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Full length Research Article

Phytochemical and *in-vitro* antibacterial effects of the partitioned portions of *Bauhinia rufescens* Lam stem bark extract

[1*]Usman H, [1]Abdulrahman F.I, [2]Abdu Kaita I, [1]Khan I.Z

[1] *Department of Chemistry, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria*
[2] *Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria*

ABSTRACT: The study into the chemical contents and *in-vitro* antibacterial effects of the portioned portions of the methanol stem bark extracts of *Bauhinia rufescens* Lam were evaluated in some Gram positive and Gram negative bacteria using the hole-in-plate disc diffusion technique. The methanol stem bark extract was successively portioned using solvents of grade polarities: n-hexane, chloroform, ethylacetate, n-butanol and water. Qualitative phytochemical components of these portions were determined using conventional protocol and the overall results indicated the presence of flavonoids, saponins, resins, cardiac glycosides, cardenolides, aloes, anthraquinones and phlobatannins in one or more of the portions. Test microorganisms include *Bacillus subtilis* and *Streptococcus pneumoniae* (Gram positive); *Escherichia coli, Klebsiella spp.* and *Salmonella typhi* (Gram negative) bacteria. The diameters of inhibition zone on the Gram positive bacteria was found in the range of 10.33 ± 0.17 (residual aqueous portion) to 28.00 ± 1.00 (n-butanol portion) while on Gram negative organism the values ranged from 10.83 ± 0.17 (residual aqueous portion) to 29.33 ± 0.17 (chloroform portion). The results revealed that most of the portions were highly sensitive on *S. typhi* with highest MIC/MBC values of 0.78/1.56 mg/ml (n-hexane and ethylacetate portions) and also 1.56/3.13 mg/ml (chloroform and n-butanol portions). The extracts showed equal MIC/MBC data of 1.56 mg/ml on *B. subtilis* exhibited by n-hexane, ethylacetate and n-butanol portions. The activity index (AI) showed that all the portions were more sensitive to *S. pneumoniae* and *E. coli* when computed with erythromycin and Gentamicin respectively. N-butanol portion had the highest AI against *B. subtilis* (77.90 %), *S. pneumoniae* (69.86 %) and *S. typhi* (57.62 %); chloroform recorded higher AI against *E. coli* (91.74 %) while ethylacetate portion had AI of 88.63 % against *Klebsiella spp*. when computed with ciprofloxacin. These portions were found to be more sensitive to Gram negative than gram positive species studied. The study further confirms the use of the part of *B. rufescens* in some parts of Northern Nigeria as a remedy against diarrhoea, dysentery and other related diseases whose causative agents are most of the organisms tested.

Keywords: *Bauhinia rufescens, organic portions, phytochemical, antibacterial, stem bark, in-vitro*.

INTRODUCTION

Briefly, the plant *Bauhinia rufescens* Lam is a scandent shrub or small tree 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 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. (FAO-UNEP 1983; Burkill 1995). The plant is deciduous in the drier area and is an evergreen in wetter areas, 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 uses – the stem bark is

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E-mail: yaroabdulng@yahoo.com

astringent and also used to cure diarrhoea, dysentery, leprosy and to reduce fever (Burkill, 1995; TopTropicals, 2009) and some socio-cultural uses . The core aim of this work was to further validate our earlier work (Usman et al., 2009b) that the crude methanol extract is active against the Gram negative organisms studied. This work however was aimed at partitioning the crude extract into various portions using solvents of graded polarities and then subject the extract on the type of organisms earlier worked on.

MATERIALS AND METHODS

Collection and Identification of Plant: The stem bark of *Bauhinia rufescens* was collected in June 2008 from Gathla village, Gwoza, Borno State, Nigeria (Long. 13^o 31.369'E, Lat. 11^o 00.562'N). The plant specimen was identified and authenticated by a Plant Taxonomist, Prof. S.S Sanusi, with the Department of Biological Sciences, University of Maiduguri. The herbarium specimen was then deposited at the Post graduate Research Laboratory, Department of Chemistry and a voucher specimen number - #003/2008 provided. The stem bark of the plant was cleaned, chopped into pieces, air-dried under shade for seven days and pulverised into fine powder and coded *"Plant material"*.

