# Antimicrobial Efficacy of *Vitellaria paradox* (Shea Butter Tree) Extracts Against *Staphylococcus aureus* and *Escherichia coli*

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DOI: 10.56201/jbgr.v9.no2.2023.pg51.58

***ABSTRACT***

*The antibacterial activities of the ethanolic extracts of leaf and stem bark of Vitellaria paradoxa were investigated. The extracts were tested against the bacterial pathogens, Staphylococcus aureus and Escherichia coli using the agar well diffusion techniques. Ethanolic extracts of the plant parts showed activity against all the bacterial pathogens tested. At the highest extract concentration (200 mg/ml), the leaf extract exhibited the highest antimicrobial activity, while no activity was detected at the lowest concentration (3.13 mg/ml) against the tested isolates. Escherichia coli and Staphylococcus aureus were more susceptible to all extracts of V.paradoxa, the efficacy of ethanolic extracts of Vitellaria paradoxa was compared to commercial antibiotic streptomycin. There were differences in the minimum inhibitory concentration (MIC) of all the Vitellaria paradoxa ethanolic extracts with respect to the type of organism. Qualitative phytochemical screening of the extracts revealed the presence of saponins, tannins and alkaloids as the active principles of Vitellaria paradoxa's antimicrobial activity. V. Paradoxa could be used as a potential source of antibiotic substance for a drug development.*

***Keywords:*** *Vitellaria paradoxa, extracts, antibacterial, bacteria, Escherichia coli , Staphylococcus aureus*

## INTRODUCTION

Traditional medicines refers to health practices, approaches, knowledge and beliefs incorporating plants, animals and mineral based medicines, spiritual therapies, manual techniques, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being (WHO, 2003).

Traditional medicines have been effectively used for thousands of years. The contribution of herbal products to modern medicine is well documented. It is reported that life in most parts of Africa is connected with herbal medicine, while 65%-80% of world's population rely on traditional medicine for their health care needs (Philips *et al.,* 2009; Calixto, 2000; Adamu*et al.,* 2013)

In Nigeria, thousands of plant species are known to have medicinal values and the use of different parts of these plants to cure specific ailments has been practiced since ancient times (Rios and

Recios, 2005). The medicinal values of plants lie in their phytochemical composition, which produce definite physiological actions on the human body (Mann *et al*., 1997).

*Vitellariaparadoxa*(formerly *Butryspermumparadoxum)* or Shea butter, is a popular tree with several applications in folkloric medicine. The tree grows naturally in the wild of the dry savannah belt of West Africa and stretches in abundance onto the foothills of the Ethiopian mountains (Adamu*et al.,* 2013).

In traditional medicine, Shea butter has been employed in the treatment of several ailments. It encourages wound healingand soothes skin irritation. Shea-butter is also used to treatinflammation, rashes in children, dermatitis, chapping and ulcers, as well as rub for rheumatism (Hong *et al*., 1996). Its leaf decoctions are used for stomach ache, headache and as an eye lotion. Roots and root bark are grounded to paste and taken orally to cure jaundice, or they are boiled and pounded totreat chronic sores. They are also used for the treatment of gastric problems as well as diarrhea and dysentery. Bark decoction is used to facilitate childbirth and to encourage lactation afterdelivery, or as a footbath neutralizes venom of the spitting cobra(Hall *et al*., 1996). Cosmetics, especially those that prevent skin drying and good looking lipsticks use Shea-butter. As a result, cosmetic industries market uses these ingredients in soaps, shampoo and skin cream preparations (Hall *et al*., 1996).

*Vitellariaparadoxa*has been studied as a potent medicinal plant(Prescott *et al*., 2002), against bacterial infections (El-Mahmoud *et al.,* 2008) and fungal infections (Ahmed and Sani, 2013). The ethanolic extraction of the active principle of this medicinal plant has been shown to be more efficacious than when water or acetone (El-Mahmoud *et al.,* 2008) and hot or cold water (Ahmed and Sani, 2013) are used as extractants. The reason for the higher antibacterial activity of the ethanolic extract has been suggested to be due to differences in the polarity of the solvents and the modulatory effect of enzyme such as phenolases and hydrolases released when plant materials are grounded in water(El-Mahmoud *et al.,* 2008). Odebiyi and Sofowora (1978) also showed that potency of a plant extract depends on both theconcentration used and the method of extraction.

