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Antibacterial activity of *Sclerocarya Birrea* on some urinary tract infections (UTI) pathogens

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Abstract
For many years, human population across the world utilizes environmental resources such as plants to treat infectious diseases. The study was aimed to determine the phytochemical constituents and antimicrobial activity of various extracts of *S. birrea* against some bacteria associated with urinary tract infections (UTIs). Four (4) clinical isolates (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella*) isolated from urine samples collected from patients with urinary tract infections attending Abubakar Imam Urology Hospital Kano, Northern Nigeria were tested against aqueous and methanol extracts of *Sclerocarya birrea* stem bark using agar well diffusion method. The qualitative and quantitative phytochemical screening of the extract was conducted using conventional laboratory methods. The result showed that the stem bark extract of *Sclerocarya birrea* contain saponin, phenol, terpenoid, alkaloid, flavonoid, anthraquinones and tannin. Quantitatively, flavonoid was found to be the most abundant constituent making about 16.5% followed by phenol 12.5%. The result of antibacterial activity of the extracts against the test isolated indicated that the extracts were active against the isolate with higher activity shown by methanol extract (13.5 mm) when compared to aqueous extract (11.4 mm). *Escherichia coli* was found to be the most sensitive isolate (13.8 mm) while *Pseudomonas aeruginosa* is the least susceptible to the isolate (12.3 mm). However, the differences in the sensitivity of the isolates to the extracts is not significant at $p<0.05$. It is concluded that the stem bark extracts of *Sclerocarya birrea* is active against some bacterial isolates associated with UTI.

Keywords: antibacterial activity, bacteria, phytochemicals, urinary tracts infections

Introduction
For many years, human population across the world utilizes environmental resources such as plants to treat themselves [1]. According to World Health Organization, an estimate of 66% to 85% of the world's population, especially from developing countries, depend directly on plants as medicines in treating diseases [2, 3]. Several studies reported that medicinal plants contain numerous biologically active compounds called phytochemicals such as alkaloid, flavonoid, steroid, phenolic compounds tannin and saponin and so on which shown medicinal properties [4]. These phytochemicals are commonly referred as secondary metabolites that were reported by several researchers to act as antimicrobial agents [5, 6].
Many human diseases are as result of infections caused by microorganisms especially bacteria pathogens, either internal or external of the human host. One of such bacterial infection is the Urinary Tract Infection (UTI), involving the presence of bacteria in the urinary tract (UT) which is naturally sterile [7]. Bacteria are the primary organisms that cause UTI. Gram positive cause 15-20% and gram negative cause 80-85%, among gram negative *Escherichia coli* is the most frequent pathogen [8] but in complicated UTI the prevalence of other antibiotic resistance organisms increases such as *Klebsiella, Proteus, Serratia, Enterobacter* and *Pseudomonas*. Among gram positives *S. saprophyticus, E. faecalis, S. pyogenes, and S. aureus* are usually prevalent and are resistant to variety of antibiotics [8].
Sclerocarya birrea is a savannah tree belonging to the family Anacardiaceae. The plant is commonly called Marula in English and *Danya* in Hausa language. It is a medium to large tree usually 9 – 18 m height. According to Agunu *et al*. [9], the plant is single stemmed with a dense, spreading crown and deciduous foliage; the bark of the plant is grey and usually peel off. The young twigs are thick and digital form with spirally arranged composite leaves at their end. The root system is tap root reaching a depth of about 2.4 meter. *Sclerocarya birrea* is one of the plant species that provide numerous benefits to human population as virtually every part of the plant is used for either medicinal or other purposes [10].
In Nigeria and other African countries, various parts of *Sclerocarya birrea* are traditionally used to treat different gastrointestinal disorder with special references to diarrhea and dysentery. The most preferred part of the plant used for treatment of different diseases includes stem bark and leaves [11]. According to Wazel [12], the stem bark of the plant is used in the treatment of diseases such as dysentery/diarrhea, hemorrhoids, stomach ulcers and pain, sore throat/mouth and toothache. In most reports, decoction as method of preparation is recommended by most traditional healers with oral drink as the main way of administration during the treatment of diarrhea/dysentery, and stomach ulcers and pain. However, for hemorrhoid a sit-bath in the decoction is best recommended [12].
In some African countries such as Nigeria, the leaves, root and stem bark of *S. birrea* used in the treatment of malaria and fever, stomach disorder, headache, sore eyes, toothache, back pain, burning urine, infertility, epilepsy, sores, boils carbuncles, abscesses and certain other bacterial infections [13]. The medicinal uses of the plant are attributed to the presence of phytochemicals it contains. The bark contains 10-20% tannin as well as traces of alkaloids [14]. The study was aimed to determine the phytochemical constituents and

antimicrobial activity of various extracts of *S. birrea* against some bacteria associated with urinary tract infections.

