Comparative Phytochemical and Antimicrobial Evaluation of Stem Bark Extracts of Bauhinia rufescens Lam (Caesalpinioideae-Leguminosae) and Sclerocarya birrea (A. Rich.) Hochst (Anarcardiaceae)

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ABSTRACT

The methanolic extracts of plants Bauhinia rufescens (BRME) and Sclerocarya birrea (SBME) were screened phytochemically and investigated in vitro against five Gram+, five Gram- bacterial isolates and three fungal species using hole-in-plate agar diffusion technique. The extractive value of BRME was 17.90% w/w (dark brown) and SBME was 15.37% w/w (reddish brown). The secondary metabolites present in both extracts were cardenolides, cardiac glycosides, flavonoids, saponins, resins, tannins and phlobatannins; anthraquinones were only present in BRME. The diameters of inhibition zones exhibited by the BRME extract against Gram+ and Gram- organisms ranged from 11.00-28.17 and 10.67-27.17 mm, respectively. SBME had values ranging from 13.00-26.50 and 11.67-26.00 mm, respectively against Gram+ and Gram- species. The overall susceptibility data revealed that BRME was more susceptible to Gram- organisms, although inhibition was in particular cases insignificant (P>0.05) against Pseudomonas aeruginosa, Klebsiella spp., Proteus vulgaris, Bacillus subtilis, Streptococcus pneumoniae (5 mg/hole). The ranges of MIC and MBC data obtained from BRME against the tested organisms were 0.78-6.25 and 1.56-12.5 mg/ml, respectively while for SBME these values were 1.56-12.5 and 1.56-25 mg/ml, respectively. BRME was more susceptible to Gram- organisms since an MIC/MBC value of 1.56 mg/ml was noted for Pseudomonas aeruginosa, Klebsiella spp., Proteus vulgaris, Bacillus subtilis, Streptococcus pneumoniae, Salmonella typhi and the MBC value for E. coli. SBME had an MIC/MBC value of 3.13 mg/ml for Corynebacterium spp. and Staphylococcus aureus and the same MBC value for Streptococcus pneumoniae. There were no antifungal activities on BRME but little activities were expressed by SBME. Finally, both plant extracts showed very good activity against the pathogenic strains tested and hence, could be a yardstick for their traditional use.

Keywords: Gram+, Gram-, microorganism

INTRODUCTION

The core aim of the present study was to screen the Bauhinia rufescens methanol extract (BRME) and Sclerocarya birrea methanol extract (SBME) phytochemically and to investigate the in vitro antimicrobial efficacy of these plants extracts with a view to finding the most active plant against a group of pathogenic organisms and to determine the minimum inhibitory and bactericidal concentrations.

Medicinal plants which are known to be the major source of medicine are fundamental to the well being of mankind the World over. These plants are cheaper and more accessible to most of the population in the World. Therefore, due to these factors, there is a need to encourage the use of medicinal plants as a potential source of new drugs (Igoli et al. 2002). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al. 2005).

In recent years, multiple drug resistance in both human and plant pathogenic microorganisms have developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Loper et al. 1991; Davis 1994; Service 1995; Güllüce et al. 2004). In addition, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-depression and allergic reactions (Ahmad et al. 1998; Güllüce et al. 2004). It is therefore, pertinent to screen medicinal plants from this part of the country which are used locally by the traditional healer for the treatment of various infectious diseases with a view to validating their traditional usage and also serve as a starting material for the development of clinically useful chemotherapeutic agents.

