Available online on 15.04.2025 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

# Phytochemical study and antibacterial activity of extracts of *Vitellaria paradoxa Gaertn* (Sapotaceae) from Chad on *Staphylococcus aureus* and *Salmonella* spp.

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## Article Info:

### Article History:

Received 13 Jan 2025
Reviewed 24 Feb 2025
Accepted 20 March 2025
Published 15 April 2025

### Cite this article as:

Martinien AD, Nadlaou B, Roberto A, Honoré H, Djimenan B, Bakarnga-Via I, Mbaïnguinam M, Phytochemical study and antibacterial activity of extracts of *Vitellaria paradoxa* Gaertn (Sapotaceae) from Chad on *Staphylococcus aureus* and *Salmonella* spp., Journal of Drug Delivery and Therapeutics. 2025; 15(4):26-34
*DOI:*
*http://dx.doi.org/10.22270/jddt.v15i4.7042*

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## Abstract

Bacterial infections are a public health problem worldwide and in Chad. *Vitellaria paradoxa* is a plant commonly used against infections in Chad. The objective of this study was to evaluate the antibacterial activity of *Vitellaria paradoxa* extracts on strains of *Staphylococcus aureus* and *Salmonella* spp.

Aqueous and ethanolic extractions of the fruits and bark of the trunk of *Vitellaria paradoxa* were carried out at the Labo-ReDES of the FSSH according to standard methods of clinical pharmacology and microbiology.

The yields of the plants ranged from (16-18%). The plant extracts inhibited the growth of *Staphylococcus aureus* and *Salmonella* spp at minimum inhibitory concentrations (MIC) ranging from 1.25 mg/mL to 20 mg/mL

The results of quantitative analysis showed that *Vitellaria paradoxa* fruits had a higher total polyphenol content (0.76 ± 0.002 mgAGE/g). Hydroethanolic extracts of *Vitellaria paradoxa* presented the higher values than aqueous extracts. The different proanthocyanidol contents of the recipe ranged from 0.22% ± 0.001 to 0.37% ± 0.003 catechin equivalents per gram.

These results demonstrate that this plant could be a potential source for developing new molecules to combat microbial infections.

**Keywords:** phytochemical study, antibacterial activity, *Vitellaria paradoxa*, Chad.

## INTRODUCTION

Infectious diseases are constantly increasing worldwide, particularly in resource-limited countries. Chad is unfortunately no exception to this reality. Millions of people become ill, and many die, after ingesting unsafe food. Among foodborne pathogens, *Salmonella* is the second leading cause of these bacterial foodborne illnesses worldwide[1]. In humans, it causes symptoms of a wide range of severity, from mild stomach aches to sepsis, and sometimes death. 2 Several foods are implicated in *Salmonella* contamination, such as meat, milk and dairy products (yogurt, cheese), and eggs. It is estimated that 1.4 million people in the United States are infected with non-typhoidal *Salmonella* each year, with 15,000 hospitalizations and 400 deaths. In humans it causes symptoms of a wide range of severity, from mild stomach aches to sepsis, and sometimes death[2]. Several foods are implicated in *salmonella* contamination, such as meat, milk and dairy products (yogurt, cheese), and eggs. It is estimated that 1.4 million people in the United States are infected with non-typhoidal *salmonella* each year, with 15,000 hospitalizations and 400 deaths. In France, the number of cases of salmonellosis is estimated at 30,000, with between 92 and 535 deaths. In sub-Saharan Africa, these infections, linked to the contamination of water and food soiled by feces, result in the death of 22 to 45% of infected people. With approximately 21.7 million cases and 217,000 deaths worldwide in 2000, typhoid fever is a global public health problem [3,4]. Multidrug resistance is one of the indicators of activity and quality in healthcare facilities. The increasing difficulties encountered in hospitals in treating certain infections resulting from multidrug-resistant bacteria are causing great concern for public health[5].

