

Phytochemical screening and evaluation of the antimicrobial properties of the hydroethanolic extracts of *Ziziphus spina-christi* L., *Cassia obovata* L., and *Euphorbia hirta* L. on the pathogenic bacteria of N'Djamena-Chad urinary tract infections

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ABSTRACT

Background: The emergence of bacteria resistant to certain classes of antibiotics poses a global public health problem, particularly in developing countries. Traditional medicine plays a crucial role in the treatment of infectious diseases, particularly urinary tract infections, in Chad. It is in this context that this research was conducted in Chad to encourage the search for new antibacterial molecules derived from three medicinal plants used in traditional Chadian medicine. **Objectives:** This research aims to study the groups of chemical compounds present in the hydroethanolic extracts of *Z. spina-christi* L., *C. obovata* L., and *E. hirta* L., and to evaluate their in vitro antibacterial activity against uropathogenic bacterial strains acquired and isolated at the bacteriology laboratory of the National University Hospital Center in N'Djamena, Chad. **Methods:** The plant samples were macerated in a hydroethanolic solvent (ethanol/water, 70/30). A phytochemical screening of the hydroethanolic extracts of *Z. spina-christi* L., *C. obovata* L., and *E. hirta* L. was performed to identify alkaloids, saponins, flavonoids, and tannins as secondary metabolites. Antibacterial activity was evaluated using the liquid microdilution method combined with the agar plate technique to determine the MIC and MBC. **Results:** Phytochemical analysis revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, and tannins in all hydroethanolic extracts of *Z. spina-christi* L., *C. obovata* L., and *E. hirta* L. All hydroethanolic extracts completely inhibited the growth of Gram-positive cocci and Gram-negative bacilli at a concentration of 50 mg/mL. The hydroethanolic extracts of *Z. spina-christi* L. and *C. obovata* L. exhibited a bacteriostatic effect against *E. coli* (MIC = 12.5 mg/mL), whereas *E. hirta* exhibited a bactericidal effect. The hydroethanolic extracts of the three plants exhibited a bactericidal effect against *K. pneumoniae*, with significant inhibition ($P < 0.05$). **Conclusion:** In summary, these three medicinal plants possess significant in vitro antibacterial activity against uropathogenic bacterial strains. The results of this study scientifically confirm their use in traditional medicine, which should be the subject of further studies aimed at identifying the toxic effects of these plants. The isolation of active compounds and an in vivo evaluation are necessary prior to any therapeutic application.

Keywords: *Ziziphus spina-christi*, *Cassia obovata*, *Euphorbia hirta*, Antimicrobial activity, Chad, Phytochemical screening

INTRODUCTION

Since prehistoric times, plants have been used by humans for nutritional and therapeutic purposes due to their richness in biologically active secondary metabolites¹. In Africa, the use of medicinal plants has been known for a long time and has been passed down from generation to generation². However, for a long time, the chemical composition and mechanisms of action of the secondary metabolites contained in these medicinal plants remained insufficiently explored scientifically. In response to microbial attacks, numerous treatments have been developed by researchers to relieve patients and reduce the incidence of infectious diseases, particularly in developing countries^{3,4}. Despite these advances, the emergence and rapid spread of increasing resistance of bacterial microorganisms to antibiotics, due to their misuse and inappropriate use, is currently a major global public health problem⁵. This situation highlights the ineffectiveness of certain existing molecules

(antibiotics) and makes it increasingly difficult to treat certain infectious diseases, particularly urinary tract infections.

In developing countries, particularly in Africa, access to quality medicines is limited due to high costs and a lack of healthcare facilities in remote areas. According to the World Health Organization (WHO), approximately 80% of Africans rely on traditional medicine for their primary healthcare⁶. However, the African continent has an exceptional botanical heritage, with nearly 5,000 species of medicinal plants, whose therapeutic efficacy is widely recognized empirically⁷. However, since this plant wealth remains largely unknown in terms of phytochemical screening and microbial activity, it constitutes a significant gap in the scientific promotion of traditional African medicine.