Briefly, the plant Bauhinia rufescens Lam is a scendant shrub or small tree (Fig. 1A) belonging to the giant family Leguminosae, subfamily Leguminosae-Caesalpinioideae; usually 1-3 m high, sometimes reaching 8 m; often scraggy, stunted and multi-stemmed. Bark (Fig. 1B) is ash-grey, smooth, very fibrous and scaly when old, slash pink, twigs arranged in 1 plane like a fishbone, with thornlike, lignified, lateral shoots, 10 cm long. Leaves are very small, bilobate almost to base, with semi-circular lobes, glaborous, with long petioles, greyish-green, less than 3 cm long. Flowers are greenish-yellow to white and pale pink, in few-flowered racemes; petals 5, spathulate, 15-20 mm long; stamens 10, filaments hairy at the base. Fruits aggregated, long, narrow...
pods, twisted, up to 10 cm long, glabrous, obliquely constricted, shining dark red-brown, with 4-10 seeds each. Pods remain on the shrub for a long time (FAO-UNEP 1983; Burkhill 1995). B. rufescens is deciduous in the drier areas and an evergreen in the wetter areas. It is often found in the dry Savannah region, especially near streams or river banks; occurring throughout West Africa and extends across Africa up to Sudan. It has wide array of medicinal and socio-cultural uses. An extract of the root is used as an astringent and anti-pyretic in local medicine. Leaves and fruits are applied for the treatment of diarrhoea, dysentery and opthalmic diseases. The bark of the roots and stem is used to cure chest complaints, syphilis and other venereal diseases, leprosy, diarrhoea and dysentery and to reduce fever (Vogt 1996).

A cold infusion of the bark is astringent and is used in Nigeria as a wound-dressing and to treat diarrhoea and dysentery (Burkill 1995); the trunk and root barks are prepared as infusions or decoctions in Senegal for treating syphilis and jaundice; also the root is used as a febrifugal, diuretic and antienteralgic. The bark or the root is widely used in decoction to treat leprosy (Nwude and Ebong 1980); the bark is also reported as a remedy against small pox. Very recently, two flavonol glycosides, namely 3,7-di-O-α-rhamnopyranosylquercetin and 3,7-di-O-α-thamno-pyranosylkaempferol have been isolated in Brazil from B. forticatata L. (Menezes et al. 2007).

Other compounds isolated from various sort of Bauhinia genus are bauhinina, 5-hydroxy-7-methoxyflavone-6-O-β-xlyopranoside, baussplendin, racemosol [preracemosol A] and des-O-methylracemosol [preracemosol B]. Other chalcone type compounds isolated are 4'-methoxyisouquerein and 2,4-di-hydroxy-4-methoxydihydrochalcone (da Silva and Filho 2002). S. birrea has been reported to contain many compounds including polyphenols, tannins, coumarins, flavonoids, triterpenoids, phytosterols, oils, organic acids, etc. (Watt and Breyer-Brandwijk 1962; van Wyk et al. 2002; Ojewole 2007).

**Materials and Methods**

**Plant Samples**

**Collection and Identification**

Fresh samples of the leaves—for identification and stem bark of B. rufescens Lam and S. birrea (A. Rich.) Hochst were collected in June 2008 from Gathala village, Gwoza, Nigeria (Long. 13° 31.369’ E, Lat. 11° 00.562’ N). Plant specimens were identified and authenticated by a plant taxonomist, Prof. S. S. Sanusi, Department of Biological Sciences, University of Maiduguri. The herbarium specimen was then deposited at the Postgraduate Research Laboratory, Department of Chemistry with voucher specimen number #003/2008 for B. rufescens Lam and #004/2008 for S. birrea (A. Rich.) Hochst. The stem bark of B. rufescens and S. birrea were cleaned, chopped into pieces, air-dried under shade for seven days, pulverised into fine powder and then coded.

**Extraction of Plant Materials**

The air-dried powdered plant material (2000 g) each was extracted exhaustively with 85% methanol in distilled water using soxhlet apparatus as described by Lin et al. (1999). This is because previous studies have reported that methanol was a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents such as water, ethanol and hexane (Ahmad et al. 1998; Lin et al. 1999). The combined methanolic extracts were concentrated to dryness at reduced pressure using rotary evaporator and the extract coded “BRME” – B. rufescens methanol extract and “SBME”- S. birrea methanol extract. BRME and SBME were then subjected to preliminary phytochemical screening and *in vitro* antimicrobial susceptibility test and their MIC and MBC determined.