In Chad, typhoid fever remains a health problem, but its prevalence is poorly understood. Along with salmonella, the *Staphylococcus aureus* bacterium is part of a group of pathogens responsible for food poisoning and intoxication [6]. Added to the health problem are socioeconomic problems, and studies have shown that ciprofloxacin, like other antibiotics, also has several disadvantages [7,8]. On the one hand, they exhibit resistance to bacterial strains and on the other hand, they present considerable side effects, the appearance and exacerbation of pruritus and various eye disorders. Given the side effects of pharmaceutical products, more than 80% of African populations resort to traditional medicine. Thus, in Chad, efforts are being made to exploit medicinal plants and prudently promote the judicious therapeutic use of traditional medicine. Some structures, such as the University of N'Djamena, have undertaken pharmacological research on medicinal plants with antiradical and antibacterial properties, such as *Anogeissus leiocarpus* and *Acacia amythethopyilla*[9,10]. Given the diversity of the Chadian flora, as well as the limited number of local plants that have been the subject of clinical investigation, it seemed important to us to study some of these medicinal plants to treat various pathologies responsible for various infections. This is how Vitellaria paradoxa, known for the treatment of dermatological diseases, intestinal infections as well as the treatment of bovine brucellosis, was chosen. In Mali this plant is used in the treatment of tuberculosis. In Chad, *V. paradoxa* is used in the treatment of female infertility[11,12,13]. In view of this scientific knowledge about this plant and its wide use in the treatment of intestinal infections and given the pressing need for an antibiotic with fewer or no side effects, safe to use, we set the objective of carrying out a phytochemical study and evaluating the antibacterial activity of *V. paradoxa* extracts.

This study has highlighted the richness of hydroethanolic and aqueous extracts of the fruits and bark of the trunk of *V. paradoxa* in compounds that could be a potential source of natural biomolecules to fight against bacterial infections in Chad, Africa and elsewhere.

## MATERIAL AND METHODS.

### Setting, period, type of study, and research procedure.

This was an observational, cross-sectional, and analytical study conducted in the Research, Diagnostics, and Scientific Expertise Laboratory (Labo-ReDES) of the Faculty of Human Health Sciences at the University of N'Djamena, Chad.

The biological material consisted of bacteria (*Salmonella* spp and *Staphylococcus aureus*) isolated from patients hospitalized at the National Reference University Hospital (CHU-RN) of Chad. And bark and fruits of *V. paradoxa* harvested in January 2023 in Goré in the Province of Eastern Logone (Southern Chad).

### Preparation of Plant Extracts

The solvents used consisted of distilled water for aqueous extracts and ethanol for ethanolic extracts. 50 g of powder from each part of the plant was left to macerate in 500 mL of 95% ethanol for 24 hours on a shaker. The mixture was filtered using cotton wool into an Erlenmeyer flask and placed in an oven at 50°C. The resulting powder was weighed, and the calculated yields ranged from 16% to 18%. 200 mg of this powder was dissolved in a solution of 1 mL of DMSO and 9 mL of distilled water. This mixture was homogenized by vortex, placed in a vial tube, and stored at +4°C away from light for further use. This extract thus constituted the mother solution with a concentration of 20 mg/mL for our extracts.

### Preparation of Dilutions

A range of concentrations was prepared from the crude extracts at a concentration of 20 mg/mL using the two-by-two dilution method (1/2, 1/4, 1/8, 1/16, 1/32). In the five test tubes, we added 10 mL of the crude aqueous extract to the first tube (1/2). Then, we homogenized to obtain a 1/2 dilution solution. From this, we removed 5 mL, placed it in the second tube (1/4), added 5 mL, and then homogenized to obtain a 1/4 dilution solution. The same steps were repeated until the final solution was obtained at 1/32 dilution. The same procedure was performed for the ethanolic extract. This dilution allowed us to obtain the following concentrations: C1=20 mg/mL, C2=10 mg/mL, C3=5 mg/mL, C4=2.5 mg/mL, C5=1.25 mg/mL.

### Disc Preparation

6 mm diameter discs, unloaded with antibiotics, were sterilized and then impregnated with 20 µL of the aqueous or ethanolic extract solution of different parts of our plants at increasing concentrations (1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, 10 mg/mL, and 20 mg/mL).

### Determination of Antibacterial Activity

The method used was that of Kirby and Bauer (1982). It is based on the diffusion of paper discs impregnated with aqueous or ethanolic plant extract on Mueller-Hinton agar. Discs, unloaded with antibiotics, 6 mm in diameter, were sterilized and then impregnated with 20 µL of aqueous or ethanolic extract solution from different parts of the plant at increasing concentrations of 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, 10 mg/mL, and 20 mg/mL. The inoculum used was 108 CFU/mL. The diffusion method was performed as follows (Hayes and Markovic, 2002).

