

Antimicrobial Activity of Extracts of *Daniella oliveri* Stem Bark on Selected Clinical Isolates

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Abstract

The utilization of medicinal plants in therapy has gained popularity due to increased trust in herbal medicine, attributed to properties such as antioxidant, antimicrobial, and antipyretic effects conferred by phytochemicals present in them. This study aimed to evaluate the antibacterial activity of *Daniella oliveri* stem bark against clinical isolates. 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*, *Bacillus* spp., *Klebsiella pneumonia* and *Planococcus glaciei* isolates. The ethanol extract of the bark was inhibitory against *Staphylococcus aureus*, *Bacillus* spp. and *Klebsiella pneumonia* with the highest zone of inhibition of 14 ± 0.00 mm, 17 ± 10.6 mm and 25 ± 10.5 mm respectively, while the aqueous extracts of bark was active against *Staphylococcus aureus*, *Bacillus* spp. and *Klebsiella pneumonia* with zone of inhibition of 14 ± 0.00 mm, 13 ± 10.4 mm and 25 ± 0.00 mm respectively. MICs ranged from 50 mg/mL to 25 mg/mL for ethanol and 100 mg/mL to 25 mg/mL for aqueous extracts, with no activity observed at 200 mg/mL. MBC results were observed at 100 mg/mL and 200 mg/mL for ethanol and aqueous extracts respectively. The

study concluded that *Daniella oliveri* possesses significant antibacterial activities, supporting its traditional use. Further research is warranted to purify and utilize the active inhibitory substance as an alternative treatment for infections associated with the test organisms.

Keywords: Antimicrobial, *Daniella oliveri*, Stem bark, *Staphylococcus aureus*, *Bacillus* spp., *Klebsiella pneumonia* and *Planococcus glaciei*

INTRODUCTION

According to Tittikpina *et al.* [1], the World Health Organization (WHO) estimated in 2010 that over 80% of people in the developing world, particularly in Africa, rely on traditional medicine for their healthcare needs. Africa, known for its rich biodiversity, is a primary source of herbal and medicinal plants used in traditional medicine. Medicinal plants are those utilized for disease treatment or pain relief, often due to the presence of phytochemicals with therapeutic properties. Many of these plants contain antimicrobial compounds effective against bacterial and fungal infections, particularly prevalent in developing countries such as Nigeria, other African nations, and India [2]. These medicinal plants are considered valuable natural resources globally, including in Nigeria, serving as sources for both traditional and modern medicines [3]. Akinyemi *et al.* [4] reported over fifteen years ago that approximately 80% of the rural population relies on herbal medicine as their primary form of healthcare.

Recognition of medicinal plants in therapy has become increasingly prevalent, driven by various factors, including growing confidence in herbal medicine. This trust in medicinal plants is often attributed to their antioxidant, antimicrobial, and antipyretic effects, which stem from the phytochemicals they contain [5]. This makes medicinal plants valuable in combating rapidly emerging resistant infections. Indeed, several studies in Africa have reported the emergence of multi-drug resistant organisms over the past decade, leading to the widespread occurrence of antimicrobial resistance to commonly used antibiotics [2].

Daniella is a genus of legumes within the Fabaceae family, characterized by trees that can grow up to heights of 30 to 40 meters [6]. Commonly known as African copaiba balsam or Ilorin balsam, this plant plays a significant role in agroforestry systems and soil and water conservation efforts. It belongs to fire-resistant savanna species and is widely distributed from Senegal to South Sudan and Uganda, mainly in the Savannah region. In Nigeria, it

goes by various local names such as "iya" in Yoruba, "maje" in Hausa, and "abwa" in Igbo. In northern Nigeria, the bark and resin of *Daniella oliveri* are utilized as a mosquito repellent, while bark extracts are employed in Burkina Faso for treating gastrointestinal parasites in small ruminants. Additionally, *Daniella oliveri* contributes to forest enrichment and serves as a timber resource [6].

