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Research Article
Antibacterial Potentials of Compounds Isolated from the Stem Bark Extract of *Sclerocarya birrea* (A. Rich, Hochst.)

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Abstract

Background and Objective: Bacterial, fungal, parasitic and viral infections are still a major threat to public health. The impact is particularly alarming in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. This has lead to the search of new antimicrobial agents mainly among plant extracts. The aim of this research was to isolate, purify and test for antibacterial activity of compounds isolated from the stem bark extract of *Sclerocarya birrea* (A. Rich, Hochst.). **Materials and Methods:** Crude methanolic extract was partitioned into solvent of varied polarity. The resulting fractions were taken for bioactivity (antibacterial) test. The most active fraction was then subjected to isolation and purification techniques. Isolation and purification were carried out using thin layer and column chromatographic techniques. The bioactivity (antibacterial) test was done using agar well diffusion method, while MIC and MBC were tested using broth dilution method. **Results:** About 4 compounds/fractions were obtained after successive thin layer and column chromatography of ethyl acetate fraction. Only compound/fraction 2 showed remarkable antibacterial activity. It showed high zone of inhibition, MIC and MBC against most of the bacterial isolates. It was found to inhibit the growth of most of the test bacterial isolates comprising of both Gram-positive and Gram-negative organisms. On the other hand, the other fractions showed little or no activity against most of the test isolates. **Conclusion:** The result of the present study signifies the potential of *Sclerocarya birrea* stem bark as a source of therapeutic agents, which may provide leads in the ongoing search for anti-microbial agents from plants.

Key words: *Sclerocarya birrea*, ethyl acetate, chromatography, anti-bacterial activity and stem bark

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nigeria is well known for its rich ethnobotanical wealth, particularly regarding medicinal plants which are traditionally used in the treatment of ailments and could be a good source for discovery of new, safe and biodegradable drugs. High population growth rate (2.8% per annum) and poverty coupled with dwindling economic reserves in the country make Nigerians resort to more affordable sources for their immediate health needs. As the population increases, demand for traditional medicine also increases[1,2].

Plants are rich in various active compounds including antimicrobial agents[3]. The recent discovery of novel drugs such as artemisinin, atropine, digitoxin, digoxin, emetine, pilocarpine, ouabain, quinidine, quinine, reserpine, vinblastine, vincristine, etc., from medicinal plants implies that vast potential still exist for the production of numerous more novel drugs. Consequently, the area of ethnopharmacology of medicinal plants has attracted increasing attention in new drugs research and development[4,5]. It is estimated that two-thirds of the world population depend on traditional medications due to the limited availability, the high prices of most pharmaceutical products and the various side effects that they cause[6]. This further justifies the search for alternative products from plants used in traditional medicine.

Sclerocarya birrae (A. Rich.) Hochst is a tree about 13 m high and up to 2.5 m girth. It is a Nigerian medicinal plant used to cure diseases and heal injuries. The plant has various effects on living system. In Nigeria and in some other African countries, the stem bark, roots and leaves of *Sclerocarya birrea* are used for an array of human ailments, including: malaria fever, dysentery, stomach ailments, headache, toothache, body pains, etc[7]. Anti-convulsant effect of aqueous stem bark of *Sclerocarya birrea* extract in mice was reported[8].

Bacterial, fungal, parasitic and viral infections are still a major threat to public health. The impact is particularly alarming in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. This has lead to the search of new antimicrobial agents mainly among plant extracts. The aim of this research was to isolate, purify and test for antibacterial activity of compounds isolated from the stem bark extract of *Sclerocarya birrea*. This is part of an ongoing research to purify, isolate and characterized antibacterial compounds from the extracts of some Nigerian medicinal plants.

MATERIALS AND METHODS

Plant sample collection and identification: Fresh disease-free stem bark of the plant was separately collected from Bodinga, Sokoto state, Nigeria in September, 2016. The sample was identified and authenticated by a Botanist at the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto, Nigeria. The plant was identified as *Sclerocarya birrea* (A. Rich, Hochst.) with voucher number UDUH/ANS/0245. The sample was shed-dried, ground and kept in air-tight containers till further use.

Preparation of plant extracts: The methanolic crude extract was prepared by soaking a sample (500 g) of powdered plant material in 90% methanol (3.0 L) for 72 h. At the end of the extraction, the extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30°C and stored in sterile sample containers at 4°C until further use.