### Preculture Preparation

The bacterial inoculum was prepared from young colonies less than 24 hours old in the exponential growth phase on Mueller Hinton (MH) agar. Culture reactivation was performed by subculture on the surface of the pre-poured nutrient agar in a Petri dish, then incubated at 37°C for 18 to 24 hours. An isolated colony from the bacterial culture was picked up using a platinum loop and homogenized in 5 mL of distilled water to prepare the inoculum.

### Inoculation Preparation

From a pure, fresh culture, we prepared a suspension with an opacity equivalent to the 0.5 Mac Farland standard.

### Plate Inoculation

The inoculum was inoculated into Mueller-Hinton agar by flooding.

### Arrangement of the discs loaded with aqueous or ethanolic extracts of each part of the plant

We placed the discs using a dispenser or tweezers, pressing them lightly, and positioned them at least 15 mm from the periphery of the dish so that the inhibition zones did not overlap. This formed a concentration gradient of the plant extract around each disc.

### Incubation

The plates were incubated at 37°C for 24 hours.

### Reading the Diameter of Inhibition Zones

No growth occurs when the plant extract is present at the minimum inhibitory concentration and is sensitive to the strain. It is then possible to measure, using a caliper, the diameter of the inhibition zone, which is directly proportional to the minimum inhibitory concentrations.

### Interpretation

After measuring the zone of inhibition, expressed as a clear zone around the aqueous or ethanolic extract of each part of the plant, it was deduced that the larger the diameter of the zone, the more sensitive the bacteria were to the plant extract. This same method was used to determine the bacteria's sensitivity to conventional antibiotics.

### Determination of Minimum Inhibitory Concentrations (MIC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration capable of inhibiting the growth of the bacteria tested[14].

Table 1: Standard used for reading the results of antibiogram tests on plant extracts

| Inhibition diameter | Degree of sensitivity of the germ |
|---|---|
| Ø < 7 mm | Insensitive |
| 7 mm ≤ Ø < 8 mm | Sensitive |
| 8 mm ≤ Ø < 9 mm | Quite sensitive |
| Ø ≥ 9 mm | Very sensitive |

### Determination of Total Polyphenols and Proanthocyanidols

The determination of total polyphenols and proanthocyanidols (condensed tannins or flavanol polymers) was determined spectrophotometrically, using the colorimetric method using the Folin-Ciocalteu reagent. This assay is based on quantifying the total concentration of hydroxyl groups present in the extract. Polyphenols represent a large family of molecules, mainly derived from plants. They exhibit antioxidant properties and limit cellular aging. Polyphenols are used in the prevention of cardiovascular, inflammatory, and neurodegenerative diseases.

## RESULTS

### Mapping of the Study Area

The different parts of the plant were collected in the province of Eastern Logone located in the South of Chad, precisely in the locality of Goré 600 km south of Chad, Department of Nya-Pendé (Figure 1). Rich in its floristic variability, the city of Goré abounds in a significant quantity of *V. paradoxa* used in the treatment of bacterial and parasitic diseases.

