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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 *Salmonella typhi* and the MBC value for *E. coli*. SBME had an MIC/MBC value of 3.13 mg/ml for *Corynaebacterium* 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
Abbreviations: **AI**, activity index; **AF**, *Aspergillus flavus*; AN, *Aspergillus niger*; **BC**, *Bacillus subtilis*; **BRME**, *Bauhinia rufescens* methanol extract; **CA**, *Candida albicans*; **CR**, *Corynaebacterium* spp.; **EC**, *Escherichia coli*; **KB**, *Klebsiella* spp.; **MBC**, minimum bactericidal concentration; **MIC**, minimum inhibitory concentration; **NS**, not susceptible; **NT**, not tested; **PS**, *Pseudomonas aeruginosa*; **PA**, percent activity; **PV**, *Proteus vulgaris*; **SA**, *Staphylococcus* aureus; **SBME**, *Sclerocarya birrea* methanol extract; **SG**, *Shigella dysenteriae*; **SII**, spectral intensity index; **SP**, *Streptococcus pneumoniae*; **ST**, *Salmonella typhi*

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 scandent shrub or small tree (**Fig. 1A**) belonging to the giant family Leguminosae, subfamily Leguminosae-Caesalpiniodeae; 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

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Research Note

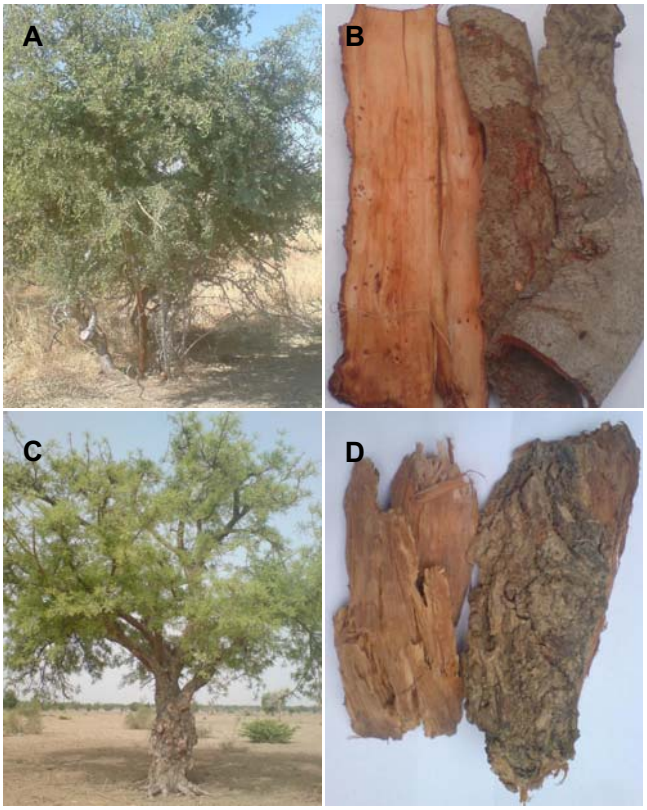

Fig. 1 Trees and stem bark of *Bauhinia rufescens* (A-B) and *Sclerocarya birrea* (C-D) in Gathla-Gwoza, Borno State, Nigeria (Pictures by H. Usman 2009).

pods, twisted, up to 10 cm long, glaborous, obliquely constricted, shining dark red-brown, with 4-10 seeds each. Pods remain on the shrub for a long time (FAO-UNEP 1983; Burkill 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; occuring throughout West Africa and extends across Africa up to Sudan. It has wide array of medicinal and socio-cultural uses.

S. birrea (A. Rich.) Hochst is a tree (**Fig. 1C-D**) about 13 m high with a short bole sometimes reaching up to 2.5 m in girth, of the drier Sahel Savannah occurring from Senegal to Niger and Northern Nigeria and found also across Africa to Ethiopia and Uganda (Burkill 1985). It is used extensively for its medicinal and socio-cultural values in Northern Nigeria.

The leaves, bark, roots of these plants have been utilised mostly for different forms of illnesses, such as treatment of diarrhoea, dysentery, leprosy, conjunctivitis, anticonvulsant, analgesic, insecticidal, stomachic, diabetes and wound healing, antibacterial, hypoglycaemic (Burkill 1985; Galvez *et al.* 1991, 1993; Burkill 1995; Eloff 2001; Ojewole 2004, 2007). The bark of the roots and stem of *B. rufescens* is used to cure chest complaints, syphilis and other venereal diseases, leprosy, diarrhoea and dysentery and to reduce fever (Vogt 1996).