Materials and Methods

Test Isolates

Four (4) clinical isolates (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella*) isolated from urine samples collected from patients with urinary tract infections attending Abubakar Imam Urology Hospital Kano, Northern Nigeria. Conventional bacteriological methods such as Gram staining, biochemical (Indole, methyl-red, Voges Proskauer, citrate, catalase and oxidase) characterization, fermentation and motility test were used for bacterial isolation and identification as described by Cheesbrough [15].

Collection and Identification of Plant Materials

The stem bark of *S. birrea* was used in this study. The plant material was collected at Ketawa village in Gezawa Local Government Area of Kano State. The stem bark of the plant was identified and authenticated at the herbarium in the Department of Plant Science, Bayero University Kano. A voucher number of BUKHAN/0125 was allotted to the plant material and a voucher specimen was deposited for future reference.

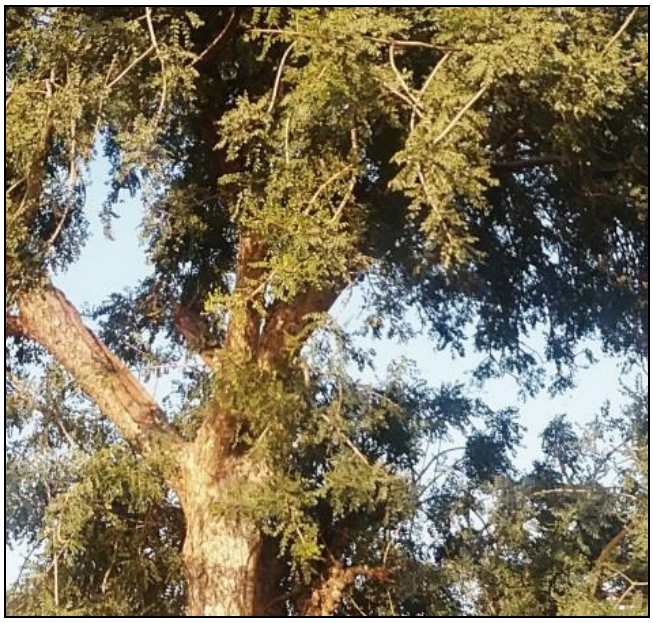

Fig 1: *Sclerocarya birrea*

Preparation of Plant extracts

Methanol and water were used in the extraction process. The fresh stem bark of *S. birrea* collected was rinsed thoroughly with distilled water and shade dried for two weeks. The material was pulverized and grounded into fine powder under laboratory condition using sterile pestle and mortar. Fifty grams (50 g) powder of the plant material was soaked in 500 ml each of distilled water and methanol respectively. The flasks were kept at room temperature for 3 days with intermittent shaking after which filtration was done using Whatman filter paper. The methanol extracts was evaporated at 60°C using rotary evaporator while the aqueous extract was evaporated at 50°C in water bath until dried extract samples were obtained. All the dried extract samples were dissolved in 20% DMSO separately to the final concentration of 200 mg/ml as a stock concentration. The stock solutions were stored in refrigerator at 4^0C for further use [16].

Qualitative Phytochemical Screening

The qualitative phytochemical screening of the stem bark extract of *S. birrea* was conducted to determine the presence of various phytochemical components such as terpenoids, flavonoids, alkaloids, steroid, phenol, anthraquinone, saponin and tannin using standard methods as described by Sofowora [17] and Trease and Evans [18].

Quantitative Phytochemical Analysis

To determine the quantitative amount of phytochemicals in the stem bark extract of *S. birrea*, different methods were employed. Spectrophotometric method was used to determine Terpenoids, alkaloid, and saponin. Folin-Ciocalteu procedure was used to determine phenol, tannin, flavonoid and anthraquinone content as described by Adeniyi *et al.* [19].