In line with the need to search for more effective and safeantibacterial drugs and to justify the traditional use of herbalpreparations in the treatment of infectious diseases, this work was designed to investigate and compare the antibacterial efficacy of the *Vitellariaparadoxa*leaf and stem bark against someclinically important isolates and to determine the phytochemical constituents present in those plant extracts.

Plants are rich in variety of secondary metabolites such as tannins, terpenoids, alkoaloids, flavonoids, phenols, steroids, glycosides and volatile oils (Cowan, 1999). Plants provide tremendous reservoir of various chemical substances with potential theraupeutic properties.

The increasing complexity in drug manufacture and agriculture worldwide, the toxicity associated with synthetic drugs and the relative tolerance as well as demand for natural products has made the need to properly identify, study and promote the conservation of plants for the benefit of humanity very important (Mann *et a*l., 2003).

Infectious diseases account for approximately one-half of all death in tropical countries due to drug resistant microorganisms and the emergence of unknown disease-causing microbes. The African system of medicine have been found to be active against a wide variety of microorganisms and these medicinal plants have shown great promise in the treatment of intractable infectious diseases including opportunistic infections (Iwu, *et al*., 1999).

Economically, traditional medicines are cheaper and more accessible to most rural populace hence its popularity (Mann *et al*., 2003). Global commercial interest in plants as a source of medicine and cosmetic is gaining prominence and creating multiple opportunities for cultivation, conservation and extraction of new plant products (Okujagu, 2006).

Works has been carried out to investigate and to determine the antimicrobial, antioxidant and cytotoxic activities of medicinal plants collected from different locations. Due to natural products of higher plants, they may give a new source of antimicrobial agents as a result of which many researchers are now engaged in medicinal plants research (Motsei, 2003).

**OBJECTIVE OF THE STUDY**

- The objective of this study is to assay the leaf and bark extract of *Vitellaria paradoxa* plant against some selected pathogens.
- To establish the antimicrobial properties of the leaf and stem bark of the plant extract.

**MATERIALS AND METHODS**

Bark of *Vitellaria paradoxa* and it leaves used in this study were collected from the garden area of Federal Polytechnic, Bida, Niger state and were properly identified in biology laboratory, the Biological science department.

**TEST ORGANISMS**

The bacterial species used in this study, *Staphylococcus aureus* and *Escherichia coli* were obtained from the Department of Biological sciences, Federal Polytechnic Bida, Niger state. Purity of the cultures was checked at regular intervals as described by Acheampong *et al*. (1988).

**PLANT COLLECTION AND IDENTIFICATION**

Samples of the bark and leaf of *Vitellaria paradoxa* were collected from the trees within the Federal Polytechnic Bida, Niger. The plant samples were identified macroscopically as described by Dalziel (1968) and confirmed at the biology laboratory. The fresh samples were dried for a week under room temperature, grounded into a fine powder and kept in plasticcontainers until further use at room temperature (28 ± 1 °C).

**PREPARATION OF ETHANOLIC EXTRACTS**

One hundred grams (100g) of each of the plant parts (leaf and stem bark) were soaked into 1000 ml of the solvent (95% ethanol) in different air-tight sterile jars respectively at room temperature. The solvents containing the extracts were decanted filtered with a muslin cloth and then with Whatman no. 1 filter paper respectively. Further extraction of the grounded samples was done with same volume of 95% ethanol, decanted and filtered two more times. The filtrates from each round of extraction were combined and were evaporated to dryness in small, open - mouth jars and then packed in separate clean dry bottles and stored at room temperature until required.

**STERILITY OF EXTRACTS**

Each of the extracts was tested for growth of contaminants. This was done by making serial dilution of 1 g of each extract up to $10^{-6}$. Twenty microliters (20 µl) of the diluents were aseptically inoculated on Nutrient Agar plates and incubated at 37 °C for 24 hours. The plates were observed for growth. Absence of microbial growth in the extract indicated their sterility. Sterile extracts were used to test for antimicrobial efficacy.

**STANDARDIZATION OF INOCULUMS**

Standardized inoculums of each tested organism was obtained by making their respective suspension up to 0.5 McFarland standard as observed in the spectrophotometer and as described by Barry *et al*. (1980).