Solvent partitioning of the crude methanolic extract: About 100 g of crude methanolic stem bark extract of *Sclerocarya birrea* was resolved in sterile distilled water (500 mL) in a separatory funnel and extracted with n-hexane. The resulting n-hexane phase was concentrated to dryness and the resulting powder was kept in a freezer in an air-tight container. The resulting aqueous phase was further extracted with ethyl acetate. The ethyl acetate fraction obtained was concentrated to dryness and the recovered powder was kept in freezer for further use. The n-butanol fraction was obtained using the above procedure. The remaining aqueous fraction was dried to powder[9]. This was also kept in freezer in an air-tight container till further use. The procedure is presented in the flowchart shown in Fig. 1.

Antibacterial screening

Preparation of inoculums of test organisms: About 0.5 McFarland turbidity standard was used to standardize the organisms. The scale was prepared by adding 0.05 mL of 1% barium chloride ($BaCl_2$) to 9.95 mL of 1% H_2SO_4. Suspensions of the organisms were made in normal saline and compared with 0.5 McFarland turbidity standard by holding the suspension and McFarland turbidity standard in front of a light against a white background with contrasting black lines. The bacterial suspension was diluted/concentrated until the density of the bacterial suspension matched with that of 0.5 McFarland turbidity standard which corresponds[10] to 1.5×10^8 CFU mL^{-1}.

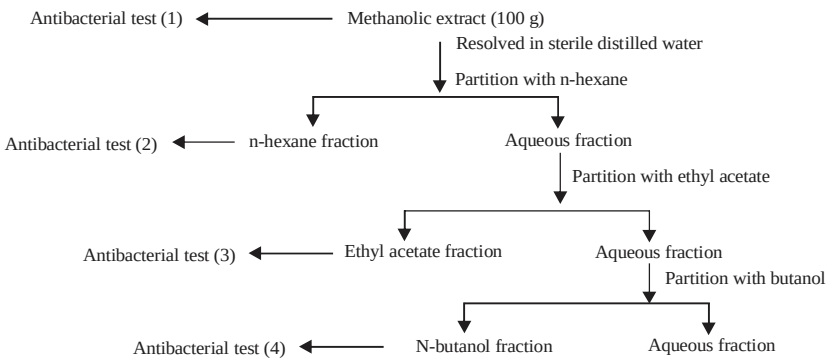

Fig. 1: Extraction and fractionation scheme of the methanolic crude extract of *A. nilotica*

Sensitivity test of the crude extracts and fractions: The antibacterial activity of the crude methanolic extract and fractions of *S. birrea* seed-less pods was determined using agar well diffusion method[11]. The standardized inocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Using a sterile cork borer (6 mm in diameter), three appropriately labelled wells were bored into each agar plate. A 0.2 mL of the appropriate extract concentrate was placed in each well and then allowed to diffuse into the agar. The plates were later incubated at 37°C for 24 h after which zone of inhibition (diameter) formed was determined as an indication of antibacterial activity. These effects were compared with that of the standard antibiotic amoxicillin at a concentration of 1 mg mL^{-1}.

Minimum inhibitory concentration (MIC): Minimum inhibitory concentration of the extract was carried out on the micro-organisms that were sensitive to the extract and was done using broth dilution method[12]. Different concentrations of the extract that exhibited antimicrobial activity against the test organisms were prepared in the test tube containing Mueller Hinton Broth (MHB). The organisms were inoculated into each tube containing the diluted extracts. The plates were incubated at 37°C for 24 h. The lowest concentrations of the extract which shows no turbidity was recorded as the minimum inhibitory concentrations.

Minimum bactericidal concentration (MBC): Minimum bactericidal concentrations of the extracts were carried out to check whether the test microbes were killed or only their growth was inhibited. Mueller Hilton agars were prepared according to the manufacturer's instruction, boiled to dissolve and were sterilized at 121°C for 15 min, the media were cooled to 45°C and the medium (20 mL) was poured in to sterile Petri dishes, the plates were covered and allowed to cool and solidify. The contents of the MIC in the serial dilution was inoculated on to the media, the plates were incubated at 37°C for 24 h, after which the plate were observed for colonies growth. The MBC was the plate with lowest concentrations of the extract without colony growth[13].

