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Full Length Research Paper

Assessing the antibacterial activity of morula (*Sclerocarya birrea*) stem bark and leaf extracts against some selected bacterial isolates in Kano, Nigeria

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Antibacterial evaluation of the methanolic extract and aqueous fraction of leaf and stem bark of the morula tree, *Sclerocarya birrea* was carried out using the agar-well diffusion method. They were tested against two gram positive organism; *Escherichia coli* and *Pseudomonas aeruginosa* and one gram negative organism *Staphylococcus aureus*. Result obtained confirmed a broad Spectrum of activity, as all the organisms were inhibited by the extract. The stem bark extract exhibited antimicrobial activities with zones of inhibition ranging from 8 - 17 and 0 - 8 mm for methanol and water extracts respectively while extracts of leaves exhibited antimicrobial activity of 6 - 16 mm and 5-8 mm for methanol and water respectively. The Minimum Inhibitory Concentration [MIC] of methanol extract of stem bark was 5 mg ml^{-1}. The minimum bactericidal concentration [MBC] was 10 mg ml^{-1}. The results of phytochemcial screening have demonstrated the presence of alkaloid, Flavonoid and tannins in some, or all the extract. Therefore, this study further confirms the traditional use of stem bark and leaf extracts of *S. birrea* in some parts of Northern Nigeria as a remedy against infectious diseases caused by most of the organism studied.

Key words: antibacterial activity, *Sclerocarya birrea,* bacterial isolate, aqueous and methanol extracts, phyto-chemicals.

INTRODUCTION

A significant proportion of indigenous plants in West African sub region are seasonal forest products harvested for consumption on site or transported to other areas particularly urban centres for sale (Nnam and Njoku, 2005). The knowledge of the nutrients composition of some of these plants enhances their use and increases their consumption which in turn improves the nutrient profile of the consuming populace (Nzeagwu and Onimawo, 2010). One of such tree is *Sclerocarya birrea* (*Anacardiaceae*) which its botanical description was reported by Moganedi et al. (2007), Hillman et al. (2008) and Ojewole et al. (2010). The tree bears pale yellow fruits with a plain tough peel and fibrous juicy sweet-sour mucilaginous flesh (Hillman et al., 2008). The bark contains 10-20% tannin as well as traces of alkaloids (Roodt 1998). It provides fibre and gum, which is mixed with salt and water to produce ink or red dye. The bark is commonly use for medicinal purpose according to Van wyk et al. (1997); Kokwaro (1993); Maydell (1986); Taylor

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et al. (1996); Galvez et al. (1991) and Iwu (1993) and (for the treatment of or to treat) variety of ailments, including fever, pile, boils, diarrhoea and blood circulation associated problems. When mixed with other medicinal plants, the bark treats various infections such as syphilis, leprosy, dysentery, hepatitis and rheumatism (Kokwaro, 1993).

Traditionally, the bark provides medicines for treating malaria, venereal diseases, diabetes, dysentery and other form of diarrhoea (Oliver, 1960; Irvine, 1961; Watt and Breyer-Brandwijk, 1962; Adjanohoun et al., 1979; Burkill, 1985; Andriamihaja, 1988). It is also used to treat haemorrhoids according to Adam et al. (1972); Oliver (1960); Sindiga et al. (1995), liver diseases by Maundu et al. (1999), inflammations of the spleen by Maundu et al. (1999), stomach ulcers and pain by Goosen (1985), gangrenous rectitis, blepharitis, skin inflammation and eruptions by Ayensu (1978), leprosy by Oliver (1960); and to ease labour pains by Wickens (1980), haemorrhagic menstruation by Oliver (1960); Adam et al. (1972), headache, fevers by Oliver (1960), sore throat/mouth and toothache by Watt and Breyer-Brandwijk (1962); Burkill (1985); Andriamihaja (1988).