**Phytochemical Screening**

A little quantity each of BRME and SBME were subjected to preliminary qualitative chromogenic phytochemical tests for the presence of the following secondary plant metabolites: alkaloids, carbohydrates, flavonoids, saponins, tannins, glycosides (cardiac, steroidal), terpenes/terpenoids, resins, aloes utilising standard conventional protocols as described by Harborne (1973), Brain and Turner (1975), Vishnoi (1979), Markham (1982), Farnsworth

**Antimicrobial studies**

**Test microorganisms**

The Gram+ organisms used in this study were: *Bacillus subtilis* (BC), *Corynebacterium spp.* (CR), *Shigella dysenteriae* (SG), *Staphylococcus aureus* (SA) and *S. epidermidis* (SP), while Gram– organisms were: *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), Pseudomonas aeruginosa (PS), *Salmonella typhi* (ST) and *Proteus vulgaris* (PV); fungal strains were: *Aspergillus flavus* (AF), *A. niger* (AN) and *Candida albicans* (CA). These organisms were clinical laboratory isolates obtained from the Department of Medical Microbiology and Department of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria. Standard susceptibility antibiotic discs used were: Ciprofloxacin (5 μg/disc); Erythromycin (5 μg/disc), Gentamicin (10 μg/disc), produced by Oxo Ltd., Hampshire, UK.

**Antimicrobial susceptibility studies**

The crude methanol extract of *B. rufoexecus* and *S. birrea* were subjected to preliminary antimicrobial evaluation on five Gram+, five Gram– and three fungal strains using the hole-in-plate disc diffusion technique as described by Forbes et al. (1990), Vletinck et al. (1995) and Usman et al. (2007a).

The extracts were made in four different stock concentrations of 50, 100, 200 and 400 mg/ml prepared by dissolving 0.5, 1.0, 2.0 and 4.0 g respectively into 10 ml each of 85% methanol in distilled water (v/v) – as vehicle. The microorganisms were main- treated by Oxoid Ltd., Hampshire, UK.

**Comparative phytochemical and antimicrobial evaluation:** B. rufescent and S. birrea stem bark extracts. Usman et al. 2008).

The minimal inhibitory concentration (MIC) was determined using the broth dilution technique as described by Volleková et al. (2001). The MIC value was determined for the microorganisms that were sensitive to the extracts under study. Each extract was first diluted to the highest concentration (100 mg/ml) in 85% methanol in distilled water (v/v), and then a two-fold serial dilution of each extract was then made to a concentration ranging from 0.098 to 50 mg/ml using nutrient broth (13 g/l). To the suspension, 5 ml of each extract concentration was added into nutrient broth and then 1.0 ml of standardized broth cultures containing 1.0 MIC 10³ CFU/ml were seeded into each test tube and then incubated at 35°C for 18–24 hrs. MIC was defined as the lowest concentration where no turbidity was observed in the test tubes.

**Determination of minimum bactericidal concentration (MBC)**

The minimal bactericidal concentration (MBC) was determined using the broth cultivation previously described by Volleková et al. (2001) as adopted by Usman et al. (2007a, 2007b) by assaying the test tubes resulting from MIC determinations. A loopful of the content of each test tube was then inoculated by streaking on a solidified nutrient agar plate and then incubated at 37°C for 24 hrs for possible bacterial growth. The lowest concentration of the sub-culture that shows no bacterial growth was considered the MBC.

**Statistical analysis of data**

Statistical analysis involved the determination of mean differences among the zone of inhibition exhibited by the extracts against each organism and the standard antibiotics. The data were analysed using one-way ANOVA with Student-Newman-Keul’s multiple comparison test performed using GraphPad InStat (GraphPad Software 1998).