Isolation/purification techniques: Thin-layer (TLC), column (CC) and preparative thin-layer chromatographic techniques were adopted for the isolation and purification processes.

Thin layer chromatography (TLC): Thin layer chromatography was carried out on aluminum TLC sheets pre-coated with silica gel 60 PF254, layer thickness of 0.2 mm.

Technique

One way ascending spotting and development: Spots were applied manually using capillary tube, plates were dried using air blower and developed at room temperature using a Shandon chromatographic tank.

Visualization of spots: Spots on TLC plates were visualized under UV light (254 and 366 nm) and spraying with 10% sulphuric acid, followed by heating at 110°C for 5-10 min.

Column chromatography of ethyl acetate fraction: The ethyl acetate extract which was the most sensitive extract from the antimicrobial screening was subjected to column chromatography (CC) for fractionation. About 3 g of the extract was chromatographed on silica gel column eluting with hexane 100%, hexane/ethyl acetate mixtures (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90), ethyl acetate 100% and ethyl acetate/methanol mixtures (98:2, 95:5, 90:10, 80:20) as solvent systems to give 110 fractions.

The 110 fractions were pooled together based on similarity in their TLC profile to give 4 sub-fractions. The 4 sub-fractions were subjected to TLC and single spot was observed for each fraction. The fractions were dried, weighed and labelled. They were then taken for bioactivity testing. The Rf values were also calculated using the Eq:

$$Rf = \frac{\text{Distance moved by spot}}{\text{Distance moved by solvent}}$$

RESULTS

Weight (g) and percentage (%) yield: The amount (weight in grams) and the percentage (%) yield of the four fractions (crude methanolic extract) are presented in Table 1. The crude methanolic extract gave the highest percentage (%) yield of 24.28% and N-hexane fraction gave the lowest percentage (%) yield of 2.36%.

Antibacterial activity: The antibacterial activities of the partitioned fractions against test isolates show different degrees of activity. Out of the four fractions derived from the crude methanolic extract of *Sclerocarya birrea* stem bark, only ethyl acetate fraction showed strong activity (with zone of inhibition ranging from 19.67±1.53-25.00±1.00 mm), while butanol, N-hexane and aqueous fractions showed little or no activity against the test isolates (with zone of inhibition ranging from N.D/not detected to 15.33±1.52 mm) (Table 2).

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of the crude methanolic extract and ethyl acetate and aqueous fractions of *Sclerocarya birrea* stem bark is presented in Table 3. The MIC exhibited by the methanolic extract, ethyl acetate and aqueous fractions were in the range of 1.56-6.25, 3.12-12.5 and 3.12-25.0 mg mL^{-1}, respectively. The MIC results showed that the test organisms were responsive to the extract/fractions of *Sclerocarya birrea* stem bark.

Minimum bactericidal concentration (MBC): The Minimum Bactericidal Concentrations (MBC) exhibited by the crude extract, ethyl acetate and aqueous fractions against the susceptible test isolates was shown in Table 4. The MBC exhibited by the crude extract, ethyl acetate and aqueous fractions against the test isolates ranged between 50 and 100 mg mL^{-1}.

TLC profile of ethyl acetate fraction: The TLC profile of ethyl acetate fraction of *Sclerocarya birrea* is presented in Table 5. Four spots/compounds were obtained from ethyl acetate fraction upon successive column and thin layer chromatography (Table 5). The highest TLC profile of 0.76 was exhibited by fraction/spot 1, while the lowest TLC profile of 0.36 was exhibited by fraction/spot 3.

Antibacterial activity of pure compounds/column chromatographic fractions: The antibacterial activity result of the 4 compounds isolated from ethyl acetate fraction against the bacterial isolates is shown in Table 6. It was only compound/fraction 2 that shows high zone of inhibition (14.00±2.65-23.33±1.52 mm) against the bacterial isolates; the other compounds/fractions showed little or no inhibition zone (from N.D/not detected-8.00±1.00 mm) against the bacterial isolate (Table 6).