Details on how the bark is used have not been systematically studied or reported, and are mostly apocryphal. Irvine (1961) reports Hausa use of a cold infusion of the bark, with mineral sodium carbonate for dysentery and applying a bark decoction to cure skin eruptions. For malaria, a brandy tincture of the bark is drunk as a prophylactic while a teaspoonful of dried powder is taken as a cure in South Africa. However, laboratory efforts to detect antimalarial activity in extracts of the bark of subsp. *caffra* failed (Spencer et al., 1947). For gangrenous rectitis, the bark decoction is drunk and used to bathe the body. A half-pint of the decoction is drunk for dysentery. For use as an analgesic for toothache, the bark (and leaves, in Madagascar: Andriamihaja, 1988) is chewed and compacted into carious tooth cavities. The paste of the bark, alone or mixed with other plants, is diluted in a drink used to treat syphilis, gonorrhoea or leprosy, in Nigeria (Adam et al., 1972) and elsewhere in Africa (Burkill, 1985). For snakebite, bark, from the trunk but especially the root, is pounded to a paste, rubbed on the bite until skin surface swells, and then a bark decoction is drunk and applied as a dressing to the wound. It is used as a general anti-inflammatory for external use. According to Burkill (1985), it is mixed with butter and applied to the forehead to relieve headache and to the eye for blepharitis. In addition, it can be used as a purgative (Burkill, 1985), and in Kenya (Pokot, Maasai) a decoction of the bark or root is added to milk to make a health drink for children (Maundu et al., 1999). It is used as an anal suppository (powder) for treating haemorrhoids (Adam et al., 1972). Powdered bark, mixed in a drink of milk or millet water, is used to reduce fevers. A similar drink, mixed with dried onion leaves and other ingredients, is used to treat haemorrhagic menstruation (Adam et al., 1972).

In Madagascar (Andriamihaja, 1988), internal (oral) use of the bark is usually as a tisane, a tea that is made from the leaves and bark and is commonly used as a sedative and antiseptic. For anal use, it is in a powder form. As a paste, powder or ointment, it is used externally as an astringent, antibiotic, antiseptic and emollient. In addition to the medical treatments mentioned above, it is used in Madagascar to treat neuralgia, rheumatism, gout and gastrointestinal disorders. In traditional dental medicine, it acts as a disinfectant, antiseptic, anti-inflammatory and analgesic; and can be used as a gargle or mouthwash.

The bark of *Sclerocarya birrea* is also used in traditional veterinary medicine (Kela et al., 1989). A decoction of the bark is used to increase the appetite of stock (Burkill, 1985), and to treat intestinal problems of horses (Adam et al., 1972). Shackleton et al. (2001) reported that anti-bacterial activity of extracts of dry bark has been demonstrated.

The objective of this research was to screen some of the phytochemicals (tannins, flavonoids and alkaloids) present in the stem bark and leave extract and assess the antibacterial effects of the stem bark and leaves extract of *Sclerocarya birrea* on some selected bacterial isolates.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant materials namely leaf and stem bark of *Sclerocarya birrea* were collected from the Botanical Garden of Bayero University, Kano Nigeria (Lat 11° 58’ 9” N, Long 8° 28’ 12”E).

The plant specimen was identified and authenticated by many plant Scientists in the Plant Science Department of Bayero University Kano as morula.

Preparation of plant sample

Each plant material was air dried under shade for 5 days and then Pulverized in a mill and stored in air tight containers and coded accordingly (i.e. leaves and stem barks separately).

Extraction of Plant sample

Exactly 20 g of the pulverized plant material was cold extracted in 200 ml of methanol and distilled water separately for 2 week with occasional shaking, as reported by Harborne (1998).

Test organisms

The test organisms used in this study were Bacterial

Table 1. Physical properties of *Sclerocarya birrea* extract of stem bark.

Properties	Methanol	Distilled water
Weight used for extraction (g)	20 g	20 g
Weight of extract (g)	3.2 g	2.7 g
Colour	Brownish	Brownish
Texture	Gummy	Gummy

isolates; *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* which were obtained from Aminu Kano Teaching Hospital, Department of Microbiology, Kano. Their Purity was confirmed by sub - culturing into nutrient broth incubated at 37°C for 18 h. The developed colonies were observed under the microscope after simple staining. Pure culture was kept on agar slopes at 4°C until needed.

Photochemical analysis of plant extract

The extract were subjected to phyto-chemical analysis for plant secondary metabolites which included Tanins, saponins, steroid, flavonoids, alkanoids and glycolsides in accordance with Trease and Evans (1989), Harbone (1998) and Oyeleke and Manga (2008). Solution which disappeared on addition of hydro acid indicates the presence of flavonoids (Oyeleke and Manga, 2008).

