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Phytochemical screening and antibacterial activities of hydro-ethanolic extracts of *Vitellaria paradoxa* and *Acacia nilotica* against *Escherichia coli* and *Klebsiella spp.*, two strains responsible for urinary tract infections

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ABSTRACT

Background: Urinary tract infections remain a major public health concern worldwide, primarily caused by *Escherichia coli* and *Klebsiella spp.* The growing emergence of multidrug-resistant bacterial strains has further complicated their treatment, making the search for new bioactive substances from medicinal plants an important alternative approach. **Objective:** This study aimed to evaluate the phytochemical composition and antibacterial activities of hydro-ethanolic extracts from the stem bark of *Vitellaria paradoxa* and the root bark of *Acacia nilotica* against *Escherichia coli* and *Klebsiella spp.*, two bacterial strains frequently implicated in Urinary tract infections. **Materials and Methods:** Plant materials were collected and authenticated (UCJ 16611 for *V. paradoxa* and UCJ 11483 for *A. nilotica*). The dried and powdered plant organs were macerated in 70% hydro-ethanol to obtain crude extracts. Phytochemical screening was carried out using precipitation and staining tests to detect the major classes of secondary metabolites. The antibacterial activities of the extracts were evaluated using the agar well diffusion method, and the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined by the broth microdilution method. **Results:** Phytochemical screening revealed the presence of several bioactive compounds, including tannins, flavonoids, saponins, alkaloids, and terpenoids, in both extracts. The hydro-ethanolic extract of *Vitellaria paradoxa* showed inhibition zones ranging from 14.00 ± 1.00 mm to 20.67 ± 0.58 mm, while that of *Acacia nilotica* ranged from 9.67 ± 0.58 mm to 13.67 ± 0.58 mm. MICs of *V. paradoxa* extract varied between 6.25 and 12.5 mg/mL, and MBCs between 12.5 and 50 mg/mL. The MBC/MIC ratio ≤ 4 indicated a bactericidal effect for *V. paradoxa*, whereas *A. nilotica* exhibited a bacteriostatic effect (MBC/MIC > 4). **Conclusion:** The study demonstrates that the stem bark extract of *Vitellaria paradoxa* exhibits stronger antibacterial activity than the root bark extract of *Acacia nilotica* against *E. coli* and *Klebsiella spp.* These findings support the potential use of *V. paradoxa* as a promising natural source for developing new therapeutic agents against urinary tract infections.

Keywords: Urinary tract infections, *Escherichia coli*, *Klebsiella spp.*, Multiresistance, *Vitellaria paradoxa*, *Acacia nilotica*.

INTRODUCTION

Urinary tract infections correspond to colonization of the urinary tract by one or more pathogenic bacteria. This attack on the urinary tract tissue is characterized by an inflammatory reaction with symptoms of varying intensity and nature depending on the pathogen responsible [1]. They are most often caused by *Escherichia coli* (75-85% of cases), followed by *Klebsiella spp* and *Proteus mirabilis* [2]. If not treated properly, these infections can lead to serious complications such as pyelonephritis, sepsis, meningitis, or infertility [3].

These conditions affect women more than men for various anatomical reasons [4]. Indeed, as the urethra is shorter in women, bacteria quickly reach the bladder and multiply there [5]. Furthermore, the anatomical proximity of the urinary meatus to the anus also facilitates the passage of bacteria at this level. However, urinary tract infections are increasingly reported in men over the age of 50 with benign prostatic hyperplasia (BPH) [6-7-8]. According to some authors, in patients with BPH, urinary tract infections are caused by chronic inflammation of the prostate (prostatitis) and poor bladder emptying, which promotes urine stasis and is conducive to the development of germs [9].

In light of the excessive and inappropriate use of modern antibiotics, which promotes the emergence of resistant bacteria, traditional medicine presents itself as a promising alternative in the search for new chemical compounds [10]. This study fits into this context, with the overall objective of testing hydro-

ethanolic extracts from the bark of *Vitellaria paradoxa* stem and the bark of *Acacia niloca* root, two plants from the Ivorian pharmacopoeia, against multi-resistant strains of *E. coli* and *Klebsiella spp.* responsible for urinary tract infections. In addition, the study also aimed to establish the phytochemical profile of these extracts.