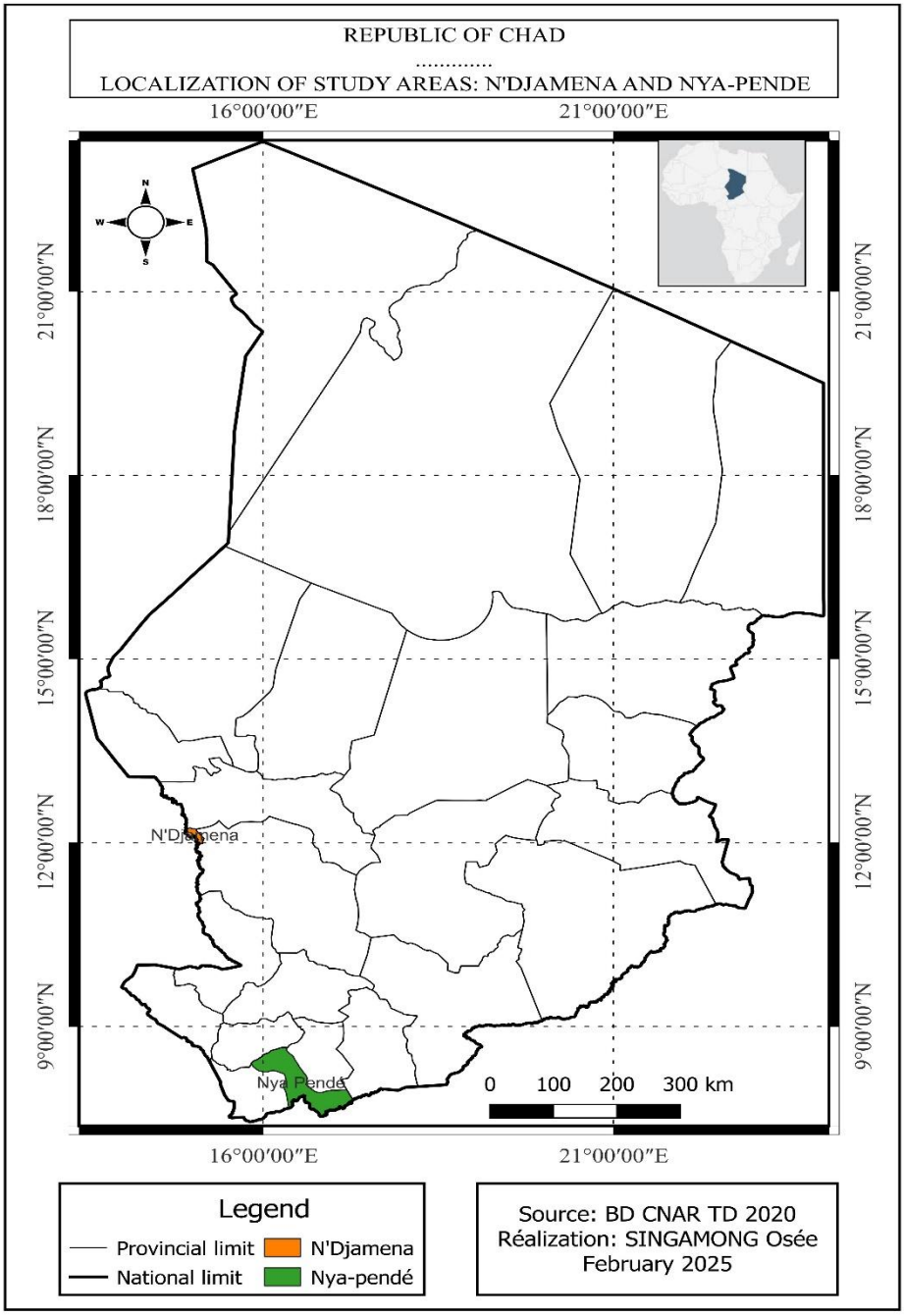



Figure 1: Map of the area of plant organ collection for the study

### Extraction Yields

After evaporating the extracts, the pastes were obtained and weighed. The mass of the resulting paste was divided by the mass of the initially weighed powder and multiplied by 100. This result is the yield (Table 2).

Table 2: Yields of ethanolic and aqueous extracts of *Vitellaria paradoxa* fruit and bark.

| Plant | Ethanolic extract | Yield |
|---|---|---|
| | Fruit | 18% |
| | Bark | 18% |
| ***Vitellaria paradoxa*** | **Aqueous extract** | Yield |
| | Fruit | 16% |
| | Bark | 16% |

### Phytochemical Screening

The identification of different classes of secondary metabolites in the extracts provided insight into their pharmacological activities. To this end, we conducted phytochemical tests on the various extracts prepared from the fruits and bark of Vitellaria paradoxa. The results are summarized in Table 3. Following photochemical analysis, extracts from different parts of *Vitellaria paradoxa* were found to be rich in tannins, alkaloids, saponins, free quinones, anthocyanins, sterols, and terpenoids; cardiac glycosides, anthraquinones, and flavonoids (Table 3).

Table 3: qualitative phytochemical screening of *Vitellaria paradoxa* extracts

| Chemical groups | Fruit | Bark |
|---|---|---|
| Alkaloids | - | + |
| Tannins | + | +++ |
| Flavonoids | + | + |
| Anthraquinones | - | + |
| Free quinones | - | + |
| Heterosides and cardiotonics | - | + |
| Saponins | +++ | + |
| Anthocyanins | + | - |
| Terpenoids and Sterols | +++ | + |

Legend: (-) = Totally absent; (+) = Weakly positive; (++) = Moderately positive; (+++) = Clearly positive.