The vegetation contains plants and herbs that have been used for their therapeutic effects in the treatment of most parasitic and bacterial diseases. In this regard, ethnobotanical surveys conducted

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among traditional healers in Chad have identified three plants that are used and most frequently cited in the treatment of urinary tract infections: *Ziziphus spina-christi*, *Cassia obovata*, and *Euphorbia hirta*. These three plants have been authenticated by a botanist from the Boitetchnopole laboratory of the Institute for Research in Animal Husbandry for Development (IRED) in N'Djamena, Chad.

In traditional Chadian medicine, *Euphorbia hirta* is particularly used in the treatment of diarrhea. However, previous studies have shown that hydroethanolic extracts of the plant inhibit the growth of *Staphylococcus saprophyticus* and *Staphylococcus aureus*⁸. However, these studies remain preliminary and were conducted in different geographical contexts. There is therefore little scientific data on the phytochemical screening and evaluation of the antibacterial activities of medicinal plants used locally against uropathogenic bacterial species in Chadian patients. In addition, few studies have compared the efficacy of the many plant species used to treat urinary tract infections. It is in this context that the present study was conducted in Chad to fill these gaps. The study aims to perform chemical screening to identify the secondary metabolites present in the hydroethanolic extracts of the leaves of these three plants: *Ziziphus spina-christi*, *Cassia obovata*, and the whole plant of *Euphorbia hirta*, as well as to evaluate their antibacterial activities on the four bacterial strains responsible for urinary tract infections isolated in the laboratory of the National University Hospital Center (CHU-RN), N'Djamena, Chad.

The results of this study will contribute to the fight against pathogenic bacteria and scientific promotion of traditional Chadian medicine in the treatment of urinary tract infections.

MATERIALS AND METHODS

Plant materials

The leaves of *Ziziphus spina-christi* L., *Cassia obovata* L., and the entire plant of *Euphorbia hirta* L. were harvested in September 2023 in the village of AdreDIR, Hadjer Lamis region, located approximately 70 km from the city of N'Djamena (Chad). The plant materials collected were authenticated by a botanist from the herbarium of the Institut de Recherche de l'Elevage pour le Développement (IRED) in N'Djamena (Chad). The reference plants were deposited in the herbarium for future reference (Figure 1).

Microbial strains used

The microbial strains used were pathogenic bacteria (strains of *S. saprophyticus*, *S. aureus*, *K. pneumoniae*, and *E. coli*) isolated and identified at the bacteriology unit of the National University Hospital Center in N'Djamena, Chad.

Methods

Plant extract preparation

The plant samples were washed with distilled water, then dried in the open air at room temperature (25°C) for 14 days in a ventilated area protected from light. After drying, the samples were ground using an electric grinder until a fine powder was obtained. For each plant studied, 500 g of powder was macerated in 5 liters of a hydroethanolic mixture (ethanol/water, 70/30) in hermetically sealed glass bottles. Maceration was carried out for 48 hours at room temperature (25°C), with frequent stirring (stirrer, ultra-mixer mod. 717).

After maceration, the hydroethanolic extracts were filtered successively using cotton wool and Whatman No. 1 filter paper. The filtrates of the hydroethanolic extracts obtained were concentrated under reduced pressure using a rotary evaporator at 40°C to remove the solvent. The dry hydroethanolic extracts obtained were weighed to calculate the extraction yield, then stored at 4°C in a refrigerator until use.

Extraction yield

The extraction yield was calculated as the ratio between the mass of dry extract obtained after evaporation and the mass of the initial plant material, which is given by the following formula: Yield (%) = $M1 / M0 \times 100$.

M1= mass of extract after evaporation. M0= mass of raw plant material.

Phytochemical screening

The extract was qualitatively tested using phytochemical screening to detect the presence of chemical components, namely alkaloids, flavonoids, tannins, and saponins. These main chemical groups were searched for in plant extracts using qualitative phytochemical analysis based on color tests⁹.

• Detection of alkaloids

The detection of alkaloids was carried out in the hydroethanolic extracts using two tests:

Method 1: using Mayer's reagent (10 g of KI and 2.70 g of HgCl₂ dissolved in 20 ml of water). Adding a few drops of this reagent to 2 ml of plant extract solution resulted in the formation of a white or white-yellow precipitate in the presence of alkaloids.

