at 50 °C for 48 hours. The aqueous and hydroalcoholic extracts obtained were then stored in hermetically sealed sterile bottles.

2.2.4. Phytochemical screening

Secondary metabolites (tannins, phenolic acids, alkaloids, polyphenols, flavonoids, saponins, sterols and polyterpenes) were screened using the tube characterization and tube precipitation techniques described by [13]

2.2.5. Isolation and confirmation of Isolates

Isolates received from the CHR were stored at -80°C at the LANADA bacteriology laboratory (korhogo). The isolates were removed from the freezer and, after a contact

time (30 min) at laboratory temperature, 10 mL of BCC medium were seeded and incubated at 37 °C for 3 h, to revive the isolates. Under sterile conditions, 0.1 mL of this preculture was spread on TBX agar in streaks, and the plate incubated at 37 °C for 24h. A suspect colony showing blue-green staining is then picked and spread on a plate containing nutrient agar to check strain purity. The plate is then incubated at 37 °C for 24 h. Gram staining and indole testing on urea-indole medium confirmed the identity of the isolates.

2.2.6-Inoculum preparation

Using a pasteur pipette, an 18 h to 24 h colony on Mueller Hinton agar is picked and placed in a test tube containing 10 mL of sterile Muller-Hinton broth. The mixture is incubated at 37 °C for 3 hours. After opalescence, 0.1 mL of this pre-culture is removed and diluted with 10 mL sterile physiological water. The bacterial suspension is homogenized and its turbidity compared with that of the 0.5 standard of the Mc Farland range. This corresponds to an *inoculum* of around $1 \text{ to } 2 \times 10^8$ CFU/mL for *E. coli* [14].

2.2.7-Susceptibility testing

Strain susceptibility testing was carried out using the agar diffusion technique. Mueller Hinton medium was inoculated by swabbing. A sterile swab was dipped into the previously prepared *inoculum*, then rubbed over the entire surface of the 4 mm-thick Mueller Hinton agar. For the plant extracts, wells approximately 6 mm in diameter were dug into the agar using a sterile punch. Each well received 80 µL of the test substance at concentrations of 200 and 100 mg/mL. After 15 min diffusion at laboratory temperature, Petri dishes were incubated at 37°C for 24 h. The presence or absence of a zone of inhibition was observed. For susceptibility to conventional antibiotics, antibiotic-impregnated discs were placed on the surface of the petri dishes using forceps that had been flamed and pressed lightly. The discs are placed approximately 3 cm apart. A maximum of six (06) discs is sufficient for a 90 mm diameter petri dish. After a contact time (5 min), the petri dishes are incubated at 37°C for 24 hours. The isolate sensitivity to plant extracts was determined according to [15]. A bacterial strain is said to be non-susceptible 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. Highly sensitive when the diameter is between 15 and 19 mm and extremely sensitive when the diameter > 20 mm. The susceptibility of isolates to conventional antibiotics was interpreted in terms of the clinical categories “susceptible”, “intermediate” or “resistant” to the various antimicrobials, in line with the critical points recommended by [15].

2.2.8- Determination of inhibitory and bactericidal concentrations of antibacterial parameters

Various concentrations were determined from a concentration range of plant extracts ranging from 200 mg/mL to 1.562 mg/mL prepared by double dilution in liquid medium according to [16]. The prepared range was autoclaved at 121°C for 15 min. The Minimum Inhibitory Concentration (MIC) corresponds to the lowest concentration inoculated without growth visible to the naked eye. A transparent medium is proof that growth has been inhibited. The Minimum Bactericidal Concentration (MBC) is determined by subculturing all the experimental tubes in the concentration range without growth visible to the naked eye. This subculture constitutes box B. The BMC is defined by comparing Box A and Box B. It corresponds to the smallest concentration in dish B whose colony count is less than or equal to the colony count of the 10⁻⁴ dilution of dish A.

2.3. Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1-Extraction yields

The highest extraction yield was obtained with *Z. zanthoxyloides* decoctate (58.44 ± 2.30%) and the lowest with ethanolic macerate of the same plant (34.22 ± 0.81%). Statistical analysis indicated a significant difference between extraction yields for *Z.*

zanthoxyloides. Between these two values, intermediate results were obtained with *C. sieberiana* extracts using the same solvents and extraction methods. Extracts from *Z. zanthoxyloides* were green in color, while those from *C. sieberiana* ranged from dark green to brown (Table 2).

Table 2: Extraction yields

Plants	Types Extraction	Solvent Extraction	Yield (%)	Colour of extracts
<i>C. Sieberiana</i>	Decoction	Aqueous	38,67 ± 0,44 ^a	Brown
	Maceration	Ethanol	39,11 ± 1,63 ^a	Dark green
<i>Z. zanthoxyloides</i>	Decoction	Aqueous	58,44 ± 2,30 ^b	Green
	Maceration	Ethanol	34,22 ± 0,81 ^a	Green

(Mean ± standard deviation of 3 trials) a, b: indicates variations in different yield classes at significant difference ($P=.62$).

