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Antibacterial Activity of Sclerocarya Birrea (a. Rich, hochst) (marula) Stem Bark extracts against Clinical Isolates of some Multi Drug Resistant Enteric Bacteria Causing Diarrhea in Children

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Abstract

The recent discovery of novel drugs from medicinal plants implies that vast potential still exist for the production of numerous more novel drugs. The study was aimed to screen for phytochemical and to determine antibacterial activity of aqueous and ethanol extract of *Sclerocarya birrea* (A. Rich, Hochst) stem bark against clinical isolates of some multi drug resistant enteric bacteria causing diarrhea in Children isolated from patients attending Murtala Muhammad Specialist Hospital Kano. Agar well diffusion method was used to determine antibacterial activity of the extract while dilution method was employed to determine the Minimum inhibitory concentration (MIC) of the extract. Preliminary phytochemical screening of the stem bark extract revealed the presence of alkaloids, flavonoids, phenol, terpenoid, anthraquinone, saponin and tannin except Steroid. The leaf has highest percentage of flavonoid with total composition of 8.5% of the extract, followed by alkaloid 6.9%, phenol 5.5%, terpenoid 3.8% Tannin 2.3 %, Anthraquinone and saponin constituting 1.4% and 1.7 % respectively. The overall sensitivity of the isolates to the extract indicated that *E. coli* was the most sensitive isolate with average zone of inhibition of 15.16 mm, followed by *Klebsiella* spp with 13.88 mm, *Salmonella* spp with 13.50 mm while least sensitivity was shown by *Shigella* spp with 12.38 mm. it is concluded that the stem bark of *Sclerocarya birrea* contained bioactive components that possess antibacterial activity.

Keywords: antimicrobial activities; diarrhea; inhibition; phytochemical; *sclerocarya birrea*

Introduction

Plants have been known to provide the basis for traditional treatment for different types of diseases with vast potential as source of new chemotherapeutic agents [1]. Medicinal plants generally contain a number of bioactive constituents, mainly the secondary metabolites such as alkaloids, flavonoids, tannins and phenolic compounds. These phytochemicals have been proven to possess immunomodulatory and antioxidant properties and may be potential natural antibacterial for the treatment of common bacterial infections [2]. Furthermore, different plant parts, plant extracts or plant species can be used in combination to achieve the same goal with great efficacy. In fact, it is thought that herbal remedies have the advantage in combining their active components to obtain synergistic or additive effects which give to the plants an efficiency superior to some of their isolated components [3]. Medicinal plants have great importance in the control of human diseases. The World Health Organization (WHO) has stated that medicinal plants are the richest and the best source for obtaining a variety of therapeutic agents [4]. Medicinal plants constitute credible sources for a huge number of modern antibiotics, several of which are usually based on their traditional folk medicine. *Sclerocarya birrea* (family: Anacardiaceae) is an important food, commercial, cultural and ethnomedicinal plant in Africa. The tree bears edible, aromatic and fleshy fruit that has constituted an essential component of the southern African diet since ancient times [5]. The tree is commonly found in semi-arid, deciduous and savannah regions of sub-Saharan Africa. It grows in wooded grasslands, riverine woodland areas and bush land, and is frequently associated with rocky hills. Its geographical distribution stretches from Gambia in West Africa, across Nigeria and Cameroon in Central Africa, to Ethiopia and Sudan in East Africa [6]. In some African countries, the stems-bark, roots and leaves of *Sclerocarya birrea* are used for an array of human ailments, including: malaria and fevers, diarrhea and dysentery, stomach ailments, headaches, sore eyes, toothache, backache and body pains, infertility, schistosomiasis, constipation, abdominal cramps and some other unspecified gastro-intestinal problems, toothaches and swollen or infected gums, cough, hypertension, arthritis, proctitis, epilepsy, diabetes mellitus, sores, boils, carbuncles, abscesses and certain other bacterial infections [5]. In traditional medicine, the bark of *S. birrea* is the part most frequently used

to treat ailments that are mostly bacteria-related (stomach-aches, diarrhea, wounds and coughs) [7]. The bark of *S. birrea* is crushed and mixed with hot, warm or cold water. The mixture is administered anally or orally. The mixture is drunk three times a day, until diarrhea subsides. Previous Studies: Previous studies have shown that extracts from the stem bark and leaves of S. birrea possess a catalogue of pharmacological activities, including analgesic, antiinflammatory, anti-diabetic and hypoglycaemic, antidiarrheal, antibacterial [8] and insecticidal properties. De Wet *et al*. [9] investigation of antidiarrheal activity of the bark of *Sclerocarya birrea* in rats revealed that the antidiarrheal activity was related to an inhibition of intestinal transit rather than to inhibition of net secretion of fluid and electrolytes provoked by the laxative agents used. In view of this, the study was aimed to determine the antibacterial activity of *S. birrea* stem bark extracts against some enteric bacteria associated with diarrhea in children.