B. rufescens and *S. birrea* are locally called in various Nigerian languages respectively as: *matsattsagi* and *danya* in Hausa, *sàsà* or *shishi* and *kēmáà* in Kanuri, *shasha* and *doko* in Waha, *nammare* and *heri* in Fulfulde, *kulkul* and *homeid* in Shuwa-Arab. These plants were known to have wide array of medicinal and socio-cultural uses (Burkill 1985, 1995). *S. birrea* (A. Rich.) Hochst is widely distributed mainly in the drier Sahel savannah regions from Senegal to Niger and Northern Nigeria and also across Africa to Ethiopia and Uganda.

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; occuring 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 or antipyretic in local medicine. Leaves and fruits are applied for the treatment of diarrhoea, dysentery and ophthalmic 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-rhamnopyranosylkaempferol have been isolated in brazil from *B. fortificata* L. (Menezes *et al.* 2007).

Other compounds isolated from various sort of *Bauhinia* genus are bauhinina, 5-hydroxy-7-methoxyflavone-6-*O*-β-xylopyranoside, bausplendina, racemosol [preracemosol A] and des-*O*-methylracemosol [preracemosol B]. Other chalcone type compounds isolated are 4′-methoxyisoquiritigenin and 2,4′-dihydroxy-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 Gathla 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 distillled 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 susceptiblity 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

(1989), Farnsworth and Euler (1962), Sofowora (1993), Silver *et al.* (1998), Trease and Evans (2002), and Ciulei (1982).

Antimicrobial studies

Test microorganisms

The Gram$^+$ organisms used in this study were: *Bacillus subtilis* (BC), *Corynaebacterium* spp. (CR), *Shigella dysenteriae* (SG), *Staphylococcus aureus* (SA) and *Streptococcus pneumoniae* (SP), while Gram$^-$ organisms were: *Escherichia coli* (EC), *Klebsiella* spp. (KB), *Pseudomonas aeruginosa* (PS), *Salmonella typhi* (ST) and *Proteus vulgaris* (PV); fungal strains were: *Aspergillus flavus* (AF), *Aspergillus 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 Oxoid Ltd., Hampshire, UK.

Antimicrobial susceptibility studies

The crude methanol extract of *B. rufescens* 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), Vlietinck *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 maintained on agar slants until use. The inocula were then prepared by subjecting the test organisms to nutrient broth and incubated for 24 hrs at 37°C. After incubation, the broth cultures were diluted to 1: 1000 for Gram$^+$ bacteria and 1: 5000 for Gram$^-$ bacteria. 1 ml of the diluted cultures was inoculated into 19 ml sterile molten nutrient agar (48°C) and Sabouraud's dextrose agar prepared according to manufacturer's specification was poured into sterile Petri dishes. These were gently swirled and allowed to solidify. Afterwards, holes of 9 mm diameter were bored onto the solidified and inoculated nutrient agar plates using a sterilised number VI cork borer. All the holes were filled with equal volumes of 0.1 ml of each extract equivalent to 5, 10, 20, 40 mg/hole. Standard discs were placed on bacterial-inoculated nutrient agar plate; the extracts were allowed to diffuse into the agar for an hour. Thereafter, plates were then incubated overnight at 35 and 37°C for fungi and bacterial strains, respectively. At the end of the incubation period, inhibition zones were recorded in mm as the diameter of growth-free zones around the bored holes using a transparent metre rule. The extract was independently tested in triplicate. Diameters of zones of inhibition ≥ 10 mm exhibited by plant extracts were considered active (Zwadyk 1972; Usman *et al.* 2007a).

Activity index (AI)

This was estimated as 100 × diameters of inhibition zone of extract ÷ diameters of inhibition zone of the standard (expressed as %) (Shahidi 2004).

Percent actvitiy (PA)

This was calculated as 100 × number of susceptible strains to a specific extract ÷ total number of tested bacterial strains. This will be expressed as % Gram$^+$, % Gram$^-$ and %T as total activity against both Gram$^+$ and Gram$^-$ (Shahidi 2004).

Spectral intensity index (SII)

This was determined as mean diameters of inhibition zones (mm) of all sensitive bacterial strains to a specific sample × %T ÷ 100 (Shahidi 2004).