Daniellia oliveri, a deciduous tree with a flat-topped crown, produces pea-shaped flowers and possesses a wide range of medicinal properties. Studies have reported its effectiveness in treating malaria, diarrhea, inflammation, pain, fever, and microbial infections. It has been traditionally used to treat gastrointestinal diseases, prevent miscarriage during pregnancy, induce sleep, and relieve rheumatism pains. Additionally, a combination of *Sarcocephalus latifolius* and *Daniella oliveri* roots aqueous extract has shown efficacy in regulating fasting blood glucose levels. With the rise of antibiotic-resistant infections and diseases, there is a growing need for alternative sources of antimicrobials. The present study aims to evaluate the antibacterial activity of *Daniella oliveri* stem bark against clinical isolates, providing insight into its potential as a natural antimicrobial agent.

MATERIALS AND METHODS

Study Area and Population

This study was carried out in Federal University Wukari, Nigeria. Wukari Metropolis is a large town which is the Headquarters of Wukari Local Government Area of Taraba State. The River Donga and River Benue passes through this area. The Local Government Area shares boundary with Benue and Nasarawa state to the south and west respectively. Geographically it is between latitude 9053'42" North and longitude 9047'59" East [7, 8]. It is one of the major towns in Taraba state and has an area of 4,308km² and a population of 241,546 in the 2006 census. The major spoken languages include, Jukun, Hausa, Fulani and Tiv. The predominant occupations of the people are agriculture, commerce and civil service [9].

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. The plant was authenticated by a botanist from

the Department of Biological Sciences, Federal University Wukari Nigeria. It was rinsed with tap water to remove some adhered salts, organism or dust [10].

Test Organisms

The bacteria isolates employed in this study were gotten from the Department of Microbiology, at University of Wukari, Nigeria from urine and wound samples collected from University Clinic Wukari.

Preparation of *Daniella oliveri* Stem Bark

The plant material (bark of the stem) was collected, washed clean with tap water, removed/peeled from the stem and then air-dried at room temperature under the shade for one week to ensure that the sample lost most of their moisture content until constant weight was obtained. The dried stem was pulverized using a mortar and pestle and then kept in a clean bottle [10].

Preparation of Extraction

A fine powder of 400 g of sample was weighed and soaked in a conical flask contain ethanol and distilled water of 1000 ml each and covered. The contents were mixture well, stirred intermittently for three days and then filtered using sterile Whatman No. 1 filter paper, 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 isolates. The method used was adopted from Temitope *et al.* [11] with some modifications, and the filtrates were evaporated using a rotary evaporator. The crude extracts were dried and stored at 4°C until screened for antibacterial activity [10].

Preparation of Extract Concentrations

The extracts were dissolved in Dimethyl Sulfoxide (DMSO). Stock solution of extracts were prepared by weighting different concentration of extract and dissolved in 10ml of (DMSO) in a container to make 100% concentration, and a consecutive dilution were made to obtain various concentration [12]

Standardization of the Test Organisms

The inoculums were standardized using 0.5 McFarland standards. The organisms were standardized using the McFarland spectrophotometer at wavelength of 625.0. 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 [13].

Determination of Antimicrobial Screening/Testing of Stem Bark

The sensitivity tests followed the agar-well diffusion method outlined by Daniels *et al.* [14]. In each sterile petri dish, 25 milliliters (ml) of molten Muller-Hinton agar were poured and left to solidify. After inverting the dishes and overnight incubation to ensure sterility, sterile cotton swabs were dipped into standardized inoculums and used to coat the agar plates' surfaces. Equidistant holes were then created on the agar using a 6-millimeter cork borer. Subsequently, reconstituted extracts of 200, 100, 50 and 25 milligrams per ml (mg/ml) were respectively applied to the bored holes. Additionally, a hole was made at the plate's center using the same cork borer, and 50% dimethyl sulfoxide was filled in as the experiment's control. After allowing 30 minutes to 1 hour for the extracts to percolate the medium, the plates were incubated at 37°C for 24 hours. Following incubation, the plates were examined for the presence of a zone of inhibition

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) assay was conducted using the agar diffusion method outlined by Coker *et al.* [15]. Two milliliters (2 ml) of the extract at varying concentrations (200, 100, 50 and 25 mg/ml) were mixed with 18 ml of pre-sterilized molten Muller-Hinton agar at 45°C. This mixture was then poured into sterile Petri dishes and allowed to solidify. Afterward, the surface of the agar was inoculated with the standardized inoculum. Subsequently, the plates were placed in an incubator set at 37°C for 24 hours. The MIC was determined as the lowest concentration of the extract that prevented or inhibited bacterial growth. Negative control plates without the extract were also included for comparison.