**Quantitative Analysis of Extracts**

The results of the quantitative analysis showed that *Vitellaria paradoxa* fruits had a higher total polyphenol content (0.76 ± 0.002 mgAGE/g). Hydroethanolic extracts of *Vitellaria paradoxa* had higher values than aqueous extracts (Table 4). The different proanthocyanidol contents in the recipe ranged from 0.22% ± 0.001 to 0.37% ± 0.003 catechin equivalents per gram.

Table 4: Quantitative analysis of extracts

| extracts | Total polyphenols (mgAGE/g) | Proanthocyanidols (mgCE/g) |
|---|---|---|
| Eaq E | 0.30±0.008 | 0.22%±0.001 |
| Eaq F | 0.52±0.006 | 0.37%±0.003 |
| Ehydroeth E | 0.50±0.006 | 0.22%±0.001 |
| Ehydroeth F | 0.76±0.002 | 0.37%±0.003 |

Eaq E = aqueous extract of *Vitellaria paradoxa* bark; Eaq F = aqueous extract of *Vitellaria paradoxa* fruit Ehydroeth E = hydroethanolic extract of *Vitellaria paradoxa* bark; Ehydroeth F = hydroethanolic extract of *Vitellaria paradoxa* fruit; mgAGE/g: milligram equivalent of gallic acid per gram of extract; mgCE/g: milligram of catechin per gram of extract.

**Antibacterial Activity of the Extracts**

Inhibition of the growth of *Staphylococcus aureus* and *Salmonella* spp. induced by aqueous and ethanolic extracts of different parts of the plant at different concentrations (1.25 to 20 mg/mL).

The zones (diameters) of inhibition of the growth of these bacteria, expressed in millimeters (mm), as a function of the concentration of the ethanolic extract of *Vitellaria paradoxa* fruit and bark, are shown in the following tables:

**Minimum Inhibitory Concentration of the Ethanolic Extract of *Vitellaria Paradoxa* Bark**

The results in Table 5 showed that *Staphylococcus aureus* and *Salmonella* spp were susceptible to a minimum inhibitory concentration (MIC) of 2.5 mg/mL of the ethanolic extract of *Vitellaria paradoxa* bark.

Table 5: Growth inhibition diameters of *Staphylococcus aureus* and *Salmonella* spp by the ethanolic extract of *Vitellaria paradoxa* bark

| Concentration (mg/mL) | *Staphylococcus aureus* | SU | *Salmonella spp* | SU |
|---|---|---|---|---|
| 20 | 15.5± 0.70 | S | 13.75±1.06 | S |
| 10 | 15±1.41 | S | 13±0.0 | S |
| 5 | 13.25±1.06 | S | 13.25±1.76 | S |
| **2.5** | **10.25±1.06** | **S** | **8.75±0.35** | **S** |
| 1.25 | 6±0.0 | I | 6.75±1.06 | I |

S= sensitive ; I= insensitive; SU= susceptibility

**Minimum Inhibitory Concentration of Ethanolic Extract of *Vitellaria paradoxa* Fruit**

The results in Table 6 showed that *S. aureus* was susceptible to a minimum inhibitory concentration (MIC) of 2.5 mg/mL and *Salmonella* spp. was susceptible to a MIC of 5 mg/mL of the ethanolic extract of *Vitellaria paradoxa* fruit.

Table 6: Growth inhibition diameters of *Staphylococcus aureus* and *Salmonella* spp. by the ethanolic extract of *Vitellaria paradoxa* fruit.

| Concentration (mg/mL) | *S. aureus* | SU | *S. spp* | SU |
|---|---|---|---|---|
| 20 | 18±1.41 | S | 16.5±0.70 | S |
| 10 | 17.75±1.06 | S | 15.75±0.35 | S |
| **5** | 15.5±0,70 | S | **14.25±1.75** | **S** |
| **2.5** | **8.75±1.06** | **S** | 6.25±0.35 | I |
| 1.25 | 6.75±1.06 | I | 6±0.0 | I |

S= sensitive ; I= insensitive ; SU= susceptibility; *S.* spp = *Salmonella* spp; *S. aureus* = *Staphylococcus aureus*

**Minimum Inhibitory Concentration of the Aqueous Extract of *Vitellaria Paradoxa* Bark**

The results in Table 7 showed that S. aureus and *Salmonella* spp. were sensitive to an MIC of 1.25 mg/mL of the aqueous extract of *Vitellaria paradoxa* bark.