Determination of minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) was determined using the nutrient broth dilution technique as described by Vollekovà *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 10^7 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 minimum bactericidal concentration (MBC) was determined using the broth dilution technique previously described by Vollekovà *et al.* (2001) as adopted by Usman *et al.* (2007a, 2007b) by assaying the test tubes resulting from MIC determinations. A 1oopful 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 17.90% w/w (dark brown in colour) and SBME 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 Ibitoye 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 (Dixcon *et al.* 1983; Al-Bayati and Al-Mola

Table 1 Phytochemical constituents of methanol stem bark extracts of *B. rufescens* and *S. birrea.*

Group Constituents	Test	Results	
		B. rufescens	***S. birrea***
Alkaloids	Dragendorff's	-	-
	Mayer's	-	-
	Wagner's	NT	NT
Aloes		+	+
Anthraquinones			
Free anthraquinones	Borntrager's	+	-
Combined anthraquinones	Borntrager's	+	-
Carbohydrates			
General test	Molisch's	+	+
Monosaccharide	Barfoed's	+	+
Free reducing sugar	Fehling's	+	+
Combined reducing sugar	Fehling's	+	+
Ketoses	Salivanoff's	+	-
Pentoses	Phloroglucinol	+	+
Cardenolides	Legal's	+	+
	Keller-Kalian's	+	+
Cardiac glycosides			
Steroidal nucleus	Salkowski's	+	-
Steroidal nucleus	Liebermann-Buchard's	+	+
Terpenoids	Liebermann-Buchard's	+	+
Flavonoids	Shinoda's	+	+
	$FeCl_3$	+	+
	Lead ethanoate	+	+
	NaOH	-	-
Phlobatannins		+	+
Resins		+	+
Saponins glycosides	Frothing's	+	+
	Fehling's	+	+
Tannins	$FeCl_3$	+	+
	Lead ethanoate	+	+
	10% HCl	+	+
	Goldbeater's	+	+

+ = present; - = absent; NT = not tested

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 studied (**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 use in 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/