Anti bacterial activity

The antibacterial activity of the crude extracts was determined in accordance with agar-well diffusion method as describe by Irobi et al. (1994). The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (100 $Cfum1^{-}$), by using inoculation loop.

A loopful of colony of test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland standards as described by the National Committee of clinical laboratory (2008).

Standard inocula of isolate were swabbed on the surface of the prepared solidified Muller Hinton agar (Oxoid). Well were then bored into the agar using a sterile 6mm diameter court borer. Approximately 0.1 ml of stock solution prepared with DMSO (dimethyl sulphoxide) was introduced into the well. It was allowed to stand at room temperate for about 2 h and then incubated at 37°C as positive control for bacteria Cipro floxacin (0.5 g/ml) was use as positive control. The control was set up in parallel using the solvent that were used to constitute the extract. The plates were observed for zones of Inhibition after 24 h. The effect was compared to those of ciprofloxacin at a concentration of 0.5 mg/ml.

Minimum inhibitory concentration

MIC was determined by preparing various concentrations of extracts by using the method of Akinpelu and Kolawale (2004). Two-fold dilutions of the crude extract was prepared and 2 ml aliquots of different concentrations were added in 18 ml of pre-sterilized molten nutrient agar at 40°C to give final concentration of 0.05 mg/ml. The medium was poured in a sterile Petri dish and allowed to set.

The surface of the medium was allowed to dry under laminar flow before streaking with 18 h old bacterial cultures. The plates were later incubated at 37°C for 24 h. After which they were examined for the presence or absence of growth. The MIC was taken as lowest concentration that prevented the growth of test organisms.

Minimum bacterial concentration

The MBC of plant extract was determined by the method of Spencer and Spencer (2004). Samples were then taken from plates with no visible growth in MIC assay and sub-cultured on freshly prepared Nutrient agar plate and later incubated at 37°C for 48 h. The MCB was taken as the concentration of the extract that did not show any growth on new set of agar plates. The MBC was not determined for water extract since the activity was low.

RESULTS

The results of extraction showed that higher yield in both leaves and stem bark extract were obtained on extraction of *Sclerocarya birrea* using methanol and distilled water (Table 1).

Results of phyto-chemical screening of the plant material indicated the presence of some secondary metabolites such as alkanoids, tannins and Flavonoids as shown in Table 2.

In-vitro inhibitory activity of the crude extract indicated that both extract showed smaller zones of inhibitions as compared to standard Ciprofloxacin, the extracts were active (even though unrefined) against the test organisms at concentration equal to that of the standard antibiotic used as control as shown in Tables 3 and 4.

Table 2. Physical properties of *Sclerocarya birrea* extract of leaves.

Properties	Methanol	Distilled water
Weight used for extraction (g)	20 g	20 g
Weight of extract (g)	3.7	4.1
Colour	Dark green	Dark green
Texture	Gummy	Dry

Table 3. Phytochemical constituent of *Sclerocarya birrea* stem barks extract.

Metabolities	Methanol	Distilled water
Tannis	+	+
Flavonoid	+	+
Alkaloids	-	+

Table 4. Phytochemical constituents of *Sclerocarya birrea* leave extracts.

Metabolies	Methanol	Distilled water
Tannins	+	+
Flavonoid	+	+
Alkanoid	-	-

Table 5. Inhibitory activity of *Sclerocarya birrea* leaves extract (mm) at different concentration (mg/ml).

Zone of inhibition (mm) at different concentration (mg/l)					
Organism	Extract	60	30	15	ciproflaxacin
	Methanol	9	8	7	24
S. aureus	Water	8	8	7	27
	Methanol	16	12	10	24
E. coli	Water	7	7	7	25
	Methanol	9	7	6	16
Pseudomonas aeruginosa	Water	6	6	5	23

DISCUSSION

The findings of this research showed that *Sclerocarya birrea* stem bark yielded more extract when subjected to methanol extraction with gummy texture and brownish appearance as indicated in Table 1 while that of *Sclerocarya birrea* leaves yielded more extract when subjected to water extraction with oily and dry texture while the appearance was dark green. These shows that water has a stronger extraction capacity than methanol which may be related to high polarity of moot compound content in the plant extract Table 2.