• Detection of saponins

Saponins: each plant extract was diluted in 10 ml of distilled water, for a total volume of 15 ml. After shaking the solution in one of the tubes for

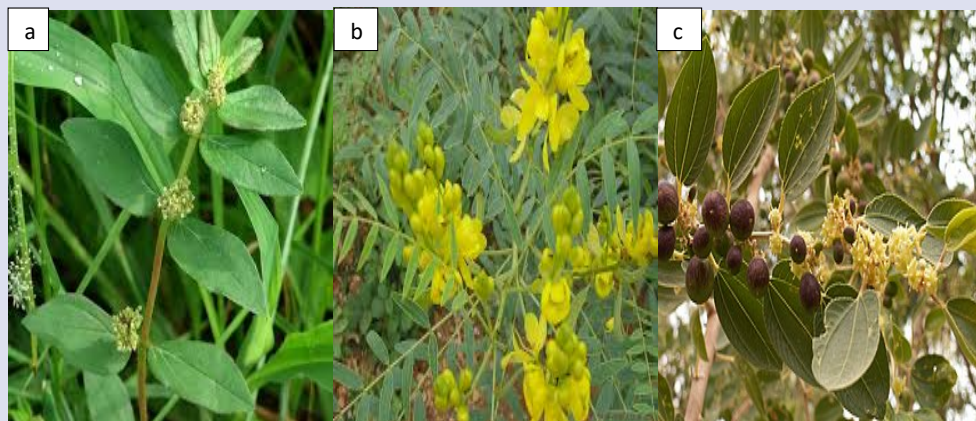


Figure 1. Medicinal plants studied: a: *Euphorbia hirta* L. (EH-01) , b: *Ziziphus spina-christi* L. (ZSC-02), and c: *Cassia obovata* L. (CO-03) .

1 minute, the solution was left to stand for 15 minutes. The formation of a persistent foam layer at least 1 cm thick indicates the presence of saponins. The second tube served as a control.

- **Detection of tannins**

Reaction with a 1% ferric chloride reagent: Each dry extract was dissolved in distilled water and divided between two tubes. In one tube, 1 to 2 drops of 1% ferric chloride reagent were added. The formation of a brown-black, blue-black, or black color indicates the presence of gallic tannins, while the formation of a green or dark green color corresponds to the presence of catechin tannins. The second tube served as a control.

- **Detection of flavonoids:** two to three drops of a diluted ferric chloride (FeCl_3) solution are added to a few ml of extract solution in a test tube. A greenish color indicates the presence of flavonoids in the samples studied.

Reaction with 10% lead acetate: each dry extract was dissolved in distilled water and divided into two tubes. In one of the tubes, 1 ml of 10% lead acetate was added. The appearance of a whitish, blue-black, or brownish precipitate indicates the presence of tannins in the plant extract. The second tube was used as a control.

Assessment of antimicrobial activity

Preparation of microbial suspension

The bacterial strains *Staphylococcus saprophyticus*, *Staphylococcus Escherichia coli*, and *Klebsella pneumonia* were cultured on nutrient agar for 24 hours at 37°C. The bacterial suspension was prepared in a sterile solution and adjusted to a turbidity equivalent to 0.5 McFarland.

Determination of antimicrobial activity

Antimicrobial activity was evaluated using the liquid microdilution method, which is the recommended reference method for determining the minimum inhibitory concentration (MIC). This method allows for a quantitative and reproducible evaluation of the antibacterial activity of hydroethanolic plant extracts.

Agar well diffusion methods. This method involves inoculating bacterial strains into a series of wells containing hydroethanolic extracts to be tested at increasing concentrations. The minimum inhibitory concentration (MIC) corresponds to the first dilution for which no bacterial growth is visible to the naked eye after 18 to 24 hours of incubation. The procedure was carried out in a microtiter plate. The culture medium used was Müller Hinton broth. Different concentrations were prepared by dilution (2-fold gradient) from a concentration of 100 mg/mL of each extract to obtain concentrations ranging from 50 mg/mL to 12.5 mg/mL depending on the plants studied. 100 μL /mL of the bacterial suspension was distributed into the test wells of the microplate containing the extracts. The control wells were composed as follows: broth alone, broth + bacterial suspension, and broth + chloramphenicol.