Materials and methods

Study Sites

The study was conducted at Microbiology Department of Murtala Muhammad Specialist Hospital (MMSH) and Laboratory of Microbiology Department of Kano University of Science and Technology Wudil. Kano State is located in the North-western Nigeria, it is coordinated at latitude 11^0 30 N and longitude 8^0 30 E. It share borders with Kaduna State to the South-West, Bauchi State to the South-East, Jigawa State to the East and Katsina State to the North. It has a total area of 20,131km^2 (7,777sqm) and estimated population of 13.4 million [10].

Collection and Identification of *S. birrea* Stem Bark

The stem bark of *Sclerocarya birrea* (A Rich, Hochst.) was collected from Kibiya town in Kibiya local Government Kano at the early hours. Identification of the plant was conducted at Herbarium unit in the Department of Plant Science Bayero University Kano with the following voucher identification number BUKHAN 0435. Voucher specimen was deposited in the Herbarium for future reference. The stem bark were washed with water to remove dust and rinsed with distilled water. Samples were air dried for two-weeks and pulverized into powder form using sterile mortar and pestle in the laboratory as described by Ali *et al.* [11]. The powdered samples were bagged in a black polythene bag and store in air tight container for further use.

Extraction of *S. birrea* Stem Bark

Ethanol and water were used as solvent in the extraction process of the stem bark. One hundred grams (100 g) of the powder were weighed out and mixed with 500 ml of distilled water and ethanol respectively in a separate sterile conical flask. The ethanol solution was extracted using Soxhlet extractor while the aqueous solution was allowed to stand for three days and extracted by maceration method. The mixtures were filtered using Whatman filter paper and the filtrates were evaporated to dryness using rotary evaporator for ethanol extract and water bath at about 40 ^{0}C for aqueous extracts respectively. The extract yields was weighted, stored in dark air tight container at 4°C [12].

Preparation of Extracts Concentrations

The stock concentration for the study was 200 mg/ml. The stock concentration was prepared by dissolving 2g of the extract in 10 ml of DMSO. Various working concentration of 100, 50 and 25 mg/ml was prepared from stock concentration by half fold dilution. The concentrations were stored until use [11].

Phytochemical Screening

Preliminary Phytochemical Screening

Phytochemical screening of the stem bark extract was conducted using the method adopted by Tiwari *et al*. [13]. Wagner's test for determination of alkaloid, Ferric chloride test for phenol, gelatin test for tannin, lead acetate test for flavonoid, foam test for saponin, acetic acid test for steroid, Salkowski test for terpenoid detection, Fehling's test for glycoside and Benzene test for determination of anthraquinones.

Quantitative Phytochemical Analysis

Various methods were employed in determining the amount of bioactive components (phytochemicals) present in the plants' stem bark. Terpenoids, steroids, and tannins were determined using Spectrophotometric method while phenol was determined using Folin Ciocalteu procedure. The alkaloids, flavonoids, and the content of saponins were evaluated using analytical method [14].

Test Isolates

Clinical isolates of *Escherichia coli, Salmonella, Klebsiella* and *Shigella* isolated from under 5 years children diagnosed with diarrhea attending Murtala Muhammad Specialist Hospital were used for the study. The isolates were screened for Multi drug resistance and extended spectrum beta lactamase production. Reference isolates *E. coli* ATCC 25922 obtained from department of Microbiology, Aminu Kano Teaching Hospital Kano was used in the study as negative control

