# ANTIMICROBIAL ACTIVITY OF *Daniella oliveri* STEM BARK EXTRACTS ON SOME SELECTED CLINICAL ISOLATES

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**Abstract:** Conventional antibiotics are gradually becoming ineffective against bacterial infections due to antibiotics resistance and bacteria mutation, Hence, the need for alternatives sources of antimicrobials. This study is aims at evaluating the antibacterial activity of stem bark cutting of *Daniella oliveri* against some clinical isolates. The plant sample was collected at agricultural research farm of Federal university Wukari and was authenticated at Forest Herbarium of Federal College of Forestry Jos. The sample was air dry at room temperature for 7 days. Exactly 400 g of the air dried samples was weighed and soaked in 1000 mL each of ethanol and water respectively for 72 hours and were extracted using decoction method. The ethanol and aqueous extracts of the bark of the *Daniella oliveri* was tested against *Staphylococcus aureus* SIRST56, *Bacillus* species B-3-35 and *Planococcus glaciei* CHR43 isolated from Urine and Wound sample at different concentrations of 200, 100, 50, and 25 mg/mL, using agar well diffusion method. The ethanol extract of the bark was inhibitory against *Staphylococcus aureus* SIRST56 and *Bacillus* sp B-3-35 with the highest zone of inhibition of 14±0.00 mm and 17±0.00 mm respectively while the aqueous extracts of bark was active against *Staphylococcus aureus* SIRST56 and *Planococcus glaciei* CHR43 with zone of inhibition of 14±0.00 mm and 13±0.00 mm for the aqueous bark respectively. The results of the Minimum Inhibitory Concentration (MIC) of the extracts reveals 50mg/ml concentration for *Staphylococcus aureus* SIRST56, 25mg/ml for *Bacillus* sp B-3-35 and *Planococcus glaciei* CHR43 respectively. The results of the Minimum Bacteriocidal Concentration (MBC) were shown at 50 mg/ml. This is an indication that the bark of *D. oliveri* have antibacterial effects against the test organisms. Hence the plant can be further harnessed and the active inhibitory substance purified and used as alternative to treat infections associated with the test organisms.

**Keywords:** Antimicrobial, Clinical isolates (Bacteria), *Daniella oliveri* Bark, and Minimum Bacteriocidal Concentration and Minimum inhibitory concentration.

## Introduction

Traditional medical knowledge is primarily transmitted orally and has little to no written record in many nations around the world (WAHP, 2020). However, since the latter half of the 20th century, there have been calls for documentation and protection of traditional medical knowledge due to the realization of the enormous potential of traditional medicine and the growing demand of indigenous knowledge holders for a fair and equitable share of benefits derived from the commercialization of their products. Long before recorded history, people employed plants for medical purposes. However, the majority of these plant substances are well-known for their ability to treat and manage a variety of ailments, either through improving human health or the immune system (Imo *et al*., 2019). Comparatively to other traditional societies throughout the world (such as China, India, and Greece), African societies did not completely employ medicinal plants.

The World Health Organization (WHO) defines traditional medicine as the body of knowledge and practical skills that are exclusively based on practical experience and observation passed down orally or in writing from one generation to the next and are used in the diagnosis, prevention, and elimination of physical, mental, or social imbalances (Gende *et al*., 2020). Besides serving as a source of new structures that could lead to medications in all major disease areas, medicinal plants are vital for the millions of people for whom traditional medicine is their only option for healthcare and for those who utilize plants for a variety of purposes on a daily basis. Over the years, scientists from all over the world have become interested in the successful use of plant extracts in the prevention, management, and treatment of both communicable and non-communicable diseases. At least 80% of Africans rely on herbal medication for their healthcare, according to a report by Ezekwesili and Ogbunugafor (2015).

According to Soro *et al*. (2016), mature and young leaves are used to cure Tuberculosis (TB) and headaches, respectively. They also reported that the plant's leaves are used in ethno medicine in Nigeria to treat diabetes, diarrhea, gastro intestinal disorders, and as a diuretic. In south-western Nigeria, the Tiv people use fresh, powdered young leaves to promote healing and stop wounds from bleeding, while they also consume leaf sap orally to treat coughs (Coker and Ogundele, 2002). Skin conditions and gonorrhea are treated with a decoction of the root. In northern Nigeria's Kano, the infusion of the stem bark of *D. oliveri* has been employed to address cancer and digestive problems (Ahua *et al*., 2007). They also reported the application of *D. oliveri* exudates for treating migraines and promoting wound healing in Mali and Nigeria. According to the research by Daniels *et al*. (2013), *Daniellia oliveri* has been shown to have high antibacterial qualities by inhibiting all of the test organisms at different dosages. The zone of inhibition against *Streptococcus pyogenes* for the ethanol extract at 100 mg/ml was 23 + 1.46. For an ethanol extract at an 80 mg/ml concentration, the lowest zone of inhibition against Pseudomonas aeruginosa was 10 + 0.84 mm. Even yet, a plant ethanol extract might stop the development of *Staphylococcus aureus* at levels as low as 50 mg/ml. The aqueous extract was not effective against