Chloramphenicol was used as a reference at concentrations between 1.5 and 2.5 μg /mL.

The plates are then incubated at 37°C for 24 hours. After incubation, any growth is revealed by the presence of cloudiness at the bottom of the well. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of extract for which no growth visible to the naked eye was observed. Wells showing no visible microbial growth at the minimum inhibitory concentration (MIC) and the following wells diluted to $\frac{1}{2}$ are re-isolated on nutrient agar. Inoculation was performed by spreading on the surface of the agar. After 24 hours of incubation in an incubator at 37°C, the culture medium was evaluated

to determine the minimum bactericidal concentration/minimum inhibitory concentration (MBC/MIC) ratio. Thus, the action of an extract will be considered bactericidal if the ratio between the minimum bactericidal concentration and the minimum inhibitory concentration (MBC/MIC) is equal to 1^{10} .

Statistical analysis of data

Our experimental data were obtained from three experiments. The results of this study were presented in tabular form. The data from this study were analyzed using SPSS version 25.0 software. A one-way ANOVA followed by an F-test was used to compare antibacterial activities, and Epi Info 7.0 software was then used to determine the significance threshold with a 5% error risk and a 95% confidence interval.

RESULTS

Extraction yields

The results presented in Table 1 show that the yields of the hydroethanolic extract from the leaves of the three plants used vary from 6 to 9.2, with a significant difference between the yields of the three hydroethanolic extracts ($P < 0.05$).

Phytochemical screening

Chemical screening tests revealed the presence of secondary metabolites (alkaloids, flavonoids, saponins, and tannins) in the hydroethanolic extract of the three plants, and the absence of saponins in *Cassia obovata* (Table 2).

Inhibitory activity of the hydroethanolic extract at 50 mg/mL

At a concentration of 50 mg/mL, the hydroethanolic extract of the leaves of *Ziziphus spina-christi* L., *Cassia obovata* L., and the whole plant of *Euphorbia hirta* L. completely inhibited the in vitro growth of all bacterial strains (*Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*). At a concentration of 25 mg/mL of hydroethanolic extracts of *Ziziphus spina-christi* and *Cassia obovata*, the bacterial strains showed variable results. These extracts of *Ziziphus spina-christi* and *Cassia obovata* completely inhibited the growth of the following hospital strains: *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The choice of these two concentrations, 50 mg/mL and 25 mg/mL, in this study is explained by the fact that, in antibacterial screening studies of crude plant extracts, it is often necessary to use proportionally high concentrations to detect biological activity. The concentration of 50 mg/mL is often reported by some authors as the reference concentration for the initial screening of the antibacterial activity of unpurified medicinal plant extracts. The second concentration of 25 mg/mL is considered an intermediate concentration, which allows the dose-dependent effect of the extracts to be evaluated and any decrease in antibacterial activity to be compared with the first concentration of 50 mg/mL and facilitate the identification of the most active extracts and guide the subsequent determination of minimum inhibitory concentrations (MICs).

Table 3 shows that *Ziziphus spina-christi*, *Cassia obovata*, and *Euphorbia hirta* at a concentration of 50 mg/mL inhibited the growth of *S. aureus* at an equivalence of 0.0025 mg/mL of the reference molecule (chloramphenicol). So these three plants showed a significant relationship with the reference molecule with $p < 0.001$

Sensitivity of bacterial strains

Our research results indicate that hydroethanolic extracts from the leaves of *Ziziphus spina-christi*, *Cassia obovata*, and the whole plant of

Table 1. Extraction yields

Plants	Extracts	Gros weights(g)	Extract weight(g)	Yield(%)	P-value
<i>Ziziphus spina-christi</i>	Hydroéthanolic	500	46	9,2	< 0,001
<i>C.assia obovata</i>	Hydroéthanolic	500	40	8	< 0,001
<i>Euphorbia hirta</i>	Hydroéthanolic	500	30	6	< 0,001

