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Sclerocary Birrea Stem Bark Extract: A Potential Source of Plant Based Antibacterial Agents

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ABSTRACT

This research was carried out to access the phytochemical constituents and antibacterial activity of *Sclerocarya birrea* stem bark found in Sokoto region, Nigeria. The plant material was collected, dried, pulverized, and extracted by maceration using ethanol. Standard qualitative phytochemical methods were employed to identify the bioactive constituents, while antibacterial activity of the extract was assessed against selected organisms including *Salmonella typhi*, *Escherichia coli, Bacillus subtilis* and *Bacillus cereus* using agar well diffusion method. The phytochemical screening revealed the presence of important secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycosides and anthraquinones. The antibacterial activity test indicated that the extract exhibited inhibitory effects on the test organism with zones of inhibition ranging between 0.02-2.60 mm. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) confirmed the sensitivity of the organisms to the plant extract, though the standard antibiotic exhibited stronger activity. The findings of this work provide preliminary scientific support for the use of *Sclerocarya birrea* stem bark in the treatment of diseases. It further shows that the stem bark contains bioactive compounds which could serve as a potential source of plant based antibacterial agents.

Keyword: Phytochemicals, Antibacteria, Stem, Bark, Bacteria

INTRODUCTION

Plants have been described as nature's gift to humanity because of their immense importance in nutrition, culture, and medicine. Since ancient times, plants have been the cornerstone of healthcare delivery systems across different civilizations, and they continue to provide essential remedies to populations worldwide. The World Health Organization (WHO) estimates that about 80% of the global population relies on plant-based traditional medicine for their primary healthcare needs, particularly in developing countries where access to conventional drugs is limited [1]. Medicinal plants are valued because they are affordable, accessible, and culturally acceptable, making them indispensable in the prevention and treatment of numerous diseases [2]. The use of medicinal plants is closely linked with their chemical constituents, known as phytochemicals. These secondary metabolites are not directly involved in plant growth and development but play vital roles in plant defense mechanisms against pathogens and environmental stress [3]. Phytochemicals such as alkaloids, tannins, flavonoids, terpenoids, phenolics, and saponins have been documented to possess various pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and antiviral effects [4]. It is these bioactive compounds that form the scientific basis for the use of plants in traditional medicine, and they also serve as lead structures for the development of modern drugs [5].

Antimicrobial resistance remains a significant cause of mortality and increase in health care cost worldwide, particularly in sub-Saharan Africa where healthcare systems face numerous challenges. In response to this, *Sclerocarya birrea* (marula) tree belonging to the family *Anacardiaceae* which can grow up to 15–20 meters in height, with a wide crown and a grey, fissured bark [6-7]. The stem bark is thick, rough, greyish in color and it used traditionally for the treatment of like diarrhea, malaria, and skin infections.

Plate 1: The *Sclerocarya birrea* tree showing stem bark and fruits

MATERIALS AND METHODS

All chemicals and solvents were obtained from LobaChemie and used as received. Mular Hilton nutrient agar and broth were used for the antibacterial analysis. The test organisms used in this study were standard laboratory strains obtained from the Department of Microbiology, Sokoto State University (or an accredited culture collection). The organisms included *Salmonella typhi* (Gram-negative), *Escherichia coli* (Gram-negative), *Bacillus subtilis* (Gram-positive) and *Bacillus cereus* (Gram-positive).

Sample collection

Fresh stem bark of *Sclerocarya birrea* was collected from matured trees during dry season within Sokoto metropolis. The collected stem bark samples were placed in clean paper bags and transported to the laboratory for further processing.

Sample treatment

The collected stem bark samples were washed with tap water to remove adhering dirt and epiphytes, and then rinsed with distilled water. The cleaned bark was dried under shade at ambient conditions for 14 days. The dried samples were pulverized to a fine powder using motor and pestle and sieved through a 1 mm mesh to obtain uniform particle size. The powdered material was stored in an airtight polyethylene bag until extraction.