Determination of Minimum Bactericidal Concentration (MBC)

Sterile Mueller-Hinton agar plates were inoculated separately with cultures from each of the MIC culture plates that exhibited no growth. These plates were then incubated at 37°C for 24 hours to ascertain the Minimum Bactericidal Concentration (MBC). The MBC is defined as the highest dilution that results in the absence of any bacterial colony on the medium [13].

Statistical analysis

The data were analyzed using SPSS software (version 10.0 for Windows, SPSS Inc, Chicago, IL) and presented as mean \pm standard error of the mean (SEM). Data were collected in triplicate, and the mean standard deviation was calculated following the method described by Steel and Torrie [12].

RESULTS

Result Summary

The antimicrobial activity of ethanol bark extract of *Daniella oliveri* was presented in Table 1. The result shows that the extract is active against *Staphylococcus aureus*, *Bacillus* spp., *Klebsiella pneumoniae*, while the high yield was obtained from ethanol extract.

Table 2 presented the antimicrobial activity of aqueous bark extract of *Daniella oliveri* showing zones of inhibition. The result shows that the extract is active against all the isolates except *Planococcus glaciei*.

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

Table 4 present the Minimum Bactericidal Concentration (MBC) of the stem bark extract of *Daniella oliveri*. The result shows the least concentration that yields no bacterial growth.

Table 1: The Antimicrobial Activity of Ethanol Bark Extract of *Daniella oliveri* Showing Zones of Inhibition in mm.

Organisms	Concentrations (mg/ml)					Negative Control	Positive Control
	200	100	50	25			
<i>Staphylococcus aureus</i>	14 \pm 0.00	13 \pm 10.6	13 \pm 0.00	12 \pm 10.4		0 \pm 0.00	22 \pm 0.00
<i>Bacillus</i> spp.	17 \pm 10.6	16 \pm 10.4	14 \pm 0.00	12 \pm 0.00		0 \pm 0.00	18 \pm 10.5
<i>Klebsiella pneumoniae</i>	25 \pm 10.5	23 \pm 10.5	20 \pm 10.6	17 \pm 0.00		0. \pm 0.00	26 \pm 0.00
<i>Planococcus glaciei</i>	0 \pm 0.00	0 \pm 0.00	0 \pm 0.00	0 \pm 0.00		0 \pm 0.00	18 \pm 10.5

Key: The values are mean of three determination \pm SD of the zone of inhibition measured in millimeter (mm). Negative control = 50% Dimethyl sulphuroxide, Positive control = Antibiotic disc of Ciprofloxacin, mg/mL = milligram per millilitre

Table 2: The Antimicrobial Activity of Aqueous Bark Extract of *Daniella oliveri* Showing Zone of Inhibition in mm.

Organisms	Concentrations (mg/ml)				Negative Control	Positive Control
	200	100	50	25		
<i>Staphylococcus aureus</i>	14 \pm 0.00	15 \pm 0.00	14 \pm 10.0	0 \pm 0.00	0 \pm 0.00	22 \pm 0.00
<i>Bacillus</i> spp.	13 \pm 10.4	11 \pm 10.4	11 \pm 0.00	0 \pm 0.00	0 \pm 0.00	18 \pm 10.5
<i>Klebsiella pneumonia</i>	25 \pm 0.00	20 \pm 10.4	21 \pm 10.6	0 \pm 0.00	0. \pm 0.00	26 \pm 0.00
<i>Planococcus glaciei</i>	0 \pm 0.00	0 \pm 0.00	0 \pm 0.00	0 \pm 0.00	0 \pm 0.00	20 \pm 10.5

Key: The values are mean of three determination \pm SD of the zone of inhibition measured in millimeter (mm). Negative control = 50% Dimethyl sulphuroxide, Positive control = Antibiotic disc of Ciprofloxacin, mg/mL = milligram per millilitre

Table 3: The Minimum Inhibitory Concentration of Both Ethanol and Aqueous Bark Extract of *Daniella oliveri*.