MATERIALS AND METHODS

Plant material

The plant material used consisted of bark from the stem of *Vitellaria paradoxa* and bark from the roots of *Acacia nilotica* (Figure 1). These organs were harvested in Korhogo (northern Côte d'Ivoire) and Daloa (Central-western Côte d'Ivoire) in February 2025, in the morning. These plants were identified at National Floristic Center of Cote d'Ivoire where specimens are kept. These samples had already been identified by Professor Aké Assi L. under the numbers UCJ 16611 (*V. paradoxa*) and UCJ 114883 (*A. nilotica*). After harvesting, the parts of both plants were dried at room temperature for 30 days, away from sunlight, in a well-ventilated room. They were then ground using an electric grinder (RETSCH, Type AS 200, Germany) to obtain a fine powder for the preparation of extracts.



Figure 1: Plant organs studied. (A) Bark of the stem of *Vitellaria paradoxa*; (B) Root bark of *Acacia nilotica*.

Bacterial strains

Bacterial support consisted of three clinical strains (*Escherichia coli* 8833, *Klebsiella spp* 8015, *Klebsiella spp* 8024) and one reference strain (*Escherichia coli* ATCC 25922).

The clinical strains were provided by the Bacteriology Laboratory of the Regional Hospital Center (RHC) in Korhogo (Côte d'Ivoire). They were isolated from urine samples from patients with urinary tract infections. The reference strain of *Escherichia coli* ATCC 25922 was obtained from the Pasteur Institute of Côte d'Ivoire (PICI). Unlike the ATCC strain, clinical strains are potentially resistant to antibiotics encountered in hospitals.

Preparation of hydro-ethanolic extracts

The hydro-ethanolic extracts at 70% of *Vitellaria paradoxa* and *Acacia nilotica* bark were obtained using the method described by Zirihi et al. [11] and employed by Ouattara et al. [12]. To do this, 100 g of powder from each plant was directly macerated in 1 L of 70 % ethanol. After homogenization using a Nasco blender (BL1008A-CB, Nigeria), the homogenate was pressed through white percale cloth and then filtered twice using cotton wool. Finally, the filtrate obtained was concentrated in an oven at approximately 60°C until the solvent had completely evaporated, resulting in a 70 % hydro-ethanolic extract.

Phytochemical screening of hydro-ethanolic extracts

Phytochemical screening was carried out using the techniques described by Békro et al. [13] and N'Guessan et al. [14]. These methods make it possible to identify the main chemical groups present in the hydro-ethanolic extracts prepared by precipitation and/or staining tests.

Evaluation of antibacterial activity of hydroethanol extracts

Preparation of bacterial inoculum

Inoculum for each bacterial strain was prepared by homogenizing two young colonies aged 18-24 h in 10 mL of Mueller-Hinton broth. The mixture was then incubated at 37°C for 3 hours. At the end of this incubation, 0.1 mL of the broth was transferred to 10 mL of sterile Mueller-Hinton broth to achieve a standard turbidity of 0.5 McFarland estimated at $1.5 \cdot 10^6$ CFU/mL [15].

Evaluation of bacterial strain susceptibility

Strain susceptibility to extracts was determined by the cookie-cutter well method in Mueller-Hinton agar medium as proposed by Ganfon et al. [16]. Indeed, Petri dishes containing Mueller-Hinton agar were flooded with each prepared bacterial inoculum ($1.5 \cdot 10^6$ CFU/mL). After drying these plates in an oven for 30 min at 37°C, 6 mm-diameter wells were made on each plate using a sterile Pasteur pipette. Each well was then filled with 80 µL of 100 mg/mL hydroethanol extract. Next to these wells filled with each extract, a control well was prepared with 80 µL of sterile distilled water. At the same time, gentamycin (30 µg) was also used as a standard positive control antibiotic. After 45 min of pre-diffusion, the whole set was incubated in the oven at 37°C for 18 hours. The effect of each extract on the strain studied was assessed by measuring the diameter of the zone of growth inhibition around the well. The strain is sensitive to the extract if this diameter is greater than or equal to 10 mm, otherwise it is said to be resistant [17].

Determination of minimum inhibitory and bactericidal concentrations

Minimum inhibitory concentrations (MICs) were determined by liquid dilution using the method of Okou [18]. The MIC corresponds to the lowest concentration that caused the absence of growth visible to the naked eye of the bacteria tested.

The MBC corresponds to the lowest concentration that allowed less than 0.01% of the germs in the starting suspension to survive after 24 hours. It was assessed using the technique described by Marmonier [19] and Okou [18]. It was determined by inoculating new Mueller-Hinton media prepared from tubes with no apparent growth. Finally, the BMC/MIC ratio was calculated to assess the antibacterial potency of each extract. An extract is considered bactericidal if this ratio is less than or equal to 4, and bacteriostatic if it is greater than 4 [20].