Extraction of Plant Materials: The air-dried powdered plant material (2000 g) was extracted exhaustively with 85 % methanol in distillled water using soxhlet apparatus as described by Lin *et al.* (1999). Report from previous studies (Ahmad *et al.* 1998; Lin *et al.* 1999) 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. The combined methanolic extracts were concentrated to dryness at reduced pressure using rotary evaporator and the extract coded "BRME" – *Bauhinia rufescens methanol extract.* About 200 g of the BRME was successively partitioned with n-hexane, chloroform, ethylacetate, n-butanol and residual aqueous portions also coded NH, CF, EA, NB and RA respectively and then evaporated to dryness and kept at 4 ^{o}C till use. The portions were then subjected to preliminary phytochemical screening and *in vitro* antimicrobial susceptiblity test while the MIC and MBC determined accordingly.

Qualitative Phytochemical Screening: A little quantity each of the partitioned portions of the methanol extracts of BRME were subjected to preliminary qualitative 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 protocol 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), Ciulei (1982).

Antimicrobial studies

Test microorganisms: The Gram positive organisms were: *Bacillus subtilis, Streptococcus pneumoniae*, Gram negative organisms used in this study were: *Escherichia coli, Klebsiella spp., Salmonella typhi*. Standard susceptibility antibiotic discs used were: Ciprofloxacin (5 µg/disc); Erythromycin (5 µg/disc), Gentamicin (10 µg/disc), produced by Oxoid Ltd., Hampshire, England. These organisms were clinical isolates obtained from the Department of Medical Microbiology and Department of Veterinary Medicine, University of Maiduguri, Maiduguri-Nigeria.

Antimicrobial susceptibility studies : The portioned portions of the methanol stem bark extract of *B. rufescens* was subjected to preliminary antimicrobial evaluation on two Gram positive and three Gram negative strains using the hole-in-plate disc diffusion technique as described by Forbes *et al.* (1990); Vlietinck *et al.* (1995); Usman *et al.* (2007a,).

The extracts were made in five different stock concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml; prepared by dissolving 0.125 g, 0.25 g, 0.50 g, 1.00 g and 2.00 g respectively into 10 ml each of 85 % methanol in distilled water (v/v) – as vehicle. The micro organisms were maintained on agar slants until use. The inocula was then prepared by subjecting the test organisms in nutrient broth and incubated for 24 hours at 37^{o}C. After incubation, the broth cultures were diluted to 1:1000 for Gram positive bacteria and 1:5000 for the Gram negative bacteria. One millilitre of the diluted cultures was inoculated into 19 ml sterile molten nutrient agar (48^{o}C) and sabaround 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 sterilized number VI cork borer. All the holes were filled with equal volumes of 0.1 ml of each portioned portion equivalent to 1.25, 2.5, 5.0, 10, 20 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 ^{o}C and 37 ^{o}C for fungi and bacterial strains respectively. At the end of

the incubation period, inhibition zones were recorded in millimeters as the diameter of growth-free zones around the bored holes using a transparent meter rule. The extract was independently tested in triplicate. Diameters of zones of inhibition $\geq$ 10 mm exhibited by plant extracts were considered active (Zwadyk 1972; Usman *et al.,* 2007a).

Activity index (AI): This was estimated as: 100 x diameters of inhibition zone of extract ÷ diameters of inhibition zone of the standard antibiotic (expressed as %) (Shahidi 2004).

Percent actvitiy (PA): This was calculated as 100 x number of susceptible strains to a specific extract ÷ total number of tested bacterial strains. This will be expressed as % Gram positive, % Gram negative and %T as total activity against both Gram positive and Gram negative (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 x %T ÷ 100 (Shahidi 2004).

Determination of minimum inhibitory concentration (MIC): The MIC was determined using the nutrient broth dilution technique as described by Volleková *et al.* (2001). The minimum inhibitory concentration value was determined for the microorganisms that were sensitive to the extract(s) under study. Each extract was first diluted to the highest concentration (50 mg/ml) in 85 % methanol in distilled water (v/v); two-fold serial dilution of each extracts were then made to a concentration ranging from 0.039 to 25 mg/ml using nutrient broth (13 g/l). To the suspension, 5ml of each extract concentration was added into nutrient broth and then 1.0 ml of standardized broth cultures containing 1.0 x 10^7 CFU/ml were seeded into each test tube and then incubated at 37^o c for 18-24 hours. MIC was defined as the lowest concentration where no turbidity was observed in the test tubes.

