Phytochemical Screening and Antibacterial Activity of Stem Bark, Leaf and Root Extract of Sclerocarya birrea (A. Rich.) Hochst

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Abstract

Background: Sclerocarya birrea is widely used in Nigerien communities for medicinal purposes to treat several gastrointestinal diseases including diarrhea. To lend credibility to its traditional use against diarrhea, laboratory studies were conducted.

Objective: The aim of this study is to investigate the antibacterial activity and the phytochemical constituents of the crude extracts of root, bark and leaf of S. birrea.

Materials and Methods: The collected different plant parts were air dried, powdered and separately extracted with ethanol and methanol. The alkaloid, flavonoid, saponin and tannin contents in all the plant parts were estimated using standard methods. The total and serially diluted fractions of the extracts were tested for antibacterial activity against selected enteropathogens by agar well diffusion and deep-well microdilution method.

Results: Phytochemical screening revealed the presence of flavonoid, saponin and tannin in all the plant extracts. The extracts from the different parts showed varied antibacterial activity against the test bacteria. The bark extracts showed superior activity against Escherichia coli (zone of inhibition = 17 mm) and Salmonella typhi (zone of inhibition = 20 mm) at 200 mg/mL.

Conclusion: The presence of important phytochemical groups and the antibacterial potential of alcoholic extracts of S. birrea could permit to justify its traditional usage against diarrhea.

Background

Since many years, human population across the world utilized elements of their environment, in particular plants, to treat themselves. To date, even with the spectacular progress accomplished in the field of science, an estimate of 66% to 85% of the world’s population, especially from developing countries, depend directly on plants as medicines in treating all sorts of diseases.¹⁻³ Various studies have reported that medicinal plants contain numerous biologically active compounds such as flavonoids, terpenoids, carotenoids, steroids, simple phenolic, glycosides, tannins, saponins, polyphenols, to mention a few, which have shown medicinal activities.⁴ Most of these phytochemicals, commonly referred as secondary metabolites, were reported to act as antimicrobials.⁵⁻⁷

Niger, a West African country as defined by the United Nations, occupy an area of 1 267 000 km² and lies between the longitudes 0° 16’ and 16° East, and the latitudes 11 °1’ and 23 °17’ North.¹ The country is rich in biodiversity with a flora of about 2124 species, of which an endemic species (Sclerocarya birrea) in the southern part⁶ is included. Many plants from the Niger flora which have been used among the common population by traditional healers for the treatment of common diseases have been significantly recorded.

Diarrhea is a gastrointestinal disorder in which there is rapid transit of gastric contents through the intestine, which is characterized by abnormal fluidity and high frequency of fecal evacuation, usually semisolid or watery fecal matter, 3 or more times a day.⁵⁻¹¹ It is a common cause of death in developing countries including Niger Republic and the second most common cause of infant deaths worldwide. The use of medicinal plants remedies to treat most of the gastrointestinal disorders including diarrhea is a common practice in many African countries.¹¹⁻¹²

Sclerocarya birrea belongs to the family Anacardiaceae and commonly called Dania by the Hausa people and Marula in English. It is a medium to large savannah tree (usually 9 to 18 m), single stemmed with a dense,
spreading crown and deciduous foliage; the bark is grey and usually peels off in flat, round disks, exposing the underlying light yellow tissue; young twigs are thick and digitiform with spirally arranged composite leaves at their ends; it has a thick, relatively short taproot reaching depths of 2.4 m, lateral roots branch at the upper 60 cm of soil; mycorrhizae are found on the fine roots.13 The leaves are divided into 10 or more pairs of leaflets, dark-green above, and with sharp point. The flowers are borne in small, oblong clusters with red sepals and yellow petals.14 The fruit is pale yellow when ripe, approximately 15 to 25 g in weight, about 30 mm in diameter, and borne in late African summer to mid-winter.15 The tree grows in a wide variety of soils but prefers well-drained soil.16 To date, the plant species remains endemic and it is widely distributed across the country.

*Sclerocarya birrea* is a ‘food plant for all seasons’ and is one of those rare plant species that provides several benefits to local communities - virtually every part of the plant is used for either medicinal or some other purposes.17

In Niger and other African countries, different parts of *S. birrea* are traditionally used to treat various gastrointestinal disorders principally diarrhea/dysentery. Almost all parts of the plant, especially the bark and leaves, are used for treatment of various diseases.8 Traditionally, the bark is used for the treatment of various gastrointestinal disorders, especially dysentery/diarrhea, hemorrhoids, stomach ulcers and pain, sore throat/mouth and toothache.14 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.18

In spite of various medicinal usages of *S. birrea* in Niger, their phytochemical constituents and/or their biological activities have not yet been fully documented. The present study aimed at evaluating the good potentials of this plant against gram-negative enteropathogenic strains most implicated in the development of diarrheal infectious diseases.