3.2 Phytochemical screening

The results in Table 3 show the presence of polyphenols, flavonoids, tannins and coumarins in extracts from both plants. However, these extracts contained no alkaloids or phenolic acids. They contained very few sterols, terpenes and saponins. The extracts (decocted and macerated) of *C. sieberiana* were rich in saponins, unlike those of *Z. zanthoxyloides*. The decocted extracts of both plants contained no sterols or terpenes. The difference in phytochemical composition of the extracts is due to the absence of saponins in *Z. zanthoxyloides* extracts.

Table 3: Phytochemical screening of secondary metabolites

Métabolites secondaires	Leaves extracts			
	<i>Z. zanthoxyloides</i>		<i>C. sieberiana</i>	
	aqueous	Hydro ethanolic	Aqueous	Hydro ethanolic
Phenolic acids	-	-	-	-
Alkaloids	-	-	-	-
Coumarins	+	+	+	+

Flavonoids	+	+	+	+
Polyphenols	+	+	+	+
Saponins	-	-	+	+
Sterols and Terpenes	-	+	-	+
Tannins	+	+	+	+

+: Presence; -: Absence

3.3. Sensitivity of isolates to the extracts studied

The results of the bacterial sensitivity test to extracts are shown in Table 4. The largest inhibition diameters were obtained at a concentration of 200 mg/mL. In the decocted extract, they ranged from 20.67 ± 0.44 to 22.33 ± 1.78 mm. The largest inhibition diameter (22.33 ± 1.78 mm) was obtained with *E. coli* (Ech Dam) and *E. coli* (Ech1) strains. At the same concentration (200 mg/mL), the inhibition diameters of our extracts are virtually identical to those obtained with gentamicin (reference antibiotic). The statistical analysis indicates that there is no significant difference between the inhibition diameters of the decocted *C. sieberiana* extract and those of gentamicin (10 µg). In the case of ethanolic extract, the largest inhibition diameters were also obtained at a concentration of 200 mg/mL. These diameters ranged from 12 ± 0.00 to 16.33 ± 0.42 mm. For this extract and at the same concentration, the largest inhibition diameters remained well below those obtained with the decocted extract at a concentration of 100 mg/mL. All germs were sensitive to gentamicin. The statistical analysis showed that there was a significant difference between the mean inhibition diameters obtained with the decocted extract and the ethanolic extract at a concentration of 200 mg/mL. The decocted and macerated extracts of *Z. zanthoxyloides* showed no inhibitory effect on the in vitro growth of the isolates tested. No zone of inhibition was determined (Table 4).

Table 4: Diameters of in vitro growth inhibition of *E. coli* by *C. sieberiana* extracts

	Extract inhibition diameters (mm)				
	Aqueous		Hydro-ethanolic		GEN
	200 (mg/mL)	100 (mg/mL)	200 (mg/mL)	100 (mg/mL)	(10 ⁻³ mg)
<i>E. coli</i> (Ech1)	22,33±1,78 ^a	16±0,67	13,33±0,89 ^{bc}	10,33±0,44	21,33±1,56
<i>E. coli</i> (Ech 6577)	21,33±2,89 ^a	18±1,33	12,33±0,44 ^{bc}	10±0,00	20,33±1,56
<i>E. coli</i> (Ech Dam)	22,33±0,89 ^a	17,67±1,56	12±0,00 ^b	9,5±0,5	21,33± 0,89
<i>E. coli</i> (Ech 528)	20,67±0,44 ^a	15,67±0,89	15,33±1,56 ^c	11,33±1,78	21,33±0,44
<i>E. coli</i> ATCC25922	22,25±0.78 ^a	18,52±0,12	16,33±0,42 ^c	10,00±0,00	21,33±0,44

GEN : Gentamycine (antibiotique de référence). a, b, c indicate the different statistical classes between the inhibition diameters of aqueous and ethanol extracts at 200 mg/ml

Table 5 : Diameters of inhibition of in vitro growth of *E. coli* by extracts of *Z. zanthoxyloides*

	Diamètres d'inhibition des extraits (mm)				
	Aqueous		Hydro-ethanolic		GEN
	200 (mg/mL)	100 (mg/mL)	200 (mg/mL)	100 (mg/mL)	(10 ⁻³ mg)
<i>E. coli</i> (Ech1)	0	0	0	0	21,33±1,56
<i>E. coli</i> (Ech 6577)	0	0	0	0	20,33±1,56
<i>E. coli</i> (Ech Dam)	0	0	0	0	21,33± 0,89
<i>E. coli</i> (Ech 528)	0	0	0	0	21,33±0,44
<i>E. coli</i> ATCC25922	0	0	0	0	21,33±0,44