*aeruginosa*. However, the plant demonstrated antifungal effectiveness against *Fusarium oxysporum* between dosages of 80 mg/ml and 100 mg/ml. The plant is therefore considered a broad spectrum antibacterial.

Temitope *et al*. (2016) claim that *Daniellia oliveri* extracts have the potential to be sources of new antimicrobial compounds, particularly those that are effective against *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa Staphylococcus*, *aureus*, *Klebsiella pneumonia*, *Bacillus cereus*, *Proteus vulgaris*, *Streptococcus viridians*, *Shigella dysenteriae*. The investigation undertaken by Coker and Ogundele (2016) uncovered that *Daniellia oliveri* evinces superior antifungal properties in its leaves and stem bark as opposed to its aqueous extract. *Candida krusei* and *Candida albicans* were found to be highly vulnerable, while *Epidermophyton floccosum* and *Trichophyton interdigitale* showed minimal impact. Apart from the above usage, Scrotal elephantiasis, diarrhea, ring worms, syphilis, typhoid fever, eye pain, and earache are all treated with the roots, stem, bark, and leaves of *Daniella oliveri* (Temitope *et al*., 2016). Some of these infections and diseases are gradually developing antibiotic resistance to some of the conventional antibiotics. Hence, the need for alternatives sources of antimicrobials. This present study is aims at evaluating the antibacterial activity of the stem bark cutting of *Daniella oliveri* against some clinical isolates.

## Materials and Methods

### *Collection and Identification of Plant samples*

The Bark of *Daniella oliveri* was obtained from the agricultural research farm of Federal University Wukari, Taraba State, Nigeria. It was authenticated by a botanist from the Forest Herbarium of the Federal College of Forestry in Jos, Plateau State, with a voucher number of FHJ082022.

### *Test Organisms*

The test organisms were isolated from urine and wound samples collected from General hospital and Kwararafa Clinic all in Wukari.

### *Preparation of Plant Samples and Extractions*

The method that was used was adopted from Temitope *et al*. (2016) with some modifications. the plant material (bark) was collected, washed thoroughly with tap water, pulverized using a mortar and pestle, and air dried at room temperature for one week to ensure that the sample lost most of their moisture content. After which, the sample was pounded to a fine powder, and 400 g was weighed and soaked in containers containing 1000 ml of Ethanol and water. The mixture was allowed to stay for three days to check for fermentation, which did not occur. After which, they were filtered using Muslin cloth, and the filtrates were evaporated to dryness. The crude extracts were reconstituted with 50% dimethylsulfoxide (DMSO), which was used to determine the antimicrobial activity against the clinical isolates.

### *Standardization of the Test Organisms*

The inoculums were standardized using 0.5 McFarland standards. A loopful of the confirmed test isolates was picked using a sterile wire loop and emulsified into 2 ml of sterile normal saline to match the 0.5 McFarland Standard as described by Magashi and Abdulmalik (2018).

### *Antimicrobial Screening/Testing*

The sensitivity tests were carried out using the agar-well diffusion method described by Daniels *et al*. (2013). 25 milliliters (ml) of molten Muller-Hinton agar were poured into each sterile Petri dish and allowed to set and gel. The Petri dishes were inverted and incubated overnight to check for sterility. Sterile cotton swabs were dipped into the standardize inoculums. The swabs were used to swab the surface of the set of agar plates. A cork borer of 7 millimeters (mm) was used to bore holes equidistant from each other on the agar. The reconstituted Extracts of 200, 100, 50 and 25 milligrams per ml (mg/ml) was used to fill the bored holes, respectively. A hole was created using the same cork borer at the center of the plate, and 50% dimethyl sulfoxide was filled in, which served as the control of the experiment. The plates were allowed to stand for 30 minutes to 1 hour so that the extracts could percolate the medium, and thereafter they were incubated at 37 °C for 24 hours, after which the plates were observed for a zone of inhibition.