**DETERMINATION OF ANTIMICROBIAL ACTIVITIES**

The Agar Well Diffusion method as described by Linoand Deogracious (2006) was used. By this method, 0.1 ml of the respective standardized inoculums (0.5 McFarland turbidity standard = 1.0 x 108 cfu/ml) of each test bacterium was spread into sterile Mueller Hinton Agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer (5.0 mm diameter) was used to bore wells aseptically in the agar plates. The extracts were prepared and serially diluted in a two-fold dilution to achieve different concentrations of 3.13, 6.25, 12.5, 25, 50, 100 and 200mg/ml respectively for each extract. Subsequently, 0.3 ml of each concentration of the extracts was introduced into the wells earlier bored Agar plates. The extracts were allowed to diffuse into the medium (kept for 1 hour on the bench before incubation) at 37 °C for 24 hours. Streptomycin was used as a positive control, while a Mueller Hinton agar plate without antimicrobials was the negative control. Antimicrobial activity of the extracts was determined by measurement of zones of inhibition produced around the wells. The diameter of the zones indicated the degree of susceptibility of the test bacteria.

**DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC)**

The Minimum Bactericidal Concentration of the extracts was determined by subculturing test solutions which showed no detectable growth (no turbidity after 24 hours incubation) onto fresh Nutrient Agar plates (the recovery medium) and incubated further for 24 hours. Absence of growth on the recovery medium indicated bactericidal effect, while the appearance of growth on further incubation indicated bacteriostatic effect.

**PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT**

The extracts were screened for the presence of carbohydrates, tannins, alkaloids, saponins, polyphenols- and other constituents successively as described by Odebiyi and Sofowora (1978) and Herbune (1973).

## RESULTS AND DISCUSSION

### RESULTS

Table 1 shows the phytochemical screening of crude extracts of *Vitellariaparadoxa*

| Phytochemicals | Bark | Leaf |
|---|---|---|
| General glycosides | + | + |
| Tannins | + | + |
| Steroids | - | + |
| Saponins | + | + |
| Carbohydrates | + | + |
| Alkaloids | + | + |
| Polyphenols | + | - |
| Terpenoids | + | + |

**KEY** +: Present -: Absent

Table 2 shows Diameter of inhibition zones of *V. Paradoxa* extracts and commercial antibiotic (mm)

| Concentration (mg/ml) | Bark | Leaf | Streptomycin |
|---|---|---|---|
| 200 | 12.5 ± 02 | 15.0 ± 03 | 17.0 ± 03 |
| 100 | 10.0 ± 01 | 8.0 ± 02 | 16.0 ± 02 |
| 50 | 8.0 ± 01 | 5.5 ± 01 | 15.0 ± 03 |
| 25 | 7.5 ± 03 | 5.0 ± 03 | 14.0 ± 01 |
| 12.5 | 4.5 ± 03 | 3.0 ± 01 | 12.0 ± 00 |
| 6.25 | 3.0 ± 01 | 3.0 ± 01 | 8.0 ± 00 |
| 3.13 | -- | -- | 3.0 ± 02 |

*Staphylococcus aureus* McFarland turbidity standard (1.0 x 108 cfu/ml)

Table 3Shows Diameter of inhibition zones of *V. Paradoxa* extracts and commercial antibiotic (mm)

| Concentration (mg/ml) | Bark | Leaf | Streptomycin |
|---|---|---|---|
| 200 | 14.0 ± 02 | 14 ± 00 | 17.5 ± 00 |
| 100 | 11.5 ± 03 | 10.5 ± 00 | 16.0 ± 02 |
| 50 | 7.5 ± 03 | 7.5 ± 02 | 15.0 ± 03 |
| 25 | 6.0 ± 03 | 6.0 ± 02 | 14.0 ± 03 |
| 12.5 | 4.0 ± 01 | 5.0 ± 02 | 12.0 ± 01 |
| 6.25 | 3.0 ± 03 | 3.0 ± 01 | 7.0 ± 00 |
| 3.13 | -- | -- | 3.0 ± 01 |

*Escherichia coli* McFarland turbidity standard (1.0 x 108 cfu/ml)

## DISCUSSION

The current experiment investigated and compared the antimicrobial activity of the ethanolic extracts of leaf and bark of *V. Paradoxa* (Shea butter tree) against selected clinical bacterial isolates (*Staphilococcus aureus*and *Escherichia coli*). *V. paradoxa* as it has recently been a research focus as potential source for drug development due to its antibacterial (El-Mahmood *et al.*,2008) and anti fungal (Ahmed and Sani, 2013) activities.