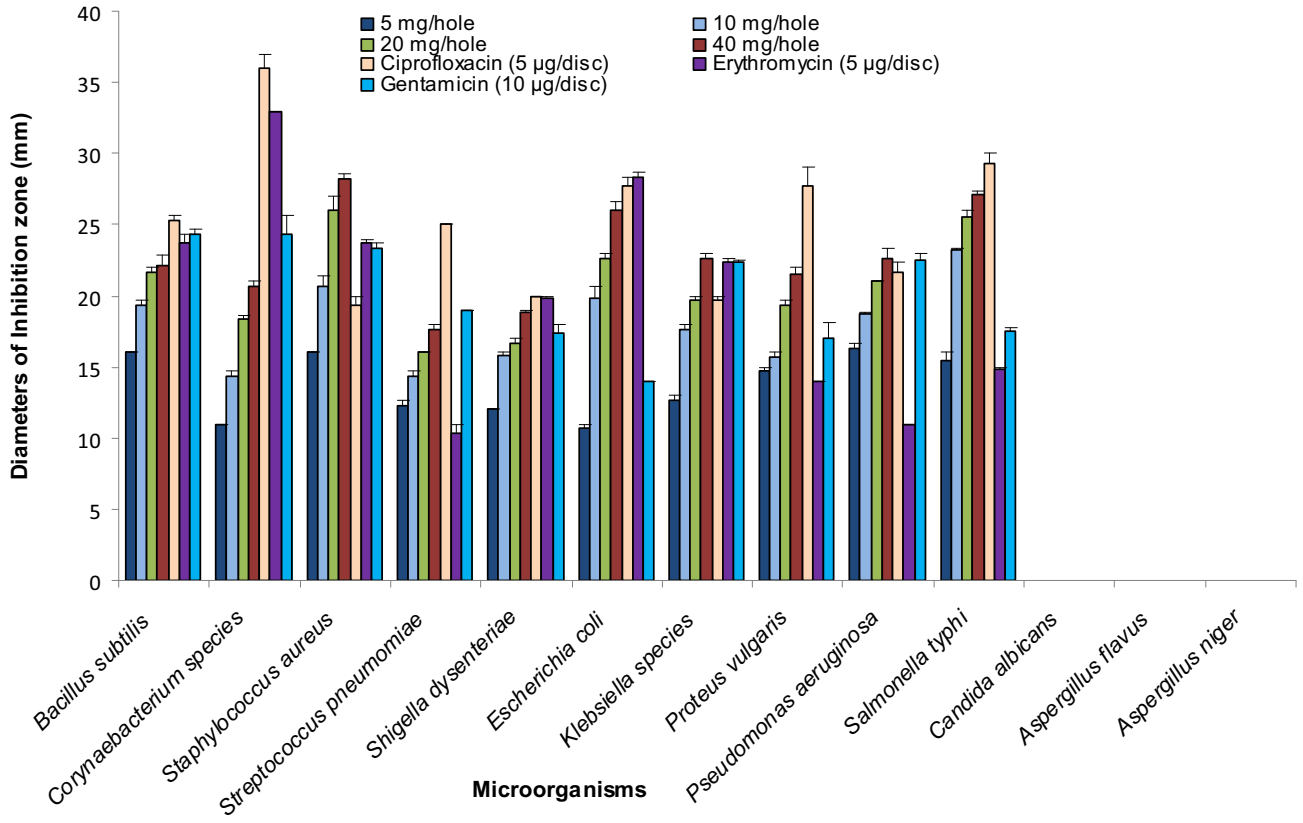

Fig. 2 Effects of methanol stem bark extract of *Bauhinia rufescens* against some pathogenic microorganisms. Values represent mean ± SEM.

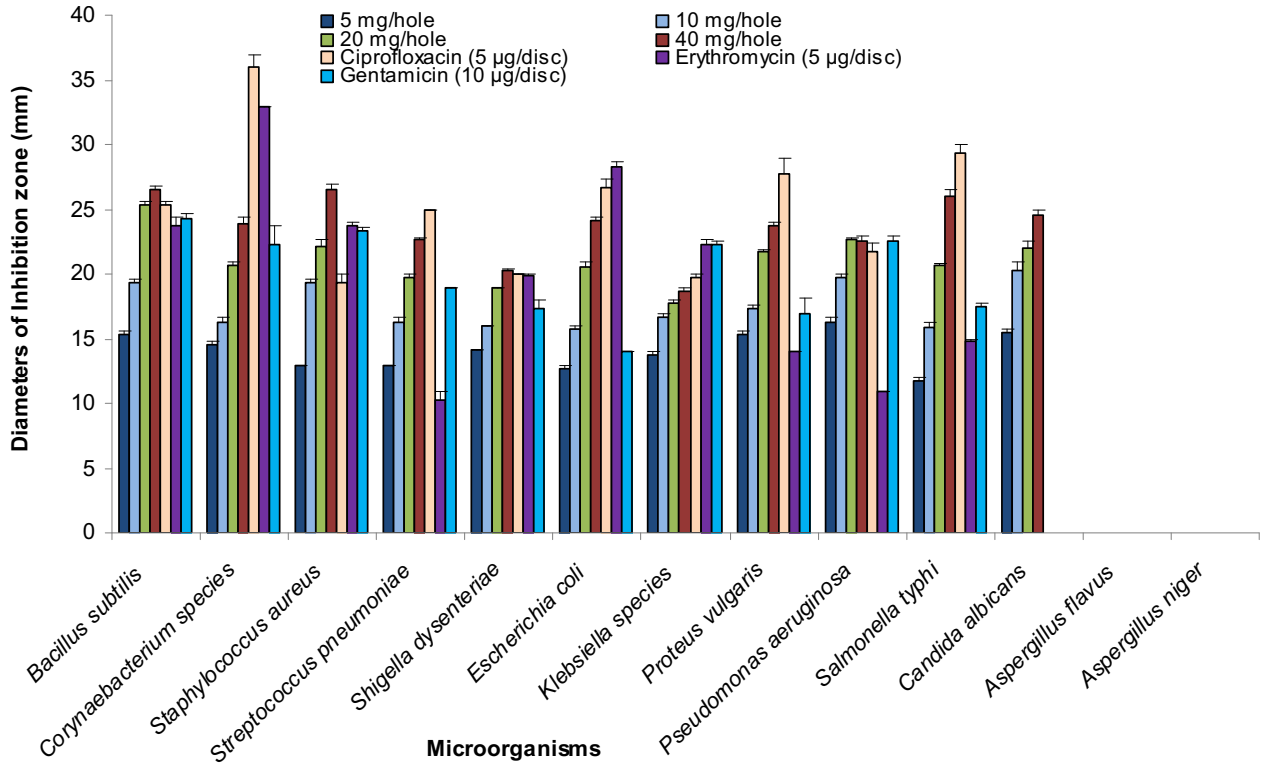

Fig. 3 Effects of methanol stem bark extract of *Sclerocarya birrea* against some pathogenic microorganisms. Values represent mean ± SEM.