Statistical analysis

Experimental data were analyzed using SPSS software. Values were presented as mean \pm standard deviation. To assess the significance of observed differences, the analysis of variance (ANOVA) method was used, followed by Tukey's multiple comparison test with a threshold of 5%. If the P value is less than 0.05, the difference between the values is considered significant; otherwise, it is not significant.

RESULTS

Phytochemical composition

Phytochemical screening revealed that the 70% hydroethanol extract of *Vitellaria paradoxa* contained all the chemical groups sought, with the exception of sterol-terpenes and alkaloids, which were absent. The same was true of the 70% hydroethanol extract of *Acacia nilotica*. In fact, the latter extract showed the presence of the phenolic compounds we were looking for (flavonoids, tannins), while sterol-terpenes and saponosids were absent (Table 1).

Table 1: Phytochemical groups of hydro-ethanolic extracts

Secondary metabolites	<i>Vitellaria paradoxa</i>	<i>Acacia nilotica</i>
Total polyphenols	+	+
Flavonoids	+	+
Tannins	+	+
Alkaloids	-	+
Sterols and terpenes	-	-
Saponosids	+	-

- : absence ; + : presence

Table 2: Diameters of growth inhibition zones for hydro-ethanolic extracts at 100 mg/mL

Bacterial strains	<i>Vitellaria paradoxa</i>	<i>Acacia nilotica</i>	Gentamycin
<i>E. coli</i> ATCC 25922	19.00 ± 1.00 ^b	9.67 ± 0.58 ^a	28.33 ± 1.52 ^a
<i>E. coli</i> 8833	18.67 ± 0.58 ^b	13.67 ± 0.58 ^b	27.33 ± 0.58 ^a
<i>Klebsiella spp.</i> 8015	20.67 ± 0.58 ^b	12.33 ± 0.58 ^b	24.66 ± 2.08 ^b
<i>Klebsiella spp.</i> 8024	14.00 ± 1.00 ^a	10.33 ± 0.57 ^a	23.33 ± 3.51 ^b

The values represent the averages of three repetitions with the standard deviation. Values followed by the same superscript letter in the same column do not differ significantly (P > 0.05).

Table 3: Comparative antibacterial parameters of hydro-ethanolic extracts

Extracts	Strains	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Antibacterial power
<i>Vitellaria paradoxa</i>	<i>E. coli</i> ATCC25922	6,25	12,5	2	Bactericidal
	<i>E. coli</i> 8833	12,5	50	4	
	<i>Klebsiella spp</i> 8015	12,5	50	4	
	<i>Klebsiella spp</i> 8024	6,25	25	4	
<i>Acacia nilotica</i>	<i>E. coli</i> ATCC25922	25	200	8	Bacteriostatic
	<i>E. coli</i> 8833	25	200	8	
	<i>Klebsiella spp</i> 8015	25	200	8	
	<i>Klebsiella spp</i> 8024	25	200	8	

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

Diameters of the growth inhibition zones

Table 2 shows the diameters of the growth inhibition zones of bacterial strains to the hydro-ethanol extracts studied. Analysis of the results revealed that *Vitellaria paradoxa* and *Acacia nilotica* extracts showed antibacterial activity on all the bacterial strains tested, with respective inhibition diameters ranging from 14.00 ± 1.00 mm to 20.67 ± 0.58 mm for *V. paradoxa* and from 9.67 ± 0.58 mm to 13.67 ± 0.58 mm for *A. nilotica*. In addition, *Klebsiella spp* 8015 (20.67 ± 0.58 mm) was the most sensitive to *Vitellaria paradoxa* extract, while *Klebsiella spp* 8024 (14.00 ± 1.00 mm) was the least sensitive. *A. nilotica* extract was more active on *E. coli* 8833 (13.67 ± 0.58 mm) and less active on *E. coli* ATCC 2592 (9.67 ± 0.58 mm). Gentamycin also showed activity on all the bacterial strains tested, with inhibition diameters ranging from 23.33 ± 3.51 mm (*Klebsiella spp* 8024) to 28.33 ± 1.52 mm (*E. coli* ATCC 25922). Figure 2 shows some of the inhibition zones observed during the experiment.