Determination of minimum bactericidal concentration (MBC): The MBC was determined using the broth dilution technique previously described by Volleková *et al.* (2001) as adopted by Usman *et al.* (2007a,b) 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 35 ^{o}C for 24 hours for possible bacterial growth. The lowest concentration of the sub-culture that shows no bacterial growth was considered the minimum bactericidal concentration.

Statistics: The statistical analysis involved the determination of mean differences among the zone of inhibition exhibited by the extracts against each organism and the standard antibiotics analysed using One-way ANOVA with Student-Newman-Keuls Multiple comparisons test performed using GraphPad InStat (GraphPad Software, 1998).

.RESULTS AND DISCUSSION

The extractive values of the extracts were found to be 2.34 % w/w (yellowish-brown viscous oil), 6.64 % (yellowish-brown mass), 2.99 % (brown mass), 12.31 % (light-brown mass), 36.71 % (reddish-brown mass) for n-hexane (NH), chloroform (CF), ethylacetate (EA), n-butanol (NB) and residual aqueous (RA) portions respectively. The results of the qualitative phytochemical analysis revealed that alkaloids was absent in all the portions while phlobatannins was present in RA portion only. Flavonoids and cardiac glycosides were present in all the portions; saponins and resins were present in EA, NB, RA only. Cardenolides was only absent in RA but present in others. Aloes was only present in NB but not found in others. Anthraquinones was found in CF, EA, NB.

These secondary metabolites were responsible for most physiological and chemotherapeutic effects exhibited by plant extractives both *in vitro and in vivo* (Hassan *et al.,* 2004; Usman *et al.,* 2009a). Most extracts under study have shown considerable amount of tannins and flavonoids; tannins have been reported to inhibit growth of microorganisms by precipitating microbial protein and making nutritional protein unavailable to them (Ogunleye and Ibitoye 2003; Idu 2007); while the antimicrobial effects of flavonoids have been attributed to their ability to complex with extra cellular, soluble protein and to complex with bacterial cell wall proteins (Cowan 1999; Musa *et al.,* 2008). The flavonoids have been known to be synthesised by plants in response to microbial infection (Dixcon *et al.* 1983; Al-Bayati and Al-Mola, 2008); thus it is not surprising to express such effects *in vitro* against wide array of microorganisms (Al-Bayati and Al-Mola 2008). Therefore, it could be probable that the flavonoidal compounds present in these extracts may equally have related mode of action. Saponins also have been reported to exhibit wide range of biological activities especially antibacterial (Al-Bayati and Al-Mola 2008) whose mode of action involves cell membrane lysis; thus the high activities exhibited by the n-butanol soluble portion could be as a results of it containing most of the secondary metabolites found in most of the extracts. The activities of these extracts were found to be dosages-dependent against Gram-positive and Gram-negative species; this could be indicative of the presence of broad spectrum

antibacterial compounds in the plant, notably anthraquinones, cardiac glycosides, saponins, flavonoids, resins, aloes, tannins, phlobatannins, cardenolides etc.

The results of the diameters of inhibition zones of the five portions (NH, CF, EA, NB, RA) and standard antibiotics were presented in Fig. 1-5. Diameters of inhibition zones exhibited by all the portions at 20 mg/hole is presented in Fig. 1; the results shows that the activities against Gram positive and Gram negative organisms ranges from 10.33 ± 0.17 to 28.00 ± 1.00 mm and 10.67 ± 0.17 to 29.33 ± 0.17 mm respectively.