### *Determination of Minimum Inhibitory Concentration (MIC)*

The Minimum inhibitory concentration (MIC) assay was determined by the agar diffusion method as described by Coker *et al*. (2020). Two milliliters (2ml) of the extract at different concentrations (50, 40, 30 and 20 mg/ml) were added to 18 ml of pre-sterilized molten Muller-Hinton agar at a temperature of 45 °C. The medium was poured into sterile Petri dishes and allowed to set and gel. The surface of the medium was inoculated with the standardized inoculum. The plates were later incubated in an incubator at 37°C for 24 hours. The lowest concentration that prevents/inhibits bacterial growth was taken as the MIC. Agar plates without extract were used as negative controls.

### *Determination of Minimum Bactericidal Concentration (MBC)*

Sterile Mueller-Hinton agar plates were separately inoculated with cultures from each of the MIC culture plates that showed no evidence of growth. The plates were further incubated at 37 °C for 24 hours to determine the Minimum Bactericidal Concentration (MBC), which is the highest dilution that yields no single bacterial colony on the medium (Magashi and Abdulmalik, 2018).

### *Statistical analysis*

The data obtained were analyzed using Variance (ANOVA) and were presented as mean ± standard deviation using statistical package for social sciences (SPSS) version 16.0.

## Results

The antimicrobial activity of ethanol bark extract of *D. oliveri* was presented in Table 1. The result shows that the extract is active against *Staphylococcus aureus* SIRST56 and *Bacillus sp* B-3-35.

Table 2 presented the antimicrobial activity of aqueous bark extract of *D. oliveri* showing zones of inhibition. The result shows that the extract is active against *Staphylococcus aureus* SIRST56 and *Bacillus sp* B-3-35.

The Minimum Inhibitory Concentration (MIC) of the ethanol bark extract of *D. oliveri* was presented in Table 3. The result shows the least concentration that inhibit the growth of the test organisms.

Table 4 present the Minimum Inhibitory Concentration of the aqueous bark extract of *D. oliveri* against the test organisms.

Table 5 present the Minimum Bactericidal Concentration (MBC) of the stem bark extract of *D. oliveri*. The result shows the least concentration that yield no bacterial growth.

**Table 1:** The antimicrobial activity of ethanol bark extract of *D. oliveri* showing zones of inhibition in mm.

| | CONCENTRATIONS (mg/mL) | | | | | |
|---|---|---|---|---|---|---|
| ORGANISMS | 200 | 100 | 50 | 25 | Negative control | Positive control |
| *Staphylococcus aureus* SIRST56 | 14±0.00 | 13±0.00 | 13±0.00 | 12±0.00 | 0±0.00 | 20±0.00 |
| *Bacillus sp* B-3-35 | 17±0.00 | 16±0.00 | 14±0.00 | 11±0.00 | 0±0.00 | 16±0.00 |
| *Planococcus glaciei* CHR43 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 18±0.00 |

KEY: The values are Mean ± Standard deviation of the zone of inhibition measured in millimeter (mm). Negative control = 50% Dimethyl sulphuroxide, Positive control = Ciprofloxacin, mg/mL = milligram per millilitre

**Table 2:** The antimicrobial activity of aqueous bark extract of *D. oliveri* showing zone of inhibition in mm.

| | CONCENTRATIONS (mg/mL) | | | | | |
|---|---|---|---|---|---|---|
| ORGANISMS | 200 | 100 | 50 | 25 | Negative control | Positive control |
| *Staphylococcus aureus* SIRST56 | 14±0.00 | 15±0.00 | 14±0.00 | 0±0.00 | 0±0.00 | 20±0.00 |
| *Bacillus* sp B-3-35 | 12±0.00 | 11±0.00 | 11±0.00 | 0±0.00 | 0±0.00 | 16±0.00 |
| *Planococcus glaciei* CHR43 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 | 18±0.00 |

KEY; The values are Mean ± Standard deviation of the zone of inhibition measured in millimeter (mm). Negative control = 50% Dimethyl sulphuroxide, Positive control = Ciprofloxacin mg/mL = milligram per millilitre

**Table 3:** the Minimum Inhibitory Concentration of the ethanol bark extract of *D. oliveri*.

| | CONCENTRATIONS (mg/mL) | | |
|---|---|---|---|
| ORGANISMS | 25 | 20 | 15 |
| *Staphylococcus aureus* SIRST56 | - | + | + |
| *Bacillus* sp B-3-35 | - | + | + |
| *Planococcus glaciei* CHR43 | + | + | + |