Table 7: Growth inhibition diameters of *Staphylococcus aureus* and *Salmonella* spp. by the aqueous extract of *Vitellaria paradoxa* bark.

| Concentration (mg/mL) | *S. aureus* | SU | *S. spp* | SU |
|---|---|---|---|---|
| 20 | 16.75±0.35 | S | 14.75±0.35 | S |
| 10 | 15.75±0.35 | S | 14.25±0.35 | S |
| 5 | 14.25±0.35 | S | 13.5±0.70 | S |
| 2.5 | 13.5±0.7 | S | 9.5±0.70 | S |
| 1.25 | **7.75±1.06** | **S** | **7.25±0.35** | **S** |

S= sensitive ; I= insensitive ; SU= susceptibility; *S.* spp = *Salmonella* spp; *S. aureus* = *Staphylococcus aureus*

**Minimum Inhibitory Concentration of *Vitellaria paradoxa* Fruit Aqueous Extract**

The results in Table 8 showed that *S. aureus* and *Salmonella* spp. were susceptible to an MIC of 2.5 mg/mL of *Vitellaria paradoxa* fruit aqueous extract.

Table 8: Growth inhibition diameters of *Staphylococcus aureus* and *Salmonella* spp. by *Vitellaria paradoxa* fruit aqueous extract.

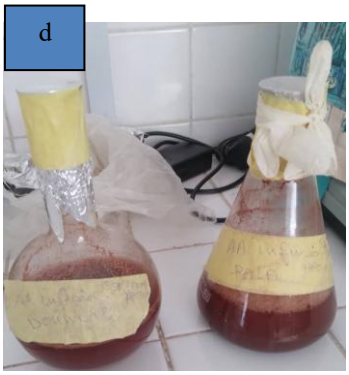
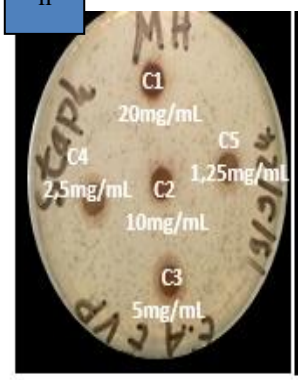
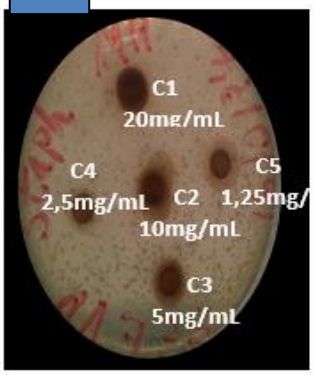
| Concentration (mg/mL) | *S. aureus* | SU | *S. spp* | SU |
|---|---|---|---|---|
| **20** | 15.75±2.47 | S | 12.75±1.06 | S |
| **10** | 15.25±1.06 | S | 12.5±0.70 | S |
| **5** | 13.75±2.47 | S | 12.25±0.35 | S |
| **2.5** | **8.25±1.06** | **S** | **8.75±0.35** | **S** |
| **1.25** | 6.75±0.35 | I | 6±0.0 | I |

S= sensitive ; I= insensitive; SU= susceptibility; *S.* spp = *Salmonella* spp; *S. aureus* = *Staphylococcus aureus*

**Biotechnological Stage of Collection and Preparation of Vitellaria Paradoxa Extracts.**

Table 9 shows the different processing steps for *Vitellaria paradoxa* organs. This table shows that ethanolic extracts had significantly higher antibacterial activity than aqueous extracts (Figures h and i).

Table 9: Biotechnological Stages of Collection and Processing of *Vitellaria Paradoxa* Bark and Fruits.

| | | | | |
|---|---|---|---|---|
| 1 | a) : bark of *Vitellaria paradoxa*<br>b) : fruits<br>c) : bark powder | a  | b  | c  |
| 2 | d) : Preparation of extracts<br>e) : filtration of extracts<br>f) : drying | d  | e  | f  |
| 3 | g) : preparation of extracts for phytochemical screening<br>h) : antibacterial activity with the aqueous extract<br>i) : antibacterial activity with hydroethanolic extract | g  | h   | i   |
| 4 | j) : *Staphylococcus aureus* colonies on Chapman agar<br>k) : *Salmonella* spp colonies on Hektoen agar<br>l) : antibiogram test | j  | k  | l  |