Antibacterial Activity of the Extract

The agar well diffusion method was used to determine the antibacterial activity of the plants' stem bark extracts as described by Ali *et al*. [11]. A 0.1 ml volume of the standardized organisms (0.5 McFarland) were introduced onto the surface of freshly prepared Mueller Hinton agar in a sterile Petri dish and allowed to set and then labeled. A 6 mm sterile Cork borer was used to punch holes (i.e. 5 wells) in the inoculated agar medium. Four of the wells were filled with four different concentrations of the extract which were labeled accordingly; 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml, while the 5th well contained 25 mg/mL of Ciprofloxacin as control for this research. The plates were left on the bench for one hour to enable proper diffusion of the extracts and then incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition around each well was measured to the nearest millimeters along straight line. The experiment was conducted in triplicate and average value was evaluated.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the micro-dilution method as described by Clinical and Laboratory Standards Institute [15]. Serial two-fold dilutions of all the extracts were prepared with sterile broth in a 96-well microtitre plate, obtaining a concentration range from 50 to 3.125 mg/mL (50, 25, 12.5, 6.25 and 3.125 mg/mL). It was followed by addition of 5 µL of the isolates suspension which was added to the wells containing the dilutions. Non-inoculated wells containing sterile broth and extract were used as negative controls. After incubation for 24 hours at 37°C, the samples were observed. MIC was recorded as the lowest concentration of each plant extract that inhibited the bacterial growth as detected by the absence of visual turbidity.

Determination of Minimum Bactericidal Concentration (MBC)

To estimate the MBC, an aliquot of each well that did not show microbial growth in the prior tests were swabbed on the entire surface of Mueller Hinton Agar plates and then incubated for 24 hours at 37°C. The lowest concentration that prevented the bacterial growth was registered as MBC [19].

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the different extracts of *S. birrea* used was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard error. Significance level for the differences was set at $p<0.05$.

Results

Phytochemical Screening

The result of phytochemical screening of *S. birrea* stem bark extracts is presented in Table 1 below. The result indicated the presence of saponin, phenol, terpenoid, alkaloid, flavonoid, anthraquinone and tannin in both aqueous and ethanol extract.

S/N	Phytochemical	Aqueous extract	Ethanol extract
1	Saponin	+	+
2	Steroids	-	-
3	Phenols	+	+
4	Terpenoid	+	+
5	Alkaloids	+	+
6	Flavonoids	+	+
7	Anthraquinones	+	+
8	Tannin	+	+

Table 1: Phytochemical Screening of *S. birrea* Stem Bark Extracts

Quantitative Phytochemical Analysis

The quantitative phytochemical analysis of the extract is presented in Table 2 below. Quantitatively, flavonoid was found to be the abundant phytochemical constituent making about 8.5% of the extract, followed by alkaloid 6.9%, phenol 5.5%, terpenoid 3.8% Tannin 2.3 %, Anthraquinone and saponin constituting 1.4% and 1.7 % respectively.

S/N	Phytochemical	Quantitative analysis (%)
1	Saponin	1.70±0.05
2	Phenols	5.50±0.00
3	Terpenoid	3.80±0.01
4	Alkaloids	6.90±0.02
5	Flavonoids	8.50±0.00
6	Anthraquinones	1.40±0.03
7	Tannin	2.30±0.03

Table 2: Quantitative Phytochemical Analysis of the Extract

Antibacterial Activity of Aqueous *S. birrea* Stem bark Extract

The antibacterial activity of various concentration of aqueous extract of *S. birrea* stem bark is presented in Table 3. The antibacterial activity of the aqueous extract depends on its concentration and types of isolates. Highest zone of inhibition is demonstrated by *E. coli* (18 mm) while *Shigella* has the least activity (15 mm) at 200 mg/mL. The extract at all concentration were active against non-ESBL producing isolate *E. coli* ATCC 25922. The zone of inhibition of the control (Ciprofloxacin 25 mg/mL) against the test isolates ranges from to 20 – 23 mm

	Concentration (mg /mL)/zone of inhibition (mm)				
Isolates	**25**	**50**	**100**	**200**	**Control**
Escherichia coli	12.00±0.3^{a}	13.00±0.5^{a}	17.00±0.0^{b}	18.00±0.5^{b}	23.00
Salmonella spp	10.00±0.0^{a}	12.00±0.3^{b}	15.00±0.5^{c}	17.00±0.3^{c}	21.00
Shigella spp	09.00±0.5^{a}	12.00±0.3^{b}	14.00±0.5^{b}	15.00±0.3^{c}	20.00
Klebsiella spp	10.00±0.0^{a}	13.00±0.5^{b}	14.00±0.3^{b}	16.00±0.5^{c}	22.00
ATCC 25922	15.00	16.00	19.00	22.00	25.00

Key: Values having different superscript on the same row are considered significantly different at $p<0.05$