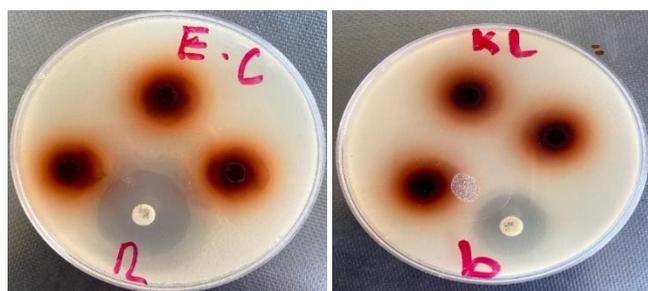


Figure 2: Some areas of inhibition observed during the experiment

Antibacterial parameters of 70% hydro-ethanolic extracts

The antibacterial parameters of the two extracts studied are summarized in Table 3. Against the different bacterial strains, the *Vitellaria paradoxa* extract recorded MICs ranging from 6.25 mg/mL to 12.5 mg/mL, while the MBCs ranged from 12.5 mg/mL to 50 mg/mL. Thus, for this extract, bactericidal activity was observed in all strains tested, as the MBC/MIC ratio was less than or equal to 4. In contrast, the *Acacia nilotica* extract revealed a bacteriostatic effect, as its MBC/MIC ratio was greater than 4.

DISCUSSION

Bioactive plant molecules play a crucial role in the treatment of various diseases. The aim of this study was to explore the antibacterial potential of the bark of the *Vitellaria paradoxa* stem and the roots of *A. nilotica* on bacteria responsible for urinary tract infections. In addition, phytochemical screening of 70 % hydro-ethanolic extracts from these two plants was essential.

Thus, phytochemical screening of 70 % hydro-ethanolic extracts from the stem bark of *V. paradoxa* and the roots of *A. nilotica* revealed the presence of a variety of chemical groups. Indeed, both extracts contain phenolic compounds (flavonoids, tannins). However, alkaloids were detected only in the *A. nilotica* extract, while saponosides were found only in the *V. paradoxa* extract. These results are similar to those of Tidiane et al. [21] and Keita et al. [22], who highlighted the predominance of these same secondary metabolites in the hydro-ethanolic extract of *V. paradoxa* bark and in the decoction of *A. nilotica* fruits.

The 70 % hydro-ethanolic extracts exhibited antibacterial activity against urinary strains of *Escherichia coli* and *Klebsiella spp.* Indeed, the diameters of the growth inhibition zones recorded ranged from 14.00 ± 1.00 mm to 20.67 ± 0.58 mm for *V. paradoxa* stem bark and from 9.67 ± 0.58 mm to 13.67 ± 0.58 mm for *A nilotica* roots.

When comparing the two extracts studied, the inhibition zones strongly correlated with the antimicrobial parameters evaluated (MIC, MBC). Indeed, for a given bacterial strain, the plant extract that induced a large inhibition zone was the most active.

However, these antibacterial activities could be explained by the presence of phenolic compounds (flavonoids, tannins) associated with other chemical groups (alkaloids, saponosids) contained in these extracts. Indeed, these secondary metabolites are well known for their antimicrobial properties, acting through various mechanisms such as cell membrane disruption and enzyme inhibition [23].

The results obtained are consistent with those of studies conducted by Olasunkanmi et al. [24] and Tordzagla et al. [25], which highlighted the marked inhibitory activity of *V. paradoxa* extracts on several other common pathogenic germs. The inhibition zones reported by these authors are comparable to those observed in this study.

The significant phytochemical composition of the two plant extracts studied, combined with their antibacterial activities, would justify their traditional use in effectively combating several conditions, including toothache, wounds, bronchitis, and sexually transmitted infections [26-27-28].

CONCLUSION

Extracts of *Vitellaria paradoxa* have shown promising bactericidal activity against strains responsible for urinary tract infections, unlike *Acacia nilotica*, whose effect remained bacteriostatic. However, *Vitellaria paradoxa* appears to be a better candidate for the treatment of urinary tract infections linked to *E. coli* and *K. pneumoniae*. *V. paradoxa* could also be used against urogenital infections in patients with benign prostatic hyperplasia (BPH) where strains of *E. coli* and *K. pneumoniae* are implicated.

Furthermore, additional studies are needed to better understand the mechanisms of action and toxicity of the extracts studied. It would also be interesting to extend this study to other multidrug-resistant pathogenic strains.

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Conflict of interest

The authors declared no conflict of interest.

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