MIC and MBC of compound II: The MIC result of compound II from ethyl acetate fraction is presented on Table 7. The MIC exhibited by the compound/fraction II against the test isolates ranged between 0.63 and 2.50 mg mL^{-1}. While the MBC

Table 1: Weight (g) recovered and percentage (%) yield of the crude methanolic stem bark extract and fractions of *Sclerocarya birrea*

Extracts	Weight (g)	Yield (%)
Crude methanolic extract	156.3	24.28
N-hexane	11.8	2.36
Ethyl acetate	42.3	8.46
Butanol	16.4	3.28
Aqueous	31.2	6.24

Table 2: Antibacterial activity of crude methanolic stem bark extract and fractions

	Zone of inhibition (mm)					
Bacterial isolates	Methanol	N-hexane	Ethyl acetate	Butanol	Aqueous	Amox.
E. coli	20.67±2.31	ND	24.33±1.53	11.33±2.08	14.33±1.53	28.67±1.15
K. pneumoniae	20.00±2.65	ND	22.33±2.52	ND	7.67±0.58	26.67±0.58
Proteus spp.	17.00±1.00	4.67±1.53	21.00±1.00	6.33±3.06	15.33±1.52	30.00±1.00
P. aeruginosa	18.67±0.58	ND	25.00±1.00	8.00±1.00	13.67±2.08	25.37±1.53
S. aureus	19.33±0.57	5.00±1.00	22.67±1.15	5.33±1.53	4.33±0.58	23.00±1.00
S. typhi	16.00±2.00	ND	19.67±1.53	ND	ND	28.00±2.00
S. pneumonia	15.67±3.06	ND	18.00±2.00	13.33±3.51	ND	26.00±1.00

Values are mean and standard deviation of 3 replicates, ND: No activity, Amox.: Amoxicillin as positive control

ranged between ND and 10.0 mg mL^{-1}. The result showed that compound II has remarkable activities against the tested bacterial isolates.

DISCUSSION

Sequential extraction involving solvent of varying polarity (n-hexane, ethyl acetate, butanol and water) was used to extract varied compounds from the stem bark of *Sclerocarya birrea*. A sequential extraction procedure was chosen mainly because the nature and polarity and hence the solubility of the bioactive compounds in the stem bark extract of *Sclerocarya birrea* were unknown[14]. In general n-hexane was used to extract hydrophobic or non-polar compounds such as fatty acids, waxes fatty acids some alkaloids and terpenoids[15]. Ethyl acetate is known to extract both medium polarities and some polar compounds such as phenols, flavonoid, tannin and some terpenoid[16,17]. On the other hand butanol and water are known to extract hydrophilic or polar compounds such as carbohydrate, amino acids and their derivatives[16].

The amount weight (g) and the percentage (%) yield of the four fractions (crude methanolic extract) are presented on Table 1. The amount of extract recovered and consequently the percentage yield depends largely on the fibre content of the plant/sample being extracted. High fibre contents gave a very low percentage yield. On the other hand, low fibre content gave a very high percentage yield[18].

The antibacterial results of the partitioned fractions against test isolates showed different degrees of activity. Out of the four fractions derived from the crude methanolic extract of *Sclerocarya birrea* stem bark, only ethyl acetate fraction showed strong activity, while the butanol, N-hexane and aqueous fractions showed little or no activity against the test isolates used (Table 2). This suggested that ethyl acetate will be good solvent for the isolation and purification of the active principles present in the stem bark of *Sclerocarya birrea*.

Different parts of this plant were previously reported to contain phytochemicals such as tannins, alkaloids, steroids, terpenoids, flavonoids and cardiac glycolside[8,16,19-22]. These bioactive compounds were established to be frequently responsible for the antimicrobial properties of most medicinal plants[23,24]. The presence of these bioactive components in the extract/fractions of *S. birrea* might therefore be responsible for the observed antibacterial activities against most of the bacterial isolates.

The minimum inhibitory concentration (MIC) was determined for the crude methanolic extract, ethyl acetate and aqueous fractions. The MIC results presented in Table 3 reflect a trend that tends to show different interactions

Table 3: Minimum inhibitory concentrations (mg mL^{-1}) of crude methanolic stem bark extract, ethyl acetate and aqueous fractions against bacterial isolates

Bacterial isolates	Methanol	Ethyl acetate	Aqueous	Amoxicillin
E. coli	3.12	6.25	6.25	1.56
K. pneumoniae	6.25	12.5	12.50	1.56
Proteus spp.	3.12	6.25	6.25	0.78
P. aeruginosa	6.25	6.25	6.25	1.56
S. aureus	1.56	3.12	3.12	0.39
S. typhi	6.25	6.25	25.00	1.56
S. pneumonia	3.12	3.12	12.50	1.56