KEYS
**(+): N**o Inhibition, (-)**:** Inhibition, mg/mL = milligram per millilitre

Table 4: the Minimum Inhibitory Concentration of the aqueous bark extract of *D. oliveri*.

| | CONCENTRATIONS (mg/mL) | | |
|---|---|---|---|
| ORGANISMS | 50 | 40 | 30 |
| *Staphylococcus aureus* SIRST56 | - | + | + |
| *Bacillus sp* B-3-35 | - | + | + |
| *Planococcus glaciei* CHR43 | + | + | + |

KEY
**(+): N**o Inhibition, (-): Inhibition, mg/mL = milligram per millilitre

**Table 5:** the Minimum Bactericidal Concentration (MBC) of the stem bark extract of *D. oliveri*.

| ORGANISMS | Stem bark extracts (mg/mL) ethanol | water |
|---|---|---|
| *Staphylococcus aureus* SIRST56 | 25 | 50 |
| *Bacillus* sp B-3-35 | 25 | 50 |
| *Planococcus glaciei* CHR43 | - | - |

KEY
**(-): N**o Inhibition (growth), mg/mL = milligram per millilitre

**Discussion**

Medicinal plants have served as the models for many clinically used antibiotics, and are now being reassessed as sources of antimicrobial agents. However, most of the uses have not been scientifically proven. It is therefore, necessary to scientifically evaluate the activity of plants before considering them as useful antimicrobial agents. The result of the antimicrobial screening showed that the tested strains were sensitive to both root and stem bark extracts of *Daniella oliveri*, as reported by Temitope *et al*. (2016). The antimicrobial activity of ethanol bark extract was tested against three clinical isolates as presented in table 1 below.

The result shows that *Staphylococcus aureus* SIRST56 and *Bacillus sp* B-3-35 are sensitive to the extract with highest zones of inhibition recorded at 200 mg/mL, 14mm and 17mm respectively. while *Planococcus glaciei* CHR43 shows no activity. This result agrees with the work of Temitope *et al*. (2016) which reported that *Staphylococcus aureus* and *Bacillus sp* are sensitive to *Daniella oliveri* bark extract. The result shows that as the concentration of the extract increases, the activity of the extract also increases and vice versa. The result also revealed ethanol to be a better solvent for extraction. At 25 mg/mL concentration there was still inhibition which shows that ethanol is a very good extraction solvent. The high antimicrobial properties of the ethanol plant extract are not surprising because previous studies have reported ethanol to be a better solvent for extraction (Daniels *et al*., 2013).

The result of the aqueous bark extract (Table 2) also shows that the *Staphylococcus aureus* SIRST56 and *Bacillus* sp B-3-35 are sensitive to the extract and *Planococcus glaciei* CHR43 is not. At 200 mg/mL concentration, the activity of the extract was measured to 14 mm and 12 mm for *Staphylococcus aureus* SIRST56 and *Bacillus* sp B-3-35 respectively. As the concentration decreases, the activity decreases. However, at 100 mg/mL concentration, *Staphylococcus aureus* SIRST56 exhibit higher activity (15 mm) than 200 mg/mL. this could be due to the fact that lower concentration (100 mg/mL) can easily penetrates the medium. The results also shows that water is also a good extraction solvent but not as ethanol. *Staphylococcus aureus* shows higher activity than *Bacillus* sp.

The results of the Minimum Inhibitory Concentration (MIC) for the ethanol stem bark is shown in table 3. The MIC was determined to be 25 mg/mL concentration for both *Staphylococcus aureus* and *Bacillus* sp. respectively for the ethanol extract, while the MIC for the aqueous stem bark was determined to be 50 mg/mL concentration for both *Staphylococcus aureus* and *Bacillus* sp. respectively. This results is in accordance with the work of Nwuche and Eze (2009).

The result of the Minimum Bactericidal Concentration (MBC) is presented in table 4. The result shows that the MBC for the ethanol is also at 25 mg/mL concentration and 50 mg/mL for the aqueous for both *Staphylococcus aureus* and *Bacillus* sp., which agrees with Nwuche and Eze (2009). This shows that the extracts are bactericidal in nature.

**Conclusions**

The present study has shown that the stem bark of *D. oliveri* could contain a wide range of phytochemicals which has been attributed to the antimicrobial property of the plants. The present study also reveals that both the ethanolic and aqueous extracts of the stem/bark exhibited antimicrobial activity against the test organisms at various concentration. Therefore, the stem bark of *Daniella oliveri* can be used as an alternatives cure for infections associated with the test organisms.

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