**RESULTS AND DISCUSSION**

**Phytochemical constituents**

The plant materials were extracted with methanol; the choice of methanol as solvent was based on the earlier works of Ahmad et al. (1998), Güllüce et al. (2004), Parekh et al. (2006) and Nebedum et al. (2009); these authors reported that an organic solvent, especially methanol, was a better solvent for consistent extraction of antimicrobial substances from medicinal plants compared with other solvents such as water, hexane and ethanol. The extractive value of BRME was 15.37% w/w (reddish-brown in colour) and SMME was 17.90% w/w (dark brown in colour) and SMME was 15.37% w/w (reddish-brown in colour). The phytochemical constituents of the plant extracts are presented in Table 1. Cardiac glycosides, saponins, flavonoids, resins, aloes, tannins, phlobatannins, cardenolides, etc., as well as carbohydrates were present in most plant extracts studied. Alkaloids were absent in both extracts but anthraquinone derivatives were present only in BRME. These secondary plant metabolites have been reported by many authors to be responsible for most pharmacological and biological effects both in vitro and in vivo exhibited by plant extracts. Both extracts showed a considerable amount of tannins and flavonoids; tannins have been reported to inhibit the growth of microorganisms by precipitating microbial protein and making nutritional protein unavailable to them (Ogunleye and Isbitoye 2003; Idu 2007); the antimicrobial effects of flavonoids have been attributed to their ability complex with extracellular, soluble protein and to complex with bacterial cell wall proteins (Cowan 1999; Musa et al. 2008). The activity of these extracts at higher doses against Gram+ and Gram– bacteria may be indicative of the presence of broad spectrum antibiotic compounds in the plants, notably cardiac glycosides, saponins, flavonoids, resins, aloes, tannins, phlobatannins, cardenolides. Flavonoids have been known to be synthesised by plants in response to microbial infection (Dixson et al. 1983; Al-Bayati and Al-Mola
2008); thus it is not surprising to express such effects in vitro against a wide array of microorganisms (Al-Bayati and Al-Mola 2008). It is therefore, probable that the flavonoids present in these extracts may behave in a similar manner. Saponins have also been reported to exhibit a wide range of biological activities, especially antibacterial (Al-Bayati and Al-Mola 2008) whose mode of action involves cell membrane lysis and thus saponins in these extracts may equally act in a similar manner.

**Effects of extracts on microorganisms**

The results of the inhibition zone diameters of the two extracts are presented in Figs. 2 and 3; the diameters of inhibition zones exhibited by BRME extract against Gram + organisms were found to be in the range of 11.00 ± 0.00 to 28.17 ± 0.44 mm while 10.67 ± 0.33 to 27.17 ± 0.17 mm was recorded as the range of values against Gram – species (Fig. 2). SBME had the ranges of 13.00 ± 0.00 to 26.50 ± 0.28 mm and 11.67 ± 0.33 to 26.00 ± 0.58 mm values for Gram + and Gram– species, respectively (Fig. 3).

The overall susceptibility data revealed that BRME was comparatively more susceptible to Gram – bacteria, although the inhibition was in particular cases insignificant (P>0.05) against PS, KB, PV, BC, SP (5 mg/hole). The extracts’ activities were found to increase significantly as the dose varied, and analysis of comparative dosages on both extracts revealed that a significant difference (P<0.05, P<0.001) was observed at 20 and 40 mg/hole for most organisms. The results from both extracts showed similar trend of activities notably due to the presence of common phytocomponents which is suggestive of the reason for their traditionally. No significant difference (P>0.05) was noted between Ciprofloxacin and BRME at 40 mg/hole against ST and SG while variable differences were observed at lower doses, a trend similar to that observed by Parekh et al. (2006). BRME was more susceptible to Gram - bacteria while susceptibility against Gram + was higher on SBME. There was no significant difference (P>0.05) as observed in Fig. 3 between Ciprofloxacin and SBME on BC, SG, or PS at 20 and 40 mg/hole and also at 10 and 20 mg/

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>5 mg/hole</th>
<th>10 mg/hole</th>
<th>Ciprofloxacin (5 μg/disc)</th>
<th>Gentamicin (10 μg/disc)</th>
</tr>
</thead>
</table>
| **Fig. 2** Effects of methanol stem bark extract of Bauhinia rufescens against some pathogenic microorganisms. Values represent mean ± SEM.
hhole between SA. Similarly, no significant effects were noted at 20 mg/hole between Ciprofloxacin and BRME against KB and PS while at 40 mg/hole the extract showed insignificant (P>0.05) different effects against SG, EC and PS (Fig. 2).

Gentamicin (a potent Gram− antibiotic) recorded insignificant activity (P>0.05) compared to BRME at 40 mg/hole against KB and PS, against PV at 5 and 10 mg/hole and at 20 mg/hole against PV SBME at 20 and 40 mg/hole exhibited a non-significant (P>0.05) activity against PV when compared with Gentamicin.