Extraction of *Sclerocarya birrea* Stem Bark

The sample was defatted by maceration using ethanol as solvent. In a typical experiment, the powdered stems bark (176 g) were weighed and placed in a clean (1000 cm^3) conical flask and then ethanol (400 cm^3) was added. The conical flask was closed with glass stopper and the mixture was left for 24 hours with intermittent shaking. After extraction the mixture was filtered through Whatman No. 1 filter paper and the filtrates were concentrated by gentle evaporation in an air-circulating oven at 40–60 °C until all the remaining solvents were removed. The percentage extract yield was calculated as:

$$\% \text{ extract yield} = \frac{\text{Weight of extract}}{\text{Initial weight of sample}}\, x\, 100 \ldots\ldots\ldots\ldots\ldots\ldots (1.0)$$

Qualitative phytochemical screening of extract

Qualitative tests for major phytochemical classes were performed on the crude extracts using standard procedures adapted from Harborne and similar protocols [8].

Antibacterial tests

Antibacterial activity was determined using the agar well diffusion method as described in the standard microbiology texts [8]. The Nutrient Agar plates prepared according to manufacturer's instruction were allowed to solidify for 15 minutes at room temperature and incubated without inoculum for 24 hours at 37 ^{0}C to ensure the sterility of the medium. The Nutrient Agar plates were flooded with 1 ml of the inoculum and the excess was removed using Pasteur pipette. 4-6 wells (cups) of about 6 mm in diameter were cut on each Nutrient Agar plate using a sterile cork borer and the agar plugs were removed using a sterile ampoule file. The extract solution (0.1 mL) was placed in each of the wells and were allowed to settle for two hours at room temperature and then incubated for 24 hours at 37 ^{0}C. The inhibition zone was observed and then recorded in millimeters using a transparent ruler. Standard antibiotics were used.

Minimum inhibitory concentration (MIC)

The MIC of each extract was determined by the broth dilution method in test tubes as described by standard protocols [9]. Extracts were serially diluted in nutrient broth to obtain a range of concentrations, inoculated with standardized bacterial inoculum and incubated at 37 °C for 18–24 hours. The MIC was recorded as the lowest concentration of extract showing no visible turbidity.

Minimum bactericidal concentration (MBC)

To determine the MBC, aliquots from tubes that showed no visible growth in the MIC assay were plated onto fresh nutrient agar plates and incubated at 37 °C for 24 hours. The MBC was recorded as the lowest concentration of extract that yielded no bacterial colonies on sub-culture, indicating bactericidal activity.

RESULTS AND DISCUSSION

Extraction of *Sclerocarya birrea* Stem Bark

The mass of the extract and percentage yield obtained from *Sclerocarya birrea* Stem Bark were given in Table 1. The extract has a mass of 32.25 g with percentage yield of 18.32 % as shown in the table below.

Table 1: Percentage Yield (%)

Extract	Original mass (g)	Mass of extract (g)	Percentage yield (%)
Ethanol	176.00	32.25	18.32

Qualitative Phytochemical Screening of *Sclerocarya birrea* Stem Bark Extract

The results of the qualitative phytochemical screening of the extract obtained were shown in Table 2. Saponins, flavonoid, tannins, alkaloid, steroids, cardiac glycosides and anthraquinones were all detected.

Table 2: Qualitative Phytochemical Screening of *Sclerocarya birrea* Stem Bark Extract

Phytochemicals	Ethanol
Saponins	
(a) Froth's test	+
Flavonoids	
(a) Alkaline test	+
(b) Ferric chloride test	+
(c) Shinoda's Test	+
Tanins	
(a) Ferric chloride test	+
(b) Lead acetate test	+
Alkaloid	
(a) Mayer's test	+
(b)Wagner's reagent	+
(c) Hager's test	+
Steroids and Inter-terpenoids test	
(a) Salkowski's test	+
(b) Libermann-Richard's test	+
Anthraquinone	
(a) Borntrager's test	+
Cardiac glycoside's test	+

Note: A single plus sign (+) indicates presence of the compound, while negative signs (-) suggest the compound is absent.

The presence of alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycosides, and *anthraquinone* demonstrates that *Sclerocarya birrea* is rich in phytochemicals with known pharmacological activities.

Antibacterial Activity of *Sclerocarya birrea* Stem Bark Extract

The antibacterial activity of the extract was tested against four pathogenic bacterial organisms: *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, and *Bacillus cereus*. The results obtained from the antibacterial test were shown in table 3a – c.