Objectives

The purpose of this study was to investigate the phytochemical contents and the antibacterial activity of ethanol and methanol extracts of different parts of *S. birrea* against *Shigella flexneri*, *Salmonella typhi*, and *Escherichia coli* by agar-well diffusion and deep-well microdilution method.

Materials and Methods

Plant Material

Plant parts (root, leaf and stem bark) of *S. birrea* were collected in Niamey city (Niger Republic) in the Botanical Garden of the Abdou Mounmouni University (UAM) and were verified by a competent botanist, a researcher at the Faculty of Science, UAM, Niger. The plant materials were rinsed, air dried under shade at room temperature and powdered by the use of metallic mortar and pestle. The obtained powders were then stored in plastic bags.

Bacterial Test Strains and Culture Media

Three clinical strains (*S. flexneri*, *S. typhi* and *E. coli*) isolated from stool samples collected from admitted patients with diarrhea episodes were obtained from the Bacteriological Laboratory, Niamey National Hospital (HNN), Niger. Conventional bacteriological methods (Clinical and Laboratory Standards Institute, CLSI, USA) were used for isolate identification and characterization. Nutrient Agar (NA) (Deben Diagnostics Ltd, UK) slant was used for the maintenance of bacterial cultures. Bacteria strains were activated by sub-culturing into fresh nutrient agar slants and then placed in an incubator (Incubator IN160, Memmert, Germany) overnight at 37°C prior to the test. Mueller-Hinton Agar (MHA) (Deben Diagnostics Ltd, UK) was used for minimum inhibitory concentration (MIC).

Preparation of Plant Extracts

Firstly, 30 g of grounded air-dried plant material was shaken (120 cycles/min) in 150 mL of each solvent (methanol and ethanol) (Blulux Laboratories Ltd-121001), at room temperature for 48 hours. The insoluble material was filtered using filter paper (Whatman No.4) (Whatman/GE healthcare, Cat No. 1004-150) and evaporated to almost dryness in a water bath at 50°C (Isotemp 210, Fisher Scientific). The crude extracts were weighed and placed in a refrigerator at -4°C in sealed glass bottles until use.

Phytochemical Tests

Standard methods were used for the screening of the plant extract for various phytochemical constituents.19,20 Phytochemical constituents tested include tannins, saponins, flavonoids and alkaloids. The reading of the results is done by direct visual observation of the coloration profile of the reactions and/or the formation of precipitates.

Antibacterial Activity Assays

**Agar Well Diffusion Method**

The agar well diffusion method was used to evaluate the antibacterial activity of the various solvent extracts of the plant. Wells of 6 mm diameter were made in MHA (Deben Diagnostics Ltd, UK) plate (90 mm diameter) using cock borer and inoculum size of 10⁶ CFU/mL of test bacteria were spread on the solid media with a sterile swab stick moistened with the bacterial suspension. The partially dried extracts were reconstituted in dimethyl sulfoxide (DMSO) to a concentration of 200 mg/mL considered as stock concentrate. About 80 µL of each
plant extract was placed in the wells. As negative control, similar experiments were also set up for comparison with 100% DMSO. Gentamicin and ciprofloxacin (Oxoid Ltd, England) were the antibiotics used as positive controls. The plates were placed in an incubator for 24 hours at 37ºC and the zone of inhibition, if any, was measured in millimeter excluding the well diameter.21

Minimum Inhibitory Concentration
The MIC value was determined for the bacteria strains that were sensitive to the extract(s) under study. Two-fold serial dilutions of each stock of extracts (at 200 mg/mL) were made in different range. Each test bacterium inoculum adjusted with an electronic Densimat (Marcy-l’Etoile, Biomerieux SA, France) to 10⁶ CFU/mL was seeded in a 96-well microplates (Sterilin Ltd, Parkway, Newport, NP11 3EF, UK) and treated with various concentrations of the plant extract and then incubated at 37ºC for 18 to 24 hours. MIC is the least concentration where no turbidity was observed in the test tubes.21

Results
The results of the qualitative phytochemical screening of crude extracts of the 3 different parts of *S. birrea* were presented in Table 1. Table 2 represents the results for the antibacterial activity of the root, bark and leaves extracts of *S. birrea* and standard antibiotics used against the test bacteria. Furthermore, Table 3 shows the results obtained by the dilution method in liquid medium for the determination of the MIC.