On the other hand, Erythromycin (a potent Gram+ antibiotic) compared with BRME at 40 mg/hole against Gram+ bacteria studied revealed non-significant (P>0.05) effects against SG; SBME at 20 and 40 mg/hole showed insignificant differences (P>0.05) in activity SG and at 20 mg/hole against SA. Overall, the remaining activity revealed significant (P<0.05, 0.01, 0.001) differences compared to all the antibiotics considered in this study. The two extracts were resistant to AF and AN but little activities were expressed by only SBME on CA.

**MIC and MBC of the susceptible microorganisms**

The MIC and MBC are presented in Table 2. The data obtained from BRME against the tested microorganisms as

### Table 2: Minimum inhibitory and minimum bactericidal concentrations of methanol stem bark extracts of *B. rufescens* and *S. birrea* against susceptible organisms.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Concentrations (mg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>50.00</td>
</tr>
<tr>
<td><strong>Bacillus subtilis</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Corynebacterium spp.</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Shigella dysenteriae</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Klebsiella spp.</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Proteus vulgaris</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Salmonella typhi</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Candida albicans</strong></td>
<td>-</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM.}

**Fig. 3** Effects of methanol stem bark extract of *Sclerocarya birrea* against some pathogenic microorganisms. Values represent mean ± SEM.
MIC and MBC ranged from 0.78-6.25 and 1.56-12.50 mg/ml respectively. Values ranging from 1.56-12.50 and 1.56-25.00 mg/ml were recorded as the MIC and MBC, respectively expressed by SBME against the organisms studied. BRME was more susceptible to Gram+ bacteria since the MIC/MBC value for ST and the MBC value for EC were both 1.56 mg/ml. SBME, on the other hand, recorded a MIC/MBC value and also an MBC value of 3.13 mg/ml against CR, SA and SP, respectively. Table 3 shows the susceptibility pattern of the two extracts, confirming that BRME was highly susceptible to Gram− than Gram+ organisms; while SBME was more susceptible to Gram+ compared to Gram− organisms. These were supported by the spectral intensity index (SII), where BRME had SII of 19.65 against Gram and 17.90 for Gram species; the SII expressed by the SBME was found to be 19.20 and 18.78 against Gram+ and Gram− organisms respectively. The AI of SBME was found to be 19.20 and 18.65 mg/ml against CR, SA, and SP, respectively expressed by SBME against the organisms studied. 25.00 mg/ml were recorded as the MIC and MBC, respectively. Values ranging from 1.56-12.50 and 1.56-25.00 mg/ml were recorded as the MIC and MBC, respectively expressed by SBME against the organisms studied. BRME was more susceptible to Gram+ bacteria since the MIC/MBC value for ST and the MBC value for EC were both 1.56 mg/ml. SBME, on the other hand, recorded a MIC/MBC value and also an MBC value of 3.13 mg/ml against CR, SA and SP, respectively.

Table 3 Susceptibility pattern of Bauhinia rufescens and Sclerocarya birrea against some pathogenic organisms

<table>
<thead>
<tr>
<th>Extract</th>
<th>EC</th>
<th>KB</th>
<th>PV</th>
<th>PS</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRME</td>
<td>74.21</td>
<td>141.38</td>
<td>92.37</td>
<td>81.37</td>
<td>64.30</td>
</tr>
<tr>
<td>SBME</td>
<td>68.44</td>
<td>130.38</td>
<td>84.75</td>
<td>74.65</td>
<td>70.47</td>
</tr>
</tbody>
</table>

a, Ciprofloxacin; b, Erythromycin; c, Gentamicin; BC, Bacillus subtilis; CR, Corynebacterium spp; SA, Staphylococcus aureus; SP, Streptococcus pneumoniae; SG, Shigella dysenteriae; EC, Escherichia coli; KB, Klebsiella spp; PV, Proteus vulgaris; PS, Pseudomonas aeruginosa; ST, Salmonella typhi; T, total; Y, mean

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