Table 4: Minimum bactericidal concentration (mg mL^{-1}) of crude methanolic stem bark extract, ethyl acetate and aqueous fractions

Bacterial isolates	Methanol	Ethyl acetate	Aqueous	Amoxicillin
E. coli	100	100	ND	12.5
K. pneumoniae	50	50	100	25.0
Proteus spp.	100	50	100	6.25
P. aeruginosa	50	100	50	12.5
S. aureus	100	50	100	6.25
S. typhi	50	100	ND	25.0
S. pneumonia	100	50	ND	12.5

Table 5: TLC profile of ethylacetate fraction of *Sclerocarya birrea* stem bark

Fraction (spot)	Colour	Rf
1	Orange	0.76
2	Green	0.48
3	Brown	0.36
4	Yellow	0.64

Table 6: Antibacterial activity of pure compounds/fractions from ethyl acetate fraction

	Zone of inhibition (mm)					
Bacterial isolates	Fraction I	Fraction II	Fraction III	Fraction IV	5% methanol	Amox.
E. coli	8.00±1.00	19.33±2.52	ND	3.67±1.53	ND	23.00±1.00
K. pneumoniae	6.33±2.31	20.67±2.08	ND	ND	ND	26.70±0.58
Proteus spp.	ND	14.00±2.65	ND	6.00±1.73	ND	21.00±1.00
P. aeruginosa	ND	23.33±1.52	ND	ND	ND	27.67±2.09
S. aureus	6.00±2.65	21.67±3.05	ND	3.33±1.53	ND	23.00±1.00
S. typhi	ND	19.00±2.65	ND	ND	ND	18.67±2.08
S. pneumonia	ND	20.67±1.14	ND	ND	ND	23.67±1.15

Table 7: MIC and MBC (mg mL^{-1}) of compound/fraction II

Bacterial isolates	MIC	MBC
E. coli	1.25	5.00
K. pneumoniae	2.50	ND
Proteus spp.	0.63	5.00
P. aeruginosa	1.25	10.0
S. aureus	0.63	10.0
S. typhi	2.50	ND
S. pneumonia	2.50	ND

among bioactive components of the stem bark extract of *Sclerocarya birrea*. Furthermore, simultaneous comparison of the MIC values exhibited by crude methanolic extract, ethyl acetate fraction and aqueous fractions against each test bacterium showed that MIC values of crude methanolic extract against test bacteria were smaller than they were for ethyl acetate and aqueous fractions. This showed that there might be synergistic antibacterial-enhancing interactions between different bioactive components of the pods extract. Antibacterial-enhancing interactions among bioactive components of plant extracts have been reported[25].

The MBC exhibited by the crude extract against the test isolates ranged between 50 and 100 mg mL^{-1}. Also, the ethyl acetate and aqueous fractions showed an MBC ranging between 50 and 100 mg mL^{-1}. Thus, the MBC exhibited by both extract and the two fractions followed the same pattern (Table 4).

A total of 4 pure compounds/fractions were obtained from the thin layer and column chromatography of ethyl acetate fraction. The antibacterial activity of the 4 compounds from ethyl acetate fraction was analysed against the bacterial isolates. It was only compound/fraction II that shows high zone of inhibition against the bacterial isolates; the other compounds/fractions showed little or no inhibition zone against the bacterial isolate (Table 6). In addition, the MIC and MBC analysis of compound II showed remarkable activities against the tested bacterial isolates (Table 7).

CONCLUSION

The result of the present study signifies the potential of *Sclerocarya birrea* stem bark as source of therapeutic agents, which may provide leads in the ongoing search for antibacterial agents from plants. Further, the activity exhibited by the isolated compound against tested bacteria species that are associated with various infectious diseases may provide scientific justification for the ethnomedicinal uses of the plant.

SIGNIFICANCE STATEMENT

This study is expected to yield important lead molecular structures for possible antibacterial drug development especially in the production of synthetically improved therapeutic agents. The identified compounds may as well be used as markers for the standardization of herbal formulations from the stem bark extract of *Sclerocarya birrea*. The bioactivity results is expected to provide preliminary scientific justification for the traditional medicinal uses of this ethno remedy, an important step towards its acceptance and development as alternative therapeutic agent.

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