3.4-Isolate resistance to conventional antibiotics

Table 6 shows the clinical categories of isolates in relation to the antibiotics tested. All germs were resistant to the Cephalosporin, Penicillin, Fluoroquinolone and Tetracycline families, with the exception of *E. coli* Ech DAM, which was sensitive to cefotaxin and ceftriaxone, and *E. coli* Ech 528, which was sensitive to levofloxacin. At the same time, all isolates were sensitive to the aminoglycoside family. *E. coli* Ech DAM was the most sensitive isolate. In addition to aminoglycosides, it was sensitive to Cefotaxime and Ceftriaxone, two Cephalosporins. *E. coli* isolates Ech 1 and Ech 6577 were the most resistant. All isolates were resistant to at least two antibiotics from two different families.

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Table 6 : Resistance profile of isolates

Clinical categories of isolats in relation with conventional antibiotics						
Familles	Antibiotiques	Code et charge	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
			Ech DAM	Ech 528	Ech 1	Ech 6577
Aminoglycosides	Gentamicine	CN (30 µg)	S	S	S	S
	Amikacine	AK (30 µg)	S	S	S	S
Cephalosporines	Cefotaxime	CTX (5 µg)	S	R	R	R
	Céfoxitine	FOX (30 µg)	R	R	R	R
	Ceftazidime	CAZ (30 µg)	R	R	R	R
	Ceftriaxone	CRO (30 µg)	S	R	R	R
Penicilline	Ampiciline	AM (10 µg)	R	R	R	R
	Amoxicilline	AMC (20 µg)	R	R	R	R
	Amoxicilline + clavulanique	AMX (20 µg)	R	R	R	R
Fluoroquinolones	Levofloxacin	LVX (5 µg)	R	S	R	R
Tetracyclines	Tétracycline	TE (30µg)	R	R	R	R

3.5-Effectiveness of extracts

Minimum Inhibitory Concentrations (MICs) of the decocted extract ranged from 6.25 to 12.5 mg/mL, while those of the 70 % ethanolic extract varied from 50 to 100 mg/mL. Minimum Bactericidal Concentrations (MBCs) ranged from 50 to 100 mg/mL for the decocted extract and from 50 to 200 mg/mL for the ethanolic extract (Tables 7 and 8) respectively. The decocted extract of *C. sieberiana* leaves exerted a bacteriostatic effect on 100 % of bacterial strains, while the ethanolic extract demonstrated a bactericidal effect on all strains tested.

The antibacterial parameters of *Z. zanthoxyloides* extracts were not determined, as all isolates remained insensitive to the action of this plant's extracts.

Table 7: Antibacterial parameters of *C. sieberiana* aqueous extract

<i>C. sieberiana</i> aqueous extract				
Isolates	CMI (mg/mL)	CMB (mg/mL)	CMB / CMI	Interpretation
<i>E. coli</i> (Ech 1)	6,25	100	16	Bacteriostatic
<i>E. coli</i> (Ech 6577)	12,5	100	8	Bacteriostatic
<i>E. coli</i> (Ech Dam)	6,25	50	8	Bacteriostatic
<i>E. coli</i> (Ech 528)	12,5	100	8	Bacteriostatic
<i>E. coli</i> ATCC 25922	3,125	25		Bacteriostatic

Table 8: Antibacterial parameters of hydroethanol extract of *C. sieberiana* *Z. zanthoxyloides*

hydroethanol extract of <i>C. sieberiana</i> <i>Z. zanthoxyloides</i>				
Samples	CMI (mg/mL)	CMB (mg/mL)	CMB / CMI	Interpretation
<i>E. coli</i> (Ech 1)	100	100	1	Bactericide
<i>E. coli</i> (Ech 6577)	100	200	2	Bactericide
<i>E. coli</i> (Ech Dam)	100	100	1	Bactericide
<i>E. coli</i> (Ech 528)	50	50	1	Bactericide
<i>E. coli</i> ATCC 25922	6,25	12,5	2	Bactericide