Antibacterial Activity of the Extracts

The antibacterial activity of the extracts was determined using agar well diffusion method as described by Ali *et al.* [16] with slight modifications. The prepared bacterial suspension equivalent to 0.5 McFarland Standard (equivalent to 1.5 x 10^6 CFU) was inoculated into sterile Mueller- Hinton agar medium in a sterile Petri-dish. A sterile 6 mm diameter sterile cork-borer was used to bore 5 wells into the agar medium at equidistance. The wells were then filled up with approximately 0.1mL of the extract solution at a concentration of 25, 50, 75 and 100 mg/L taking care to prevent spillage onto the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the extract into the medium after which the plates were incubated at 37^0C for 24 hours, and thereafter the plates were observed for zones of inhibition and measured. Amoxicillin 50 mg/mL (Pal Pharmacy) was used as a positive control in the experiment. The experiment was conducted in triplicate and the average zone of inhibition was calculated.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2mL of 100mg/mL of the extract into a test tube containing 2mL of Nutrient broth, thus producing solution containing 50mg/mL of the extract. The process continue serially up to test tube No. 5, hence producing the following concentrations; 50, 25, 12.5, 6.25 3.125 mg/mL. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5 mL of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37^0 C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity [20].

Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration of the extracts was determined using procedure of Ahmed and Beg [20]. From each tube that did not show visible growth in the MIC, 0.1mL was aseptically transferred into extract free Mueller Hilton agar plates. The plates were incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration of the extract that had less than 99% growth on the agar

plates.

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 deviation. Significance level for the differences was set at $p<0.05$.

Results

Phytochemical screening

The qualitative and quantitative phytochemical screening of is *S. birrea* stem bark extracts presented in Table 1. The result indicated the presence of saponin, phenol, terpenoid, alkaloid, flavonoid, anthraquinones and tannin. Quantitatively, flavonoid was found to be the abundant constituent making about 16.5 %, followed by phenol 12.5 %, alkaloid 5.6 %, terpenoid 3.8 % Tannin and saponin constituting 2.3 % and 1.7 % respectively.

Table 1: Qualitative and quantitative phytochemical screening of *S. birrea* stem bark extracts

S/N	Phytochemical	Aqueous extract	Methanol extract	Quantit analysis (%)
1	Saponin	+	+	1.70±0.05
2	Steroids	-	-	0.00±0.00
3	Phenols	+	+	12.50±0.00
4	Terpenoid	+	+	3.80±0.01
5	Alkaloids	+	+	5.60±0.02
6	Flavonoids	+	+	16.50±0.00
7	Anthraquinones	+	+	1.40±0.03
8	Tannin	+	+	2.30±0.03

Antibacterial Activity of aqueous extract

The antibacterial activity of various concentration of aqueous extract of *S. birrea* stem bark is presented in Table 2. The antibacterial activity of the extract depends on its concentration and types of isolates. Highest zone of inhibition is demonstrated by *E. coli* (15.80±0.20 mm) at 100 mg/mL. The zone of inhibition of the control (Amoxicillin 50 mg/mL) ranges from to 20.00 – 23.67 mm.

Table 2: Antibacterial activity of *S. birrea* aqueous extract

Concentration (mg /mL)/zone of inhibition (mm)					
Isolates	**25**	**50**	**75**	**100**	**Control**
Klebsiella sp	9.00±0.00^{a}	10.20±0.20^{b}	13.70±0.15^{c}	14.40±0.15^{c}	21.34±0.23
Escherichia coli	10.40±0.18^{a}	10.70±0.19^{a}	14.50±0.20^{b}	15.80±0.20^{b}	23.67±0.17
Staphylococcus aureus	9.40±0.15^{a}	10.10±0.14^{a}	13.80±0.12^{b}	14.20±0.35^{b}	20.00±0.00
Pseudomonas aeruginosa	8.80±0.00^{a}	9.70±0.16^{b}	13.50±0.15^{c}	14.60±0.15^{c}	21.67±0.19

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

Antibacterial activity of methanol extract

The antibacterial activity of various concentration of methanol extract of *S. birrea* stem bark is presented in Table 3. The antibacterial activity of the extract depends on its concentration and types of isolates. Highest zone of inhibition is demonstrated by *Escherichia coli* (18.70±0.24 mm) at 100 mg/mL. The zone of inhibition of the control (Amoxicillin 50 mg/mL) ranges from to 20.00 – 23.67 mm.