Table 1:
Phytochemical constituents of partitioned portions of *B. rufescens* methanol stem bark extract

S/N	GROUP CONSTITUENTS	TEST	RESULTS NH	CF	EA	NB	RA
1	Alkaloids	Dragendorff's	-	-	-	-	-
		Mayer's	-	-	-	-	-
		Wagner's	α	α	α	α	α
2	Aloes		-	-	-	+	-
3	Antraquinones						
	Free	Borntrager's	-	+	+	+	-
	Combined	Borntrager's	-	+	+	+	-
4	Carbohydrates						
	General test	Molisch's	-	-	+	+	+
	Monosaccharide	Barfoed's	-	-	-	+	+
	Free reducing sugar	Fehling's	-	-	+	+	+
	Combined reducing sugar	Fehling's	-	-	+	+	+
	Ketoses	Salivanoff's	-	-	+	+	+
	Pentoses		-	-	-	+	+
	Soluble starch		+	-	-	+	+
5	Cardenolides	Legal's	-	-	-	+	+
		Keller-Kiliani's	+	+	+	+	-
6	Cardiac glycosides						
		Salkowski's	+	+	+	+	-
	Steroidal nucleus	L-Buchard's	+	+	+	+	+
	Terpenoids	L-Buchard's	+	+	+	+	+
7	Flavonoids	Shinoda's	+	+	+	+	+
		$FeCl_3$	-	-	+	+	+
		Lead ethanoate	-	-	-	+	+
		NaOH	-	-	-	-	-
8	Phlobatannins		-	-	-	-	+
9	Resins		-	-	+	+	+
10	Saponins	Frothing's	-	-	-	+	+
		Fehling's	-	-	+	+	+
		Heamolysis	-	-	+	+	+
11	Tannins	$FeCl_3$	-	+	+	+	+
		Lead ethanoate	-	-	-	+	+
		10% HCl	-	-	-	-	+
		Goldbeater's	-	-	-	+	-

Key: + = present; - = absent; α = not tested; NH=n-hexane portion, CF=chloroform portion, EA=ethylacetate, NB=n-butanol portion, RA=residual aqueous portion