Organisms	Concentrations (mg/ml)							
	Ethanol				Aqueous			
	200	100	50	25	200	100	50	25
<i>Staphylococcus aureus</i>	-	-	+	+	-	+	+	+
<i>Bacillus</i> spp.	-	-	+	+	-	+	+	+
<i>Klebsiella pneumonia</i>	-	-	+	+	-	+	+	+
<i>Planococcus glaciei</i>	-	-	-	-	-	-	-	-

Key: + = Indicates growth, - = Indicates no growth, mg/mL = milligram per millilitre

Table 4: The Minimum Bactericidal Concentration (MBC) of the Stem Bark Extract of *Daniella oliveri*

Organism	Concentrations (mg/ml)	
	Ethanol	Aqueous
<i>Staphylococcus aureus</i>	100	200
<i>Bacillus</i> spp.	100	200
<i>Klebsiella pneumonia</i>	100	200
<i>Planococcus glaciei</i>	-	-

Key: - = No Inhibition (growth), mg/mL = milligram per millilitre

DISCUSSION

Medicinal plants have long been studied as potential sources of antibiotics and are currently being re-evaluated for their antimicrobial properties. However, many of their purported uses lack scientific validation. Therefore, it is essential to scientifically assess the effectiveness of plants before regarding them as viable antimicrobial agents. According to a study by Temitope *et al.* [11], the antimicrobial screening results revealed sensitivity of the tested strains to both root and stem bark extracts of *Daniella oliveri*.

Medicinal plants play crucial roles as both traditional and modern medicine sources, as highlighted by the World Health Organization (WHO) [16] in 2019. In today's context, approximately 80% of individuals residing in rural areas rely on medicinal plants as herbal alternatives for treating diverse ailments. Herbal treatments primarily involve utilizing plant extracts and other plant-derived products, as noted by Djordjevic [17]. It's therefore plausible that these plants contain specific bioactive (phytochemical) compounds, which might be accountable for alleviating various diseases, as suggested by Bamisaye *et al.* [3].

The choice of plants was guided by an ethnobotanical survey conducted by various researchers, indicating the antimicrobial potential of the selected plant. According to findings reported by Coker *et al.* [2], all parts of *Daniella oliveri* exhibit biological activities. This study specifically aimed to investigate the antimicrobial activity of extracts derived from the stem bark of *Daniella Oliveri* against selected clinical isolates. The results of the

antimicrobial activity assessment revealed that approximately 75% of the tested isolates demonstrated sensitivity to the extract.

The findings indicate that *Staphylococcus aureus*, *Bacillus* spp., and *Klebsiella pneumoniae* display sensitivity to the extract, with the highest zones of inhibition recorded at 200 mg/mL, measuring $14\pm 0.00\text{mm}$, $17\pm 10.6\text{mm}$, and $25\pm 10.5\text{mm}$ respectively. However, *Planococcus glaciei* showed no activity against the extract. This aligns with the findings reported by Temitope *et al.* [11], which demonstrated the sensitivity of *Staphylococcus aureus* and *Bacillus* spp. to *Daniella oliveri* bark extract, corroborating the results of the current study. The results also indicate that as the concentration of the extract increases, its activity likewise increases, and vice versa. Furthermore, the study revealed that ethanol serves as a superior solvent for extraction. Even at a concentration of 50 mg/mL, inhibition was still observed, indicating the efficacy of ethanol as an extraction solvent. The notable antimicrobial properties of the ethanol plant extract are consistent with previous studies that have identified ethanol as an effective solvent for extraction, as reported by Daniels *et al.* [14].

The findings of Ahmadu and colleagues are consistent with the current discovery. Their study examined the antibacterial activity of the aqueous ethanolic extract of *Daniella oliveri* leaves using the agar well diffusion assay. Results from their research demonstrated that the methanolic raw extract of the leaves exhibited greater activity against *S. aureus* compared to *P. aeruginosa* and *E. coli*, across concentrations ranging from 5 mg/mL to 50 mg/mL [18, 19]. Similarly, other researchers have investigated the activity of both leaves and trunk barks using a similar methodology. For instance, in one such study, the ethanolic extract of *Daniella oliveri* leaves and trunk barks inhibited bacterial growth at a concentration of 60 mg/mL [1]. These collective findings further support the antimicrobial potential of *Daniella oliveri* across various parts of the plant and highlight its effectiveness against a range of bacterial strains.