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

Organisms	**Concentrations (mg/ml)**									
	50.00	**25.00**	**12.50**	**6.25**	**3.13**	**1.56**	**0.78**	**0.39**	**0.20**	**0.01**
Bacillus subtilis	-	-	-	-	-	δ α	+	+	+	+
	-	-	µ	π	+	+	+	+	+	+
Corynaebacterium spp.	-	-	-	-	α	α	+	+	+	+
	-	-	-	-	µ	π	+	+	+	+
Shigella dysenteriae	-	-	δ	α	+	+	+	+	+	+
	-	-	µ	π	+	+	+	+	+	+
Staphylococcus aureus	-	-	-	δ	α	+	+	+	+	+
	-	-	-	-	µ π	+	+	+	+	+
Streptococcus pneumoniae	-	-	-	-	-	δ α	+	+	+	+
	-	-	-	-	µ	π	+	+	+	+
Escherichia coli	-	-	-	-	-	δ	α +	+	+	+
	-	-	-	-		µ π		+	+	+
Klebsiella spp.	-	-	δ -	α	+	+	+	+	+	+
	-	-		-	µ π	+	+	+	+	+
Proteus vulgaris	-	-	-	δ	α	+	+	+	+	+
	-	µ	π	+	+	+	+	+	+	+
Pseudomonas aeruginosa	-	-	-	-	α δ	+	+	+	+	+
	-	-	-	-	µ	π	+	+	+	+
Salmonella typhi	-	-	-	-	-	δ α	+	+	+	+
	-	-	µ	π	+	+	+	+	+	+
Candida albicans	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

α = MIC value for *B. rufescens*; δ = MBC value for *B. rufescens*; π= MIC value for *S. birrea*; µ= MBC value for *S. birrea*; - = not turbid/no growth; + = turbid/growth; NS= not susceptible; NT=not tested.

hole 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 3 Susceptibility pattern of *Bauhinia rufescens* and *Sclerocarya birrea* against some pathogenic organisms.

Extract	Percent activity			Spectral intensity index		
	$\%G^+$	$\%G^-$	%T	G^+	G^-	Y
BRME	100	100	100	17.90	19.65	18.78
SBME	100	100	100	19.20	18.65	18.93

Table 3 (Cont.)

Extract	Activity index (%)									
	BC		CR		SA		SP		SG	
BRME	[a]78.14	[b]83.62	[a]44.68	[b]48.74	[a]87.08	[b]71.12	[a]60.32	[b]71.12	[a]79.16	[b]79.84
SBME	[a]83.35	[b]91.35	[a]52.31	[b]57.07	[a]104.76	[b]85.55	[a]71.67	[b]176.45	[a]86.68	[b]87.40

Table 3 (Cont.)

Extract	Activity index (%)									
	EC		KB		PV		PS		ST	
BRME	[a]74.21	[c]141.38	[a]92.37	[c]81.37	[a]64.30	[c]104.66	[a]90.76	[c]87.41	[a]77.86	[c]130.46
SBME	[a]68.44	[c]130.38	[a]84.75	[c]74.65	[a]70.47	[c]114.71	[a]93.64	[c]90.19	[a]63.22	[c]105.96

a, Ciprofloxacin; b, Erythromycin; c, Gentamicin; BC, *Bacillus subtilis*; CR, *Corynaebacterium* 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

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 an 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 18.65 against $Gram^+$ and $Gram^-$ organisms respectively. The AI was designed to express the relation of the inhibition zone of the extract to that of standard antibiotics; both extracts showed varying levels of comparisons to either the broad spectrum antibiotic (Ciprofloxacin), $Gram^-$ susceptible antibiotic (Gentamicin) or $Gram^+$ susceptible antibiotic (Erythromycin) with BRME showing a much stronger relation to broad spectrum antibiotic while the AI of SBME showed close resemblance to Erythromycin. Finally, the two extracts showed very good activity against the pathogenic strains tested and hence could be a yardstick for their traditional use.

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