DISCUSSION

In this study, the decoction with water and maceration in a hydroethanolic mixture were used to extract the active principles from the leaves of *Cassia sieberiana* and *Zanthoxylum zanthoxyloides*. These classic methods were used to get as close as possible to traditional uses. A difference in extraction yield was observed between the extracts of the two plants. The decoction method obtained the highest extraction yield for *Z. zanthoxyloides*. As for *C. sieberiana*, there was no statistical difference between the yields of the two extraction methods. These results indicate that the leaves of *Z. zanthoxyloides* contain more water-soluble substances than those of *C. sieberiana*. The difference in extraction yield between the plant extracts studied could depend on several parameters such as: solvent, pH, temperature, extraction time, organ maturity and sample composition [17]. Several authors have reported that the combined use of water and organic solvent can facilitate the extraction of chemicals that are soluble in water and/or organic solvent [17]. In this case, the maceration extraction method is expected to achieve the highest extraction yields. In the present study the higher extraction yield obtained with *Z. zanthoxyloides* decoctate could be explained in part by the difference in genetic composition of the plant matrices. The phytochemical screening carried out during this study highlighted the presence of several phytochemicals such as polyphenols; flavonoids; saponins; coumarins and tannins. The study also showed that extracts from *C. sieberiana* and *Z. zanthoxyloides* leaves contained no alkaloids or phenolic acids. The difference in composition between these extracts is due to saponins, which are absent in *Z. zanthoxyloides* extracts. Similar results have been reported by several researchers [18 [19] [20]. These results show that climatic, geographical and genetic factors, as well as extraction methods and the organs used, can influence the composition of plant extracts [21]. The absence of alkaloids in *Z. zanthoxyloides* leaf extracts could influence the antibacterial activity of these extracts, thus justifying the choice of this plant's roots in traditional environments. The bioactive molecules present in plant species are of great importance in the treatment of certain pathologies. The richness of *C. sieberiana* in these major groups of active chemical compounds could therefore justify the traditional use of this plant to treat numerous illnesses (constipation, hypertension, malaria and female sterility) [22]. Several scientific publications have shown that different types of chemical compounds found in the bark, roots and leaves of *C. sieberiana* and *Z. zanthoxyloides* have proven antibacterial effects [23]. Antibacterial tests carried out as part of the present study demonstrated the in vitro growth-inhibiting activity of 4 clinical isolates of *E. coli*. All the strains tested were resistant to the beta-lactam family but sensitive to aminoglycosides. These ESBL phenotype isolates thus rule out the use of several molecules

such as ceftazidime, cefotaxime, cefoxitine, ceftriaxone, amoxicillin and amoxicillin + clavulanic acid in the probabilistic treatment of *E. coli* urinary tract infections. Similar results have been reported by several researchers [24]. Multidrug resistance may be linked to the excessive or inappropriate use of antibiotics in livestock farming, fish farming or other agri-food sectors with a direct or indirect impact on human and animal health. In order to control the spread of antibiotic resistance, it is essential to raise awareness of the correct use of antibiotics, coupled with increased surveillance. The search for solutions to this phenomenon has turned to medicinal plants, a powerful reservoir of new bioactive molecules. The sensitivity of the isolates studied to plant extracts varied from one bacterial strain to another. The most sensitive strains to the decocted extract were *E. coli* (Ech1) and *E. coli* (Ech Dam). On the other hand, with ethanolic extract, *E. coli* (Ech 1) and *E. coli* (Ech 528) were the most sensitive. *E. coli* Ech 1 was therefore the most sensitive strain, and *C. sieberiana* decoctate the most effective extract. These results are similar to those obtained by [25]. These authors obtained zones of inhibition of between 2 and 20 mm at lower concentrations (30, 25, 20 and 15 mg/mL) on the growth of 03 reference strains (*Escherichia coli* ATCC 25922) with methanol and dichloromethane extracts of *C. sieberiana* root bark. Furthermore, no in vitro growth inhibition of the isolates studied was observed with *Z. zanthoxyloides* extracts. The sensitivity of the strains to the extracts may be due to the presence of flavonoids, tannins, polyphenols, coumarins and especially saponins. The absence of saponins in *Z. zanthoxyloides* extracts is thought to be partly responsible for the ineffectiveness of extracts from this plant. These phytochemical compounds act either by modifying the bacterial cell membrane, by acting as an antimetabolite, or by inhibiting nucleic acid, protein and/or cell wall synthesis [26].

CONCLUSION

The phytochemical screening of *C. sieberiana* and *Z. zanthoxyloides* extracts revealed the presence of polyphenols, flavonoids, tannins, coumarins, saponins, sterols and terpenes, and absence of alkaloids and phenolic acids. The isolates studied were resistant to the beta-lamine family but sensitive to aminoglycosides. All of the *Escherichia coli* isolates were BLSE phenotypes. Aqueous and hyro-ethanolic extracts of *C. sieberiana* were effective on all isolates studied. *Z. zanthoxyloides* extracts showed no inhibitory action on the in vitro growth of any of the isolates studied. This result justifies that the leaves must not be use in traditionnal medecine to treat urinary infection. The richness of the extracts in secondary metabolites such as saponins may underlie the antibacterial activity inherent in *C. sieberiana* leaf extracts. In view

of these results, *C. sieberiana* could offer real hope in the treatment of bacterial diseases, particularly urinary tract infections, which are a real public health threat.

Competing interests

Authors have declared that no competing interests exist.

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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