(Photo : Martinien Atakewang Djetoloum et *al.*, 2024)

## DISCUSSION

The study first involved harvesting the bark and fruit of *V. paradoxa*. These different parts of the plant were extracted using both aqueous and alcoholic methods. The yields of the extracts were: aqueous extracts of *V. paradoxa*, fruits (16%) and bark (16%), and ethanolic extracts of *V. paradoxa*, fruits (18%) and bark (18%), respectively. The results of this study showed that ethanolic extracts produced higher yields than aqueous extracts. These results corroborate those of Kamagaté et al. (2021) in Côte d'Ivoire, where the yield of ethanolic extracts of *V. paradoxa* was higher than that of aqueous extracts (22.1% and 11.3%)[15]. This could be explained by the fact that ethanol would be the ideal solvent for extracting a large majority of chemical constituents from medicinal plants compared to water. The antibacterial activity of ethanolic and aqueous extracts of *V. paradoxa* was evaluated on two (02) bacterial strains (*Staphylococcus aureus* and *Salmonella* spp). The tests showed that the ethanolic and aqueous extracts inhibited the growth of these bacterial strains at MICs between 1.25 mg/mL and 20 mg/mL. The synergy of action of secondary metabolites at the level of the major groups including flavonoids, tannins, alkaloids and saponosides present in the extracts of *V. paradoxa* would be responsible for the inhibition of bacterial growth[16]. Our results are similar to previous work that showed that *V. paradoxa* extracts inhibit the growth of *Mycobacterium tuberculosis* at a concentration of 125 µg/mL. However, we observed that among the bacteria tested, Gram-positive bacteria (*Staphylococcus aureus*) showed high sensitivity compared to Gram-negative bacteria. This difference in sensitivity observed between the two bacterial strains to the two extracts of the plant studied could be explained by the fact that the wall of Gram-negative bacteria contains a lipid layer that is more resistant than Gram-positive bacteria, which lack this lipid layer [17,18]. This could be explained by the fact that the ethanolic extract easily penetrates into the bacterial cell, whether Gram-negative or Gram-positive bacteria, due to its polarity. Furthermore, some reference antibiotics show greater antibacterial activities than the tested plant substances, with larger inhibition zone diameters. This could be explained by the fact that the reference antibiotics are isolated, pure molecules of known concentrations, while the ethanolic and aqueous extracts are unpurified mixtures of active substances that are compounds resulting from secondary metabolism[18].

The results of the quantitative analysis showed that *V. paradoxa* had a higher total phenol content (0.76 ± 0.002 mgAGE/g). Previous studies have shown the presence of phenolic compounds in different plant organs[19,20]. Phenolic compounds are the main characteristic molecules of the plant kingdom. Their role is to defend plants against pathogens. They ensure human and animal nutrition and health. They are able to eliminate free radicals and inhibit lipid peroxidation by reducing hydroxyl, superoxide and pyroxyl radicals. They are also able to trap metal ions, as they have chelating properties. Polyphenols have significant antioxidant activity, superior for example to that of vitamins[21, 22, 23].

## CONCLUSION

This study demonstrated the antibacterial activity of ethanolic and aqueous extracts of *V. paradoxa* fruits and barks. *V. paradoxa* extracts inhibited the growth of *Staphylococcus aureus* and *Salmonella* spp. at minimum inhibitory concentrations (MIC) ranging from 1.25 mg/mL to 20 mg/mL. The results obtained confirm that *V. paradoxa* fruit and bark extracts possess antibacterial activity. All these cumulative results indicate that ethanolic and aqueous extracts of *V. paradoxa* possess pharmacological activity and represent potential for developing pharmaceutical products based on natural products. They confirm their use in traditional medicine.

**Conflicts of interest:** The authors declare that they have no conflicts of interest.

**Author Contributions:** MAD designed and ensured the field sample collection, performed the laboratory manipulation, and wrote the first draft. RA and HH contributed to the manipulation. NB and BVI led the laboratory manipulation and contributed to the scientific editing and guidance of the draft. MM coordinated the entire work.

**Acknowledgments:** The authors thank the staff of the Bacteriology Unit and the Toxicology/Pharmacology Unit of the Research, Diagnostics, and Scientific Expertise Laboratory of the Faculty of Human Health Sciences in N'Djamena, Chad.

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