The observation that ethanol yielded the highest extract suggests that the plant contains relatively more polar constituents. This finding aligns with the results reported by Coker *et al.* [15], who also noted higher yields of ethanol and methanol extracts from *Daniella oliveri* compared to those of n-hexane, dichloromethane, and ethyl acetate. This consistency indicates that polar solvents such as ethanol and methanol are more effective in extracting compounds from *Daniella oliveri*, likely due to the presence of polar phytochemicals in the plant material.

The results of the aqueous bark extract mirrored those of the ethanol extract. At a concentration of 200 mg/mL, the activity of the extract measured 14 ± 0.00 mm, 13 ± 10.4 mm, and 25 ± 0.00 against *Staphylococcus aureus*, *Bacillus* spp., and *Klebsiella pneumoniae* respectively. As the concentration decreased, so did the activity of the extract. Additionally, the results indicate that water is also an effective extraction solvent, although not as potent as ethanol. Interestingly, *Klebsiella pneumoniae* exhibited greater sensitivity compared to *Staphylococcus aureus* and *Bacillus* spp. This finding contrasts with the study by Bamisaye *et al.* [3], which reported that the aqueous extract did not exhibit a bacteriostatic effect on all tested organisms at the concentrations used. The observed antibacterial potentiality in this study may be attributed to the presence of glycosides and steroid compounds in the ethanolic extract, which were not detected in the aqueous extract counterpart.

Researchers have commonly utilized the concept of Minimum Inhibitory Concentration (MIC) values to assess the effectiveness of antibacterial agents. MIC represents the lowest concentration, or highest dilution, of the extract that prevents visible bacterial growth (absence of turbidity) when compared to the control tube. The pattern of MIC results obtained on the test organisms suggests that the extracts exhibit bacteriostatic activities, as reported by Bamisaye *et al.* [3].

The results of the MIC for the ethanol stem bark are presented in Table 3. The MIC was determined to be 50 mg/mL concentration for both isolates, with the exception of *Planococcus glaciei* for the ethanol extract. Similarly, the MIC for the aqueous stem bark was determined to be 100 mg/mL concentration for the clinical isolates, with the exception of *Planococcus glaciei*.

The results of the Minimum Bactericidal Concentration (MBC) are displayed in Table 4. The findings indicate that both the ethanol and aqueous extracts at concentrations of 100 mg/mL and 200 mg/mL exhibited bactericidal effects against *Staphylococcus aureus*, *Bacillus* spp., and *Klebsiella pneumoniae*. This suggests that the extracts possess bactericidal properties, effectively eliminating these bacterial strains.

The aqueous and ethanolic stem bark extracts of *Daniella oliveri* hold promise in alleviating health issues. Saponins, identified as phytochemical constituents of *Daniella oliveri*, exhibit antibacterial and antiviral properties when ingested by humans, as highlighted by Bamisaye *et al.* [3]. This action is attributed to their ability to enhance the immune system and provide protection against viruses and bacteria, as reported by Bertero *et al.* [20]. Consequently, the

aqueous and ethanolic stem bark extracts of *Daniella oliveri* may serve as valuable treatments for bacterial and viral diseases.

However, it's essential to note that certain saponins can be toxic to cold-blooded organisms and insects at specific concentrations. Therefore, caution should be exercised when consuming the aqueous and ethanolic stem bark extracts of *Daniellia oliveri*, especially at high concentrations, as mentioned by Bamisaye *et al.* [3].

CONCLUSION

The antibacterial assay of this study revealed that both the ethanol and aqueous extracts of the stem bark of *Daniella oliveri* showed bacteriostatic effects and exhibited antimicrobial activity on *Staphylococcus aureus*, *Bacillus* spp. and *Klebsiella pneumonia* in concentrations (200, 100 and 50 mg/mL) used only. These observed bacteriostatic activities on the organisms are high concentration dependent. 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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