Table 3: Antibacterial Activity of Aqueous *S. birrea* Stem bark Extract

Antibacterial activity of Ethanol *S. birrea* Stem Bark Extract

The antibacterial activity of various concentration of ethanol extract of *S. birrea* stem bark is presented in Table 4 below. The antibacterial activity of the ethanol extract depends on its concentration and types of isolates. Highest zone of inhibition is demonstrated by *E. coli* (19 mm) while *Shigella* and *Salmonella* have the least activity (16 mm) each at 200 mg/mL. The extract at all concentration were active against non-ESBL producing isolate *E. coli* ATCC 25922. The zone of inhibition of the control (Ciprofloxacin 25 mg/mL) against the test isolates ranges from to 20 – 23 mm

	Concentration (mg /mL)/zone of inhibition (mm)				
Isolates	**25**	**50**	**100**	**200**	**Control**
Escherichia coli	12.00±0.3^{a}	14.00±0.5^{a}	16.00±0.5^{b}	19.00±0.0^{c}	23.00
Salmonella spp	10.00±0.0^{a}	13.00±0.3^{b}	15.00±0.0^{b}	16.00±0.5^{c}	21.00
Shigella spp	11.00±0.3^{a}	12.00±0.3^{a}	14.00±0.5^{b}	16.00±0.0^{c}	20.00
Klebsiella spp	11.00±0.5^{a}	13.00±0.5^{a}	16.00±0.3^{b}	18.00±0.5^{b}	22.00
ATCC 25922	16.00	19.00	22.00	23.00	25.00

Table 4: Antibacterial activity of Ethanol *S. birrea* Stem Bark Extract

Key: Values having different superscript on the same row are considered significantly different at $p<0.05$

MIC and MBC of the Extracts

The MIC and MBC of *S. birrea* stem bark extracts are represented in Table 5. The result showed dilutions of various concentrations of aqueous and ethanol extracts can inhibit and/or kill the isolates. Lower MIC (6.25 mg/mL) was shown by ethanol extract than aqueous extract (12.5 mg/mL). MBC of the extracts ranges between 25 - 50mg/mL.

	Aqueous extract		Ethanol extract	
Isolates	**MIC (mg/mL)**	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
Escherichia coli	12.5	50	6.25	25
Salmonella spp	25	50	12.5	25
Shigella spp	25	50	12.5	50

Klebsiella spp	12.5	50	6.25	50

Table 4: Minimum Inhibitory Concentration (MIC) and MBC of *S. birrea* Stem bark extracts