Discussion
In this study, the phytochemical constituents and antibacterial potentials of alcoholic extracts of *S. birrea* were investigated. Ethanol and methanol were used for the extraction of different plant materials, since most studies have reported that organic solvents were better chemical reagents for consistent extraction of antimicrobial substances from medicinal plants.23,24 The results of the phytochemical screening revealed the presence of tannin, flavonoid and saponin in the 2 extracts of roots, barks and leaves of *S. birrea*. However, alkaloid was not detected in any of the plant parts. Studies conducted by various investigators have also reported the presence of both tannins, flavonoids, saponins in the alcoholic bark extracts of *S. birrea*.23-29 Various pharmacological and biological studies (both in vitro and in vivo) conducted elsewhere have reported the wide ranging antimicrobial

### Table 1. Compounds Identified in the Phytochemical Screening of the Ethanol and Methanol Extracts

| Phytochemicals | Ethanol | Methanol |
|----------------|---------|----------|
| Root           | Bark    | Leaf     | Root    | Bark    | Leaf     |
| Alkaloids      | -       | -        | -       | -       | -        |
| Saponins       | +       | +        | +       | +       | +        |
| Tannins        | +       | +        | +       | +       | +        |
| Flavonoid      | +       | +        | +       | +       | +        |

+, Presence; -, Absence.

### Table 2. Results of Antibacterial Sensitivity Test (Agar-Well Diffusion Method) of the Ethanol and Methanol Extracts of *Sclerocarya birrea* Against the Test Organisms

| Test Organism  | Control          | Zone of Inhibition Diameter (mm) |
|----------------|------------------|----------------------------------|
|                |                  | Root | Bark | Leaf |
| *Escherichia coli* | Ethanol extract | 3   | 13   | 5   |
|                 | Methanol extract | 5   | 17   | 7   |
|                 | Ciprofloxacin    | 18  |      |     |
|                 | Gentamicin       | 24  |      |     |
|                 | DMSO             | 0   |      |     |
| *Salmonella typhi* | Ethanol extract | 6   | 6    | 5   |
|                  | Methanol extract | 6   | 20   | 9   |
|                  | Ciprofloxacin    | 25  |      |     |
|                  | Gentamicin       | 24  |      |     |
|                  | DMSO             | 0   |      |     |
| *Shigella flexneri* | Ethanol extract | NI  | 5    | NI  |
|                   | Methanol extract | 3   | 7    | 4   |
|                   | Ciprofloxacin    | 26  |      |     |
|                   | Gentamicin       | 24  |      |     |
|                   | DMSO             | 0   |      |     |

NI, No inhibition.
activities of these secondary metabolites produced by some ethno-medicinal plant including *S. birrea*.

Test results for antibacterial activity of the root, bark and leaves extracts of *S. birrea* and standard antibiotics used are presented in Table 2. The ethanol and methanol extracts of the bark of *S. birrea* were found to be effective in inhibiting the growth of at least 2 amongst the three test bacteria, as demonstrated by an agar well diffusion assay (micrograph images not shown). For this method of diffusion, a plant extract is considered active when it induces an inhibition zone superior or equal to 10 mm. The methanol extract of the plant bark demonstrated profound activity against *S. typhi* (zone of inhibition diameter = 20 mm), and *E. coli* (zone of inhibition diameter = 17 mm). None of the different extracts of root and leaf were found significantly effective against any of the test bacteria (zone of inhibition diameter <10 mm). However, the diameters of the inhibition zones induced by all these extracts remained inferior to those of the reference antibiotics, gentamicin and ciprofloxacin, for all the tested bacteria.

The MIC values obtained (shown in Table 3), in general, significantly matched with those of the diameters of the inhibition zones; plant extracts that induced an important zone of inhibition presented the smallest MIC value with respect to the correspondent test bacteria. It is the case of the alcoholic extracts of *S. birrea* (bark) on *S. typhi* and *E. coli*.

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.

### Conclusion

The results of the present study reported the presence of important phytochemicals with antibacterial activities. This might explain the observed antibacterial activity against test enteropathogens. The results also indicate that both alcoholic bark extracts demonstrated wider antibacterial activities against the test enteropathogens with the least MIC.

Taken together, this study has attempted to justify the ethnomedicinal use of *S. birrea* to treat infectious diseases such as diarrhea of bacterial origin which call for further research on identification, isolation and characterization of the active components.

### Authors’ Contributions

LMM, IM and KI designed the study. LMM designed and performed the laboratory experiments. LMM and IM analyzed the data. LMM drafted the manuscript. LMM, IM and KI revised and approved the manuscript.

### Ethical Approval

This study was approved by Abdou Moumouni University (UAM), Niamey, Niger. All procedures performed in this study were in accordance with the ethical standards of the national research committee.

### Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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**Table 3. Minimum Inhibitory Concentration of the Ethanol and Methanol Extracts of the Bark of *Sclerocarya birrea* Against the Test Organisms**

| Solvents | Concentration (mg/mL) | *Shigella flexneri* | *Salmonella typhi* | *Escherichia coli* |
|----------|-----------------------|---------------------|-------------------|-------------------|
| **Ethanol** | 200 | - | - | - |
| | 100 | + | - | + |
| | 50 | + | + | + |
| | 25 | + | + | + |
| | 12.5 | + | + | + |
| **Methanol** | 200 | NT | - | - |
| | 100 | NT | - | - |
| | 50 | NT | - | + |
| | 25 | NT | + | + |
| | 12.5 | NT | + | + |

NT, Not tested.