Both plants part were found to contain some secondary metabolites. The stem bark was found to contain Tannins flavonoid and trace of alkaloids (Table 3) while the leaves contains only tannins and flavonoids but absence of alkaloids in Table 4. Some of these metabolites particularly the flavonoids were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plant (Singh and Biant, 2003). All the two extracts of the plant tested showed varying degree of antibacterial activities against the test bacterial species (Table 5).

The antibacterial activities of the methanol and water extract of *S. birrea* stem bark (Table 6) compared with standard antibiotic (Ciprofloxancin) and have appeared to be broad spectrum as its activities were independent on gram reaction.

Table 6. Inhibitory activity of *Sclerocarya birrea* of stem bark extract (mm) at different concentration (mg/l).

Zone of inhibition (mm) at different concentration (mg/L)					
Organism	Extract	60	30	15	ciproflaxacin
	Water	8	7	7	26
S. aureus	Methanol	8	8	7	27
	Water	17	16	15	23
E. coli	Methanol	8	7	0	30
	Water	7	6	6	25
Pseudomonas aeruginosa	Methanol	12	10	8	22

Table 7. Minimum Inhibitory Concentration [MIC] and Minimum Bactericidal concentration (MBC) of methanol extracts of *S. birrea* and Ciprofloxacin on test organism.

Organism	Methanol[mgml^{-1}]		ciprofloxacin [mgml^{-1}]
	MIC	MBC	
P. aeruginosa	5.00	10.00	0.396
S. aureus	5.00	10.00	0.0625
E. coli	5.00	10.00	0.5

The inhibition of *E. coli* was much less (0 - 8 mm) as compared to other bacteria. The methanol extraction (Inhibition zone of 8 - 17 mm) was found to be more effective than the water extract (Inhibition zone 0 - 8 mm) against all organisms, water extract showed low antibacterial activity with inhibition zones ranging between 6 and 8 mm for different bacterial tested.

The minimum inhibitory concentration [MIC] of methanol extract ranged between 0.5 and 10 mgml^{-1}. Also the MIC of Ciprofloxacin control ranged between O.396 and 0.5 mgml^{-1} (Table 7). The minimum bacterial activity of the extracts for different bacterial is 10.00 mg ml^{-1}. Water extract was not active against any of the organism at 10 mg ml^{-1}. Which was the highest concentration tested. The methanol extract was more active than other extracts. This may be attributed to the presence of soluble phenolic and polyphenolic compound (Kowalski and Kedzia, 2007).

The inhibitory effects of the extract of *S. birrea* stem bark against pathogenic bacterial strains can introduce the plant as potential candidate for drug development for treatment of ailments caused by these the pathogens. The non activity of bacterial strains investigated in this study is in agreement with previous works which shows that aqueous extracts of plant generally showed little or no antibacterial properties (Koduru et al., 2006; Aliero et al., 2006; Ashafa et al., 2008).

While the antibacterial activities of the methanol and water extracts of *S. birrea* leaves (Table 6) compared to standard antibiotics (ciprofloxacin) shows broad spectrum as its activity were independent on gram reaction. All the extract tested showed antimicrobial activity against both gram positive (S. aureus) and gram negative organism (P. aeruginosa and *E. coli*).

The Present investigation confirms therefore the antibacterial activity of extracts of *S. birrea* leaves.

The methanol extracts of *S. birrea* shows a wider spectrum of activity than the water extract. Thus *S. birrea* may be of value in reducing the high incidence of enterobacteria casual infectious especially in Northern Nigeria.

But the plant showed greater activity on P. aeruginosa when extracted with methanol than when extracted with water from this *in-vitro* study. It can be deduced that *S. birrea* may be used to fight opportunistic infections caused by *Escherichia coli* as reported by Belemtougri et al. (2001).

CONCLUSION

The activity of the plant parts is confirmed to be due to the secondary metabolites tannins, saponins and flavonoid. All the extracts inhibited both gram positive and gram negative organism used confirming them to have broad spectrum.

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