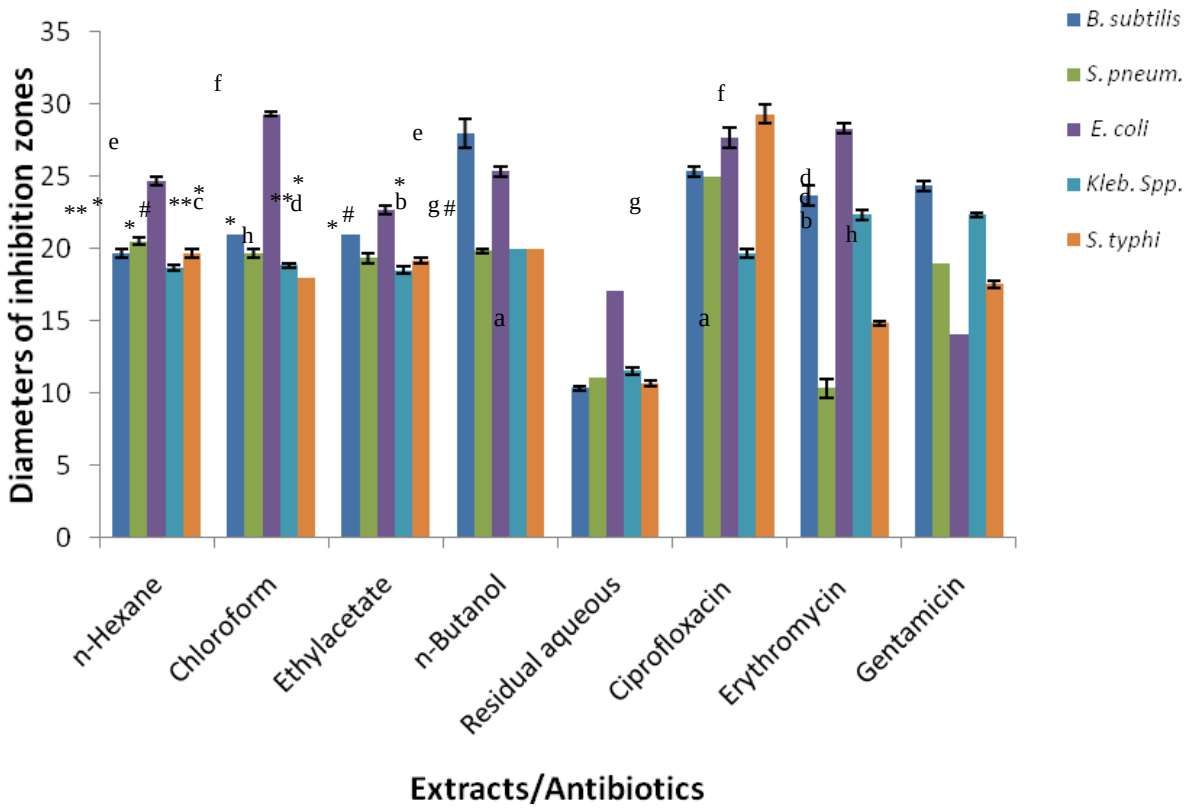

Fig. 1. Diameters of inhibition zones pattern of the partioned portions of *B. rufescens stem bark* extract against some pathogenic bacteria at 20 mg/hole; Same letters or asterisk on same organism's activity are insignifcantly (P>0.05) different

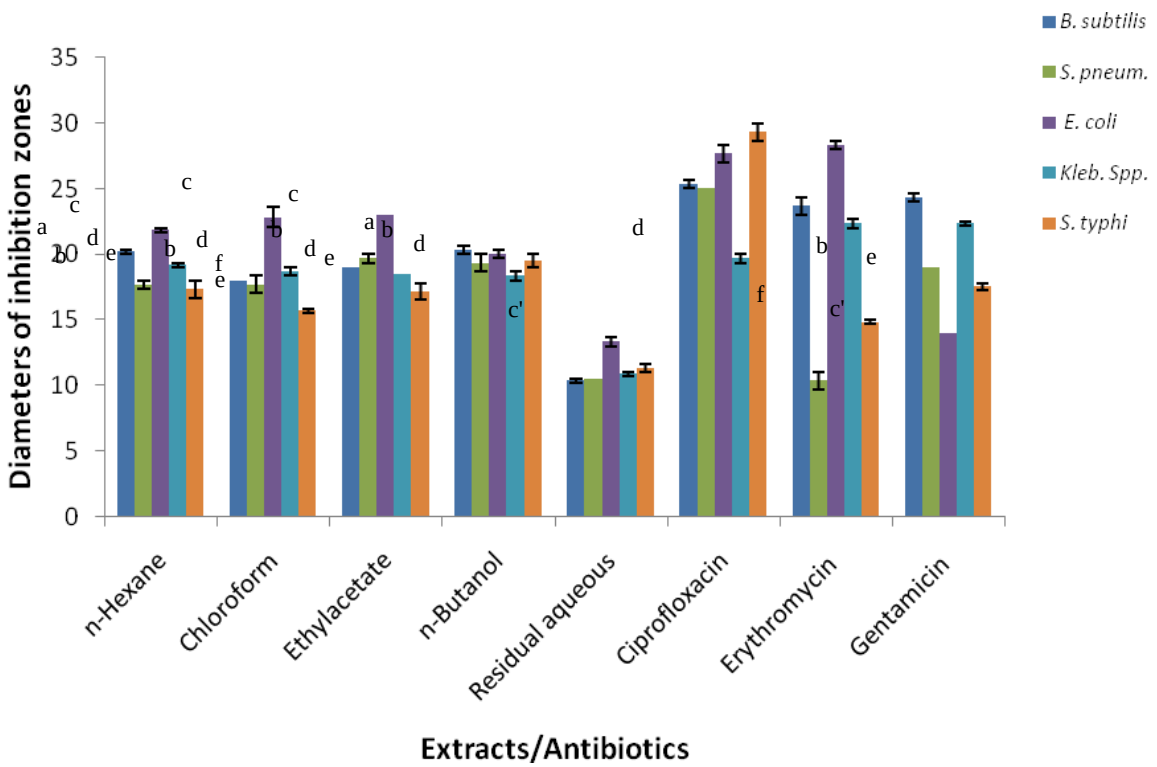

Fig. 2. Diameters of inhibition zones pattern of the partioned portions of *B. rufescens stem bark* extract against some pathogenic bacteria at 10 mg/hole; Same letters on same organism's activity are insignifcantly (P>0.05) different