Table 2. Chemical screening of hydroethanolic extracts

Plants	Alkaloids	Flavonoids	Saponisides	Tannins
<i>Ziziphus spina-christi</i>	+	+	+	++
<i>Cassia obovata</i>	++	++	0	++
<i>Euphorbia hirta</i>	+++	+++	+	+++

Legend: Absent =0; Weak presence = +; Moderate presence = ++; Strong presence = +++

Table 3. Activity of hydroethanolic extracts (MIC in mg/mL) on hospital bacterial strains

Extract	<i>S. aureus</i>	<i>E. coli</i>	<i>S. saprophyticus</i>	<i>K. pneumoniae</i>	P-value
<i>Ziziphus spina-christi</i>	50	12,5	50	50	< 0,001
<i>Cassia obovata</i>	50	12,5	50	50	< 0,001
<i>Euphorbia hirta</i>	50	50	50	50	< 0,001
Chloramphénicol	0,0025	0,0015	0,0025	0,0025	1,0000

Table 4. Antibacterial parameters of *Ziziphus spina-christi*, *Cassia obovata* and *Euphorbia hirta*

Germes	Plant extract et timer	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI	Effets	P-value
<i>S. aureus</i>	<i>Z. spina-christi</i>	50	50	1	Bactéricidal	1 ,0000
	<i>C. obovata</i>	50	50	1	Bactéricidal	1,0000
	<i>E.hirta</i>	50	50	1	Bactéricidal	1 ,0000
<i>E. coli</i>	<i>Z. spina-christi</i>	12,5	25	2	Bactériostatic	0,001
	<i>C. obovata</i>	12,5	25	2	Bactériostatic	0,001
	<i>E.hirta</i>	50	50	1	Bactéricidal	1,0000
<i>S. saprophyticus</i>	<i>Z. spina-christi</i>	50	50	1	Bactéricidal	1,0000
	<i>C. obovata</i>	50	50	1	Bactéricidal	1,0000
	<i>E.hirta</i>	50	50	1	Bactéricidal	1,0000
<i>K. pneumoniae</i>	<i>Z. spina-christi</i>	50	50	1	Bactéricidal	1,0000
	<i>C. obovata</i>	50	50	1	Bactéricidal	1,0000
	<i>E.hirta</i>	50	50	1	Bactéricidal	1,0000
	<i>Z. spina-christi</i>	50	50	1	Bactéricidal	1,0000

Euphorbia hirta inhibit the growth of the bacteria tested. It should be noted that the antimicrobial activity of these plants was evaluated using hydroethanolic extracts. These plants had an effect on the Gram-positive cocci and Gram-negative bacilli tested. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts of *Ziziphus spina-christi*, *Cassia obovata*, and *Euphorbia hirta* had a bacteriostatic effect on the strains of *Escherichia coli* strains (Table 4). Hydroethanolic extracts of *Ziziphus spina-christi*, *Cassia obovata*, and *Euphorbia hirta* had a bactericidal effect on strains of *Staphylococcus aureus* and *Staphylococcus saprophyticus*, while *Euphorbia hirta* had a bactericidal effect on the *Escherichia coli* strain. All three plants reacted positively on the *K. pneumoniae* strain, with a bactericidal effect comparable to that of the reference chloramphenicol. Hospital strains of *Staphylococcus saprophyticus* and *Staphylococcus aureus* are all sensitive to hydroethanolic extracts of *Ziziphus spina-christi* and *Cassia obovata*, with a minimum inhibitory concentration (MIC) of 12.5 mg/mL. For the hydroethanolic extract of *Euphorbia hirta*, we obtained a minimum inhibitory concentration (MIC) of 12.5 mg/mL.

Table 4 shows that the three plants have bactericidal effect (CMB/CMI=1) against the germs responsible for urogenital infections, particularly on the *Escherichia coli* strain, which has a bacteriostatic effect (CMB/CMI=2) effect against both *Ziziphus spina-christi* and *Cassia obovata* extracts, with a significant difference (P<0.05).