### Phytochemical composition

Phytochemical composition of *V. paradoxa*is shown in Table 1. General glycosides, tannins, saponins, carbohydrates, alkaloids and trepenoids were present in all plant parts (stem bark and leaf) of *V. paradoxa*ethanolic extracts. Steroid was found to be in absent in the stem bark , while the seed was shown to lack polyphenols. These bioactive compounds have been demonstrated to be responsible for the antimicrobial activity of medicinal plants (Mathias *et al.*,2007).

### Effect of ethanolic extracts of *V. paradoxa* parts against selected organisms

As shown in Tables 2 and 3 all, the plant parts tested, the bark and leaf respectively, showed antibacterial activity against the selected clinical isolates. The leaf extract showed the highest activity against all tested organisms and its efficacy was comparable to the commercial antibiotic (streptomycin) at the highest concentration (200 mg/ml) against test organisms. This higher activity is thought to be premised upon the presence of steroids, which was absent in the bark

extract. The absence of steroids in the bark of *V.paradoxa*has been reported by El-Mahmood*et al*., 2008. No activity was shown at the lowest concentration (3.13 mg/ml) of all extracts.
Antibacterial activity of the ethanolic extracts of *V. paradoxa* is organisms dependent. Each tested organism showed varying response to the respective extracts at different concentrations. Bark extracts were more potent against *E. coli* and *S. aureus*. Among the two isolates, *E. coli* was the most susceptible organism to all *V. Paradoxa* extracts (Tables 2 and 3). *Staphylococcus aureus* was susceptible, but slightly less susceptible as *E. coli*. The differences in the susceptibility of the tested organisms and variations in the specific activity of each extract may be due to the physiological properties of the clinical isolates and the presence or absence of some active principles in the extracts. More so, the sensitivity of the organisms, as indicated by the diameter of the inhibition zones (Tables 4 and 5) was proportional to the concentration of the respective extracts. At the highest concentration (200 mg/ml), all organisms were sensitive to the *V. paradoxa*extracts. Progressive decrease in respective extract concentration leaded to a proportionate reduction of the inhibition zone around each organism. Similar reports (Adamu*et al.,* 2013; Arekemase *et al*.,2013) have shown that higher concentrations of antimicrobial substances showed appreciable antimicrobial activity. All *V. Paradoxa* extracts exhibited bacteriostatic effects (Table 4) on all tested clinical isolates, but showed different minimum inhibitory concentration (MIC) which was also different with respect to each organism tested in the experiment. The bacteriostatic effect of these plant parts could possibly be due to the presence of saponins, which demonstrate remarkable physiological activity and forms lather, responsible for wound and skin protection (Ahmadu *et al*., 2006). Specifically, saponins have been suggested to exhibit greater antimicrobial effect and could serve as a precursor of steroidal substances with a wide range of physiological activities. The MIC of the stem bark extract was 6.25 mg/ml against all the organisms. However, at 12.5 mg/ml, all *V. Paradoxa* extracts showed antibacterial activity against all the clinical isolates. The variation in the MIC may be due to the phytochemical composition of the respective ethanolic extracts and the genetic make-up of each test organisms. Different organisms have been shown to respond differently to different and same concentrations of a specific medicinal plant (Philip *et al.*,2009).

## CONCLUSION

The ethanolic extracts of the bark and leaf of *Vitellariaparadoxa*have demonstrated antimicrobial activities against the tested clinical isolates (*E. coli* and *S. aureus*), thus justifying its use in traditional medicine for treating different diseases associated with the tested isolates, as it also could serve as a new and cheaper alternative for antibiotic sources. The clinical isolates used for this investigation are associated with various human diseases like gastrointestinal tract infections and body superficial wound infections. As shown in this study, *V. paradoxa* could be used as a potential source of antibiotic substance for a drug development against the diseases caused by this group of both superficial and enteric organisms. Further toxicological, purification and identification studies could be carried out to investigate the general effects of the use *V. paradoxa* for drug development.

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