Table 3: Antibacterial activity of *S. birrea* methanol extract

Concentration (mg /mL)/zone of inhibition (mm)					
Isolates	**25**	**50**	**75**	**100**	**Control**
Klebsiella sp	10.20±0.10^{a}	12.60±0.15^{b}	14.20±0.18^{c}	15.50±0.12^{c}	21.34±0.23
Escherichia coli	11.50±0.15^{a}	13.80±0.14^{b}	15.40±0.15^{b}	18.70±0.24^{c}	23.67±0.17
Staphylococcus aureus	10.70±0.21^{a}	13.00±0.20^{b}	14.50±0.22^{b}	17.70±0.21^{c}	20.00±0.00
Pseudomonas aeruginosa	9.40±0.19^{a}	12.20±0.15^{b}	13.90±0.11^{b}	16.40±0.20^{c}	21.67±0.19

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

MIC and MBC of the extract

The MIC and MBC of *S. birrea* stem bark extracts are represented in Table 4. The result showed dilutions of various concentrations of aqueous and methanol extracts can inhibit and/or kill the isolates. Lower MIC (12.5 mg/mL) was shown by methanol extract than aqueous extract. MBC of the extracts ranges between 25 - 50mg/mL.

Table 4: Minimum Inhibitory Concentration (MIC) and MBC of the extracts

Isolates	Aqueous extract		Methanol extract	
	MIC (mg/mL)	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
Klebsiella sp	25	50	12.5	25
Escherichia coli	12.5	25	6.25	25
Staphylococcus aureus	12.5	50	12.5	25
Pseudomonas aeruginosa	25	50	12.5	50

Discussion

In the present study, the qualitative, quantitative and antimicrobial activity of *S. birrea* stem bark extracts against some urinary tract bacterial pathogens were investigated. In the study, water and methanol were used for the extraction of bioactive components of the plant materials. The result of qualitative phytochemical screening revealed the presence of saponin, anthraquinones, saponin and tannin, reducing sugars, alkaloid, terpenoids, flavonoids, steroid and glycoside. Several studies were conducted on phytochemical screening and antibacterial efficacy of *S. birrea* extracts [21, 22, 23, 24, 25, 26]. The result of phytochemical screening of the

present study was consistence with that of Sasidharan *et al*. [21] Azmir *et al*. [22] who also reported the presence saponin, tannin, flavonoid on stem bark extract of *S. birrea*. On the other hand, this finding contradicts that of Manzo *et al*. [27] who found no trace of alkaloids in *S. birrea* extracts. Some of these metabolites particularly the flavonoids, tannin and alkaloid were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plant such as *S. birrea* [28].

The test result for antibacterial activity of stem bark extract of *S. birrea* (Table 2 and 3) indicated that both aqueous and methanol extracts were effective against the tested isolates. However, the finding reveals that the methanol extract (with average zone of inhibition against all the isolates of 13.5 mm) was found to be more effective than aqueous extracts (with average zone of inhibition of 11.4 mm) and this justified several studies conducted involving aqueous and organic extract [16, 21, 29] since most studies have reported that organic solvents were better chemical reagents for consistent extraction of antimicrobial substances from medicinal plants. The antibacterial efficacy of the extracts appeared to be broad spectrum with *E. coli* being the most susceptible isolate (13.4 mm) to the extract while the zone of inhibition recorded by *P. aeruginosa* was much less (12.3 mm) as compared to the rest. However, the result is not significant at $p<0.05$. From the result, the zones of inhibition produced by the isolates against the extracts remain inferior to that of standard antibiotics (Amoxicillin) for all the tested bacteria. This finding justifies the findings of several researchers [23, 24, 25, 26] who found the stem bark of *S. birrea* effective against some pathogenic bacteria. The antibacterial activity of the extracts is attributed to the presence of bioactive components of the extracts such flavonoid, tannin and alkaloid.

The MIC and MBC values obtained in the present study was consistent with the result of antibacterial activity. Lowest MIC is recorded in methanol extract (6.25 mg/mL) when compared to aqueous extract (12.5 mg/mL). Lower MIC in methanol extract may be due to better solubility of phytochemicals in organic solvent than aqueous solution. The extracts kill the tested isolates at a concentration of 25 – 50 mg/mL.

Conclusion

The findings of the present study revealed the presence of phytochemicals such as tannin, saponin, flavonoid, alkaloid and anthraquinone in the stem bark extracts of *S. birrea*. It was also found that the extracts exhibited antibacterial activity against some isolates associated with urinary tract infection. From the result, methanol extract was to be more effective than aqueous extract. The study justifies the ethno-medicinal use of the *S. birrea* against some opportunistic pathogens associated with urinary tract infections. Therefore, the plant is recommended as a potential candidate for developing drugs for treatment of urinary tract infections.

Conflict of Interest

The authors declare no conflict of interest.

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