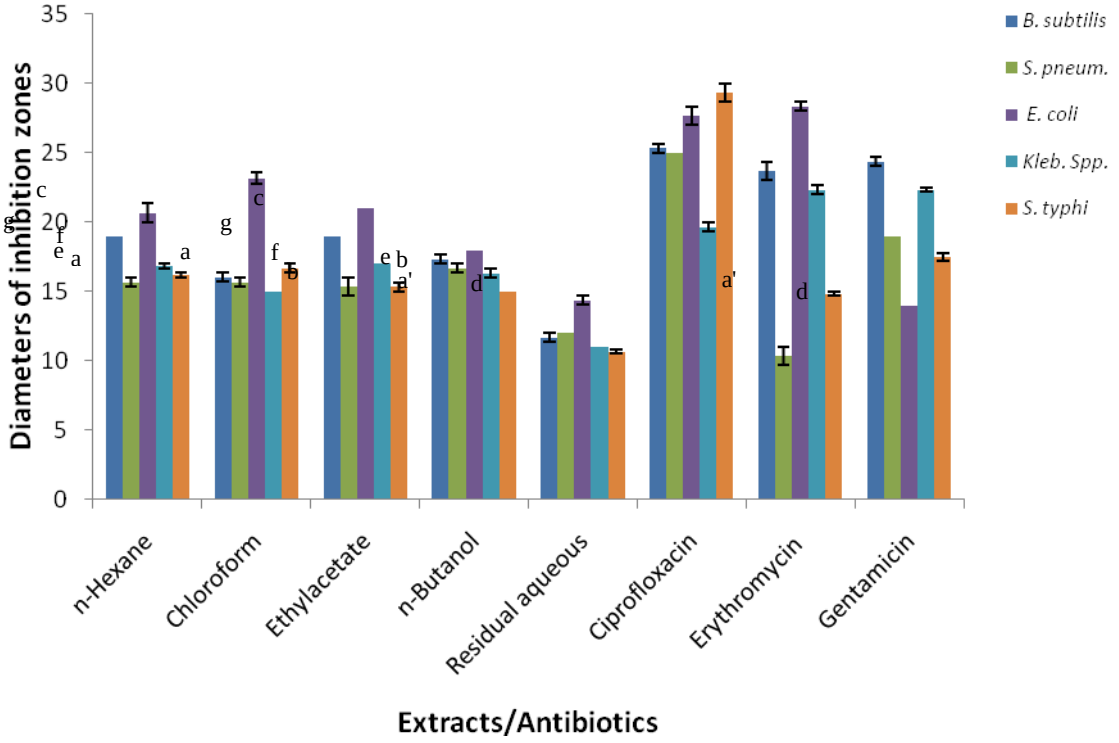

Fig. 3. Diameters of inhibition zones pattern of the partioned portions of *B. rufescens stem bark* extract against some pathogenic bacteria at 5 mg/hole; Same letters on same organism's activity are insignifcantly (P>0.05) different