Discussion

The uses of herbal treatment are one of the possible ways to treat diseases caused by multi drug resistant bacteria. The study was aimed to screen for phytochemical and to determine antibacterial activity of aqueous and ethanol extract of *Sclerocarya birrea.* The preliminary phytochemical screening of the extracts revealed the presence of saponin, phenol, terpenoid, alkaloid, flavonoid, anthraquinone and tannin in both aqueous and ethanol extract. The flavonoid was found to be the abundant phytochemical constituent making about 8.5% of the extract, followed by alkaloid 6.9%, phenol 5.5%, terpenoid 3.8% Tannin 2.3 %, Anthraquinone and saponin constituting 1.4% and 1.7 % respectively. Phytochemical screening of the stem bark of S. birrea in previous work has been reported to contain tannins, flavonoids, alkaloids, and steroids [17]. Previous work of Kutama *et al*. [18] showed that *S. birrea* is a good source of alkaloids, flavonoids, phenol, tannins, terpenoids and saponins. This work was also in conformity with that of Abdulhamid *et al*., [19] who reported the presence of tannin, saponin, alkaloid, terpenoid and anthraquinone in stem bark extract of *S. birrea*. However, the phytochemical screening in the present study was in contrast with that of Manzo *et al*. [20] who reported the absent of alkaloid in *S. birrea* stem bark extract in Niger Republic. Phytochemicals exert antimicrobial activity through different mechanisms. For instance, flavonoids possess a wide range of biological activities which include antimicrobial, anti-inflammatory, analgesic, anti-allergic effects, cytostatic and antioxidant properties [21]. The antibacterial activity of flavonoids had been shown to be a result of their ability to form complexes with bacterial cell walls' extracellular and soluble proteins [19]. Tannins act by iron deprivation, hydrogen bonding or specific interaction with proteins such as enzymes, cell envelopes and complex formation with polysaccharides [22]. Herbs that have tannins as their components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [23]; thus exhibiting antimicrobial activity. Thus these plants are traditionally used in treating diarrhea and dysentery among communities in Northern Nigeria. Saponins are known to produce inhibitory effects on inflammatory processes [24]. They were also reported to possess antibacterial property. Alkaloids are another kind of phytochemicals detected in most of the plant extracts tested. Alkaloids have been associated with medicinal uses for centuries. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines [25]. The antimicrobial activities of the plant stem bark extracts showed that both aqueous and ethanol extracts were active against the tested isolates, However, the activity differ according to the concentrations and solvent of extraction. The activities of all the plant extract were observed to increase with an increase in the concentration. However, the activity of control (Ciprofloxacin) against the test isolates organism was more than that of the extracts tested on the organism. From the result, ethanol extract was more active against the isolate than aqueous extract. This finding agreed with that of Ali *et al*. [16] who reported higher antibacterial activity in organic extract than aqueous extract. The higher activity of the ethanol extract may be attributed to better solubility of the bioactive components of the extract in organic solvent than in water. Manzo *et al.* [17] reported that the extracts from the different parts of *S. birrea* showed varied antibacterial activity against the enteric bacteria associated with diarrhea and the stem bark extracts showed superior activity against *Escherichia coli* and *Salmonella typhi* at 200 mg/mL. This finding correlates with the finding of Mai *et al*. [26] who reported the activity of aqueous extracts was active against *E. coli* and *Klebsiella* spp obtained from Microbiology Department of Gombe State University. Based on the sensitivity of the isolates to the stem extracts, the results of antibacterial activity of the extracts was more pronounced in *E. coli*, followed by *Klebsiella, Salmonella* and less active on *Shigella* spp. The antimicrobial activity of *S. birrea* stem bark extracts on the bacteria isolates was attributed to the presence of some phytochemicals such as tannins, terpenoid, alkaloid, saponin and flavonoids in the stem bark.Tannins and flavonoids are known for their laxative effect and are thought to be responsible for antidiarrheal activity by increasing colonic water and electrolyte reabsorption [27], which explains why the bark of *S. birrea* is used traditionally in treating diarrhea. Terpenoids have been shown to be active against bacteria, fungi, viruses and protozoa and their mechanism of action is speculated to involve membrane disruption by the lipophilic compounds [28]. Alkaloids have been found to have antimicrobial properties with microbicide effects against *Giardia* and *Entamoeba* species, as well as antidiarrheal effects which are probably due to their effects on transit time in the small intestine [28]. Saponins have several biological effects, some of which are antibacterial, antifungal, antiparasitic, cytotoxicity, antiviral and antioxidant activities [27]. The result showed dilutions of various concentrations of aqueous and ethanol extracts can inhibit and/or kill the isolates. Lower MIC (6.25 mg/mL) was shown by ethanol extract than aqueous extract (12.5 mg/mL). MBC of the extracts ranges between 25 - 50mg/mL. The result of MBC of this study was in conformity with the finding reported by Louis *et al*. [29] that the MBC of stem bark extract of *S. birrea* against some pathogenic bacteria including *E. coli* was 50 mg/mL. Eloff [8] reported antibacterial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* using acetone extracts of bark and leaves of *Sclerocarya birrea* with MIC values from 0.15 to 3 mg/ml. The result of the present study is in agreement with those of antibacterial and phytochemical studies on *S. birrea* against various enteropathogens that are implicated in the development of diarrhea and/or other gastrointestinal disorders [18,20,30,31].

Conclusion

Preliminary phytochemical screening of Stem bark extract of *S. birrea* showed the presence of saponin, phenol, terpenoid, alkaloid, flavonoid, anthraquinone and tannin in both aqueous and ethanol extract. Flavonoid was found to be the abundant phytochemical constituent in the stem bark making about 8.5% of the extract, followed by alkaloid 6.9%, phenol 5.5%, terpenoid 3.8% Tannin 2.3 %, Anthraquinone and saponin constituting 1.4% and 1.7 % respectively. The antimicrobial activity of the extracts showed that the ethanol extract of the stem bark showed higher antibacterial activity against the test isolates when compared to aqueous extract. However, the antibacterial activity of the extracts was more pronounced in *E. coli*, followed by *Klebsiella, Salmonella* and less active on *Shigella* spp. The antibacterial activity of the extracts is attributed to the presence of phytochemicals in it. It is recommended that there is need to exploit the potentials of the stem bark especially in areas of traditional medicine and pharmaceutical industries.

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