DISCUSSION

Extraction yields

The hydroethanolic extracts of *Z. spina-christi*, *C. obovata*, and *E. hirta* yielded extraction yields ranging from 6 to 9.2 for the leaves of the three plants used, with a significant difference (P < 0.05). This yield is quite high for crude plant extracts and is explained by the use of a hydroethanolic solvent, known for its intermediate polarity, which allows for the simultaneous extraction of hydrophilic and lipophilic compounds. Somo (2002) in Burkina Faso reports a lower yield than ours (3.13%) during methanolic extraction of the whole *E. hirta* plant. This could be due to the methods used, the solvent ratios, and the plant parts used, as well as ecological variations affecting the biosynthesis of secondary metabolites.

Phytochemical screening

Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, and tannins in the hydroethanolic extract of the studied species (*Z. spina-christi*, *C. obovata*, and *E. hirta*). These results corroborate several previous studies conducted on similar chemical compounds and plant species^{5,11}. The presence of tannins in all extracts may confer antibacterial activity due to their ability to form complexes with bacterial membrane and enzymatic proteins¹². However, some discrepancies arise regarding the absence of saponins¹³ in the methanolic

and aqueous extracts of *Euphorbia hirta* leaves. The presence of these chemical compounds could explain their antimicrobial potential but may also be related to the nature of the solvent and ecological conditions. Data^{2,14} on the leaves and fruits of *Ziziphus spina-christi* indicate the presence of flavonoids, tannins, saponins, and alkaloids⁸ in whole-plant extracts of *Euphorbia hirta*.

Sensitivity of bacterial strains and possible mechanisms of action

Regarding the susceptibility of bacterial strains and possible mechanisms of action, the hydroethanolic extracts of *Ziziphus spina-christi*, *Cassia obovata*, and the whole plant *Euphorbia hirta* exhibit antibacterial activity against the tested Gram-positive cocci and Gram-negative bacilli. The hydroethanolic extracts of the three plants used in this study had bactericidal effects on strains of *Staphylococcus aureus* and *Staphylococcus saprophyticus*, while *E. hirta* had a bactericidal effect on the *E. coli* strain. All three plants showed positive activity against the *K. pneumoniae* strain, with a bactericidal effect comparable to that of chloramphenicol, thus confirming the broad spectrum of action of these hydroethanolic extracts. The results of the study conducted by⁸ on the methanolic extract of *Euphorbia hirta* leaves against the *Staphylococcus aureus* strain are similar to our results. This activity could be attributed to the presence of tannins and leucocyanidin¹⁹. The antibacterial effect of tannins is due to their ability to react with proteins to form stable, water-insoluble compounds, thanks to their cell walls composed of proteins. They act as detoxifying agents by precipitating protein components and inhibiting their growth²⁰. In addition, they can bind to proline-rich proteins and interfere with protein synthesis²¹. It has also been reported that they inhibit microbial enzymes²². According to some studies^{23,24}, the complex formation with metal ions may be due to their toxicity as tannic acid, which chelates iron present in the environment to render it unavailable to aerobic microorganisms. The antimicrobial properties of saponins are also attributed to the leakage of proteins and certain enzymes out of the cell. They possess detergent properties capable of increasing the permeability of bacterial cell membranes without destroying them. The antibacterial effect of alkaloids is due to their ability to bind to the DNA of Gram-positive and Gram-negative bacteria and to interfere with cell division²⁵. The activity of flavonoids is likely due to their ability to form complexes with cellular and soluble proteins as well as with bacterial cell walls²⁶, to inhibit the synthesis of bacterial DNA or RNA, protein, and lipid synthesis²⁷, and to interfere with energy metabolism²⁸.