Table 3a: Zone of Inhibition (mm) of *Sclerocarya birrea* Stem Bark Extract against Test Organisms

Concentration (mg/ml)	**Zone of Inhibition (mm)**			
	Salmonella typhi	*Escherichia coli*	*Bacillus subtilis*	*Bacillus cereus*
25	1.35	1.40	0.82	0.86
50	1.40	2.05	0.02	0.84
75	2.15	2.50	1.06	1.03
100	2.25	2.60	1.46	1.13

Table 3b: Zone of Inhibition (mm) of standard antibiotic (Ceftriaxone)

Antibiotic Conc. (mg/mL)	**Zone of Inhibition (mm)**			
	S. typhi	***E. coli***	***B. subtilis***	***B. cereus***
Ceftriaxone (50 mg/mL)	4.00	4.15	4.10	3.18

Table 3c: MIC and MBC of *Sclerocarya birrea* Stem Bark Extract (mg/mL)

Test Organism	MIC (mg/mL)	MBC (mg/mL)
Salmonella typhi	50	75
Escherichia coli	75	75
Bacillus subtilis	75	75
Bascillus cereus	75	75

Discussion

The results obtained from the solvent extraction of *Sclerocarya birrea* stem bark revealed that the mass of the extract obtained was 32.25 g, with a percentage yield of 18.32 % (Table 1). This indicates that ethanol as a solvent is effective for extracting the bark. It also shows that the stem bark contain appreciable amount of polar compounds as ethanol is a polar solvent. The results for the phytochemical screening of *Sclerocarya birrea* stem bark extract (Table 2) revealed that the plant contains a wide range of secondary metabolites known for their pharmacological activities. The presence of flavonoids and saponins in relatively high concentrations is significant, as these compounds have been reported to exhibit antioxidant and antibacterial activities by disrupting microbial membranes and interfering with nucleic acid synthesis [10]. Alkaloids and tannins are also well documented for their antimicrobial properties, often acting through protein precipitation, enzyme inhibition, and interference with bacterial cell wall synthesis [11]. The antibacterial analysis demonstrated that *Sclerocarya birrea* stem bark extract possesses measurable inhibitory activity that range between 0.02 mm to 2.60 mm against all tested organisms, although the degree of activity varied (Table 3a). The result shows that the extract exhibited greater activity

against Gram-negative organisms (*Escherichia coli* and *Salmonella typhi*) compared to Gram-positive organisms (*Bacillus subtilis* and *Bacillus cereus*). This observation may be attributed to structural differences in bacterial cell walls. Gram-negative bacteria possess an outer membrane that can act as a permeability barrier, but certain phytochemicals such as flavonoids and saponins have shown higher affinity for disrupting Gram-negative bacterial membranes [12].

The MIC and MBC results (Table 3c) further confirm that the extract is both bacteriostatic and bactericidal. *Salmonella typhi* showed higher sensitivity with a MIC of 50 mg/mL, which is of particular relevance considering the prevalence of typhoid fever in Africa. The MBC values indicate that higher extract concentrations (75 mg/mL) were required to completely kill all test organisms, consistent with earlier findings on medicinal plant extracts [13]. These findings are in agreement with previous studies that reported the antibacterial potential of *Sclerocarya birrea* extracts against both Gram-positive and Gram-negative organisms [14, 15]. The presence of multiple phytochemicals acting synergistically may explain the broad-spectrum antibacterial activity observed. Importantly, the results provide scientific evidence supporting the traditional use of *Sclerocarya birrea* stem bark in the management of diarrhea, dysentery, fever, and other diseases.

CONCLUSION

From the findings of this research, it can be concluded that the stem bark of *Sclerocarya birrea* is rich in secondary metabolites, including alkaloids, flavonoids, tannins, saponins, steroids, glycosides and anthrquinones. The antibacterial activity observed in this study further validates the ethnomedicinal use of the plant in the treatment of infectious diseases. The extract showed stronger activity against Gram-negative bacteria compared to Gram-positive bacteria, with *Escherichia coli* and *Salmonella typhi* being the most susceptible organisms. This suggests that the phytochemicals present in *Sclerocarya birrea* stem bark may have specific mechanisms of action more effective against Gram-negative pathogens. Overall, the study demonstrates that *Sclerocarya birrea* stem bark possesses significant antibacterial properties and supports its traditional use as a natural remedy for microbial infections. The results also provide a scientific basis for considering this plant as a potential source of novel antibacterial agents.

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CONFLICTS OF INTEREST

The authors declare that there is no any conflict of interest.

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