For the hydroethanolic extract of *Euphorbia hirta*, we obtained an IC₅₀ of 12.5 mg/mL. Our results are consistent with those reported by^{6,13,18,29}, who studied the susceptibility of *Staphylococcus aureus* to *Euphorbia hirta* extract with an MIC of 12.5 mg/mL. For hospital strains of *E. coli*, the MIC ranges from 25 to 50 mg/mL, depending on the part of the plant used. Numerous studies worldwide, such as^{6,18,30}, confirm the susceptibility of *Escherichia coli* to *Euphorbia hirta* extract. The sensitivity observed in *E. coli* and *Staphylococcus aureus* could be explained by the presence of bioactive compounds in the plant extracts used. In this antibacterial screening study, which utilized extracts from three unpurified raw plants, high concentrations are often used to detect potential biological activity. A concentration of 50 mg/mL is often cited in the literature as the threshold concentration for initial screening to determine whether the extract has antibacterial activity, and 25 mg/mL to assess a potential dose-dependent effect and guide the MIC (the minimum inhibitory concentration to observe whether the effect decreases with dilution).

The bactericidal and bacteriostatic effects observed in these studies explain the use of these plants in the treatment of bacterial diseases. The results show that all three plants have a bactericidal effect against the *E. coli* strain, with a statistically significant difference. In this study, which

used extracts of these three unpurified raw plants, high concentrations were employed to detect potential biological activity. The results showed that all three plants have a bactericidal effect against pathogens responsible for urogenital infections, specifically against *E. coli*, with a statistically significant difference.

This study provides scientific evidence demonstrating the antibacterial efficacy, in Chad, of hydroethanolic extracts of *Ziziphus spina-christi*, *Cassia obovata*, and *Euphorbia hirta* against bacteria isolated from urine samples of patients treated at the CHU-RN. During this study, bactericidal activity was observed against *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and *Klebsiella pneumoniae*, while both bactericidal and bacteriostatic effects were observed against *E. coli* strains, suggesting a broad spectrum of activity for the three plants used. This activity is believed to be due to the combined effects and synergy of the identified secondary metabolites. This study also demonstrated a bactericidal effect comparable to that of chloramphenicol in three plants against *K. pneumoniae*, highlighting their therapeutic importance.

The results of our study could help bridge the scientific gap regarding local medicinal plants and serve as an alternative treatment against antibacterial-resistant pathogens responsible for urinary tract infections.

CONCLUSION

This study provides new and original data on the antibacterial potential of hydroethanolic extracts of *Ziziphus spina-christi*, *Cassia obovata*, and *Euphorbia hirta*, three plants widely used by traditional healers in Chad. The extracts demonstrated significant activity against uropathogenic bacterial strains isolated from patients treated at the bacteriology laboratory of the CHU-RN in N'Djamena, Chad.

The three medicinal plants used in our study exhibit statistically significant antibacterial activity in vitro, with bactericidal effects against Gram-positive cocci and Gram-negative bacilli involved in urinary tract infections. Among the plants used, *Euphorbia hirta* demonstrated bactericidal activity against *Escherichia coli*, while *Ziziphus spina-christi* and *Cassia obovata* exhibited bacteriostatic effects (MIC = 2). In contrast, all three plants exhibited comparable bactericidal activity against *Klebsiella pneumoniae*, which is important given the increasing resistance of this strain.

Thus, the presence of secondary metabolites—namely alkaloids, flavonoids, saponins, and tannins—may be responsible for this antibacterial activity. Hydroethanolic extracts have demonstrated in vitro antibacterial activity against bacterial strains responsible for urinary tract infections, and the focus on identifying bioactive secondary metabolites supports the hypothesis that the observed antibacterial activity results from synergistic interactions between these compounds. While it should be noted that these results scientifically confirm the therapeutic value of these three plants and support their empirical use in traditional Chadian medicine for the treatment of urogenital infections, they also demonstrate their potential as alternative phytotherapeutic sources in the context of growing antibiotic resistance.

Nevertheless, further studies are still needed, particularly regarding the isolation and characterization of the active compounds responsible for the antibacterial activity, as well as the assessment of toxicity and safety. These studies will then contribute to the development of pharmaceutical formulations and the confirmation of their efficacy through in vivo studies, prior to any therapeutic application. This study represents an important step toward promoting these three Chadian medicinal plants and integrating them into evidence-based herbal medicine for the treatment of urinary tract infections.

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