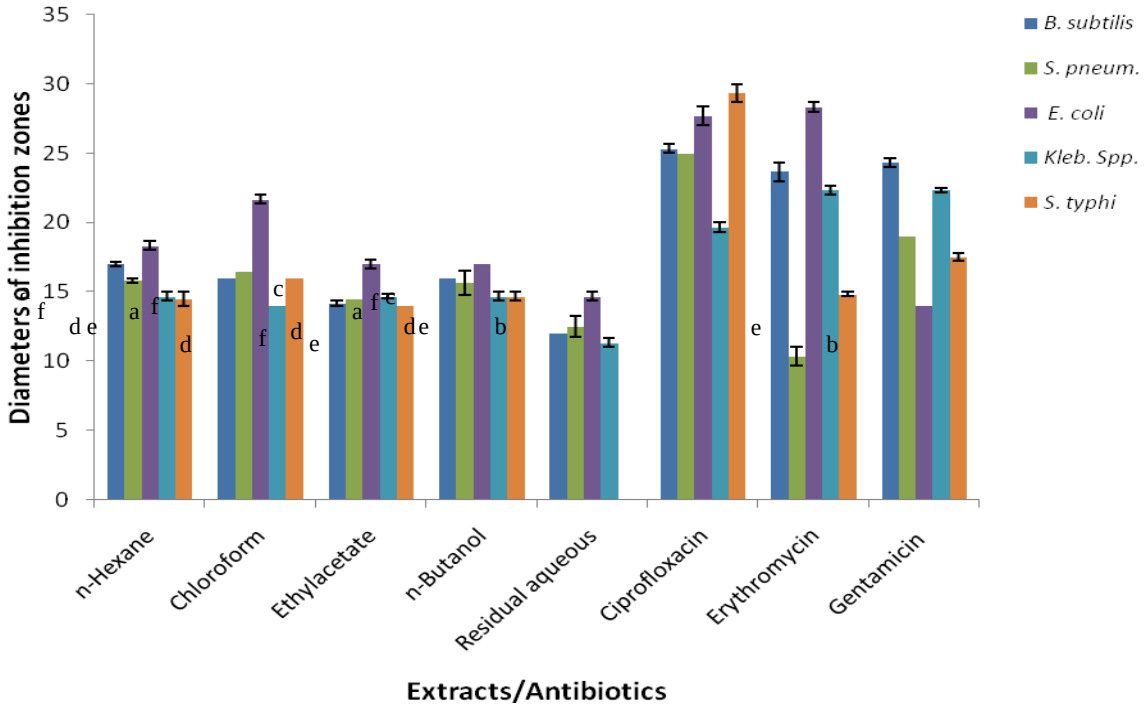

Fig. 5. Diameters of inhibition zones pattern of the partioned portions of *B. rufescens stem bark* extract against some pathogenic bacteria at 1.25 mg/hole; Same letters on same organism's activity are insignifcantly (P>0.05) different

Table 2:
Minimum inhibitory and minimum bactericidal concentrations of partitioned portions of methanol stem bark extract of *B. rufescens*

		Partitioned portions				
		NH	**CF**	**EA**	**NB**	**RA**
		Concentrations (mg/ml)				
S/No.	**Organisms**					
1	*B. subtilis*	6.25** 6.25*	12.5** 6.25*	1.56** 1.56*	1.56** 1.56*	12.5** 6.25*
2	*S. pneumoniae*	12.5** 6.25*	12.5** 6.25*	6.25** 3.13*	12.5** 6.25*	12.5** 6.25*
3	*E. coli*	6.25** 3.13*	3.13** 3.13*	3.13** 1.56*	6.25** 3.13*	12.5** 6.25*
4	*Klebsiella spp.*	6.25** 6.25*	12.5** 6.25*	6.25** 3.13*	3.13** 3.13*	ND** 3.13*
5	*S. typhi*	1.56** 0.78*	3.13** 1.56*	1.56** 0.78*	3.13** 1.56*	12.5** 6.25*

Key: **=MBC value, *=MIC value, ND=Not determined, NH=n-hexane portion, CF=chloroform portion, EA=ethylacetate, NB=n-butanol portion, RA=residual aqueous portion

The data further revealed that the chloroform extracts was more susceptible to *E. coli* while n-butanol portion was effective on *B. subtilis*, which showed significant pattern of activities on these organisms. The activities of the NH, CF and EA were insignificant (P>0.05) different compared to each other. At this dose the inhibition were not significant (P>0.05) compared to Gentamicin (GEN) against *S. pneumoniae*. Similarly, when Erythromycin (ERY) was compared with CF; likewise no significant (P>0.05) between NH and NB against *E. coli*. The broad spectrum antibiotic tested ciprofloxacin CIP) was significantly not different (P>0.05) with NB. Other comparisons between means were significantly (P<0.05, 0.01) different.

Fig. 2 shows the susceptibility pattern of the portions at 10 mg/hole dose, it was observed that the n-butanol portion happened to have a broad spectrum activity against the microorganisms studied. The activities on Gram positive species ranges from 10.33 ± 0.17 to 23.00 ± 0.00 mm; while 10.83 ± 0.17 to 22.83 ± 0.73 mm were recorded as the diameters of inhibition zones against Gram negative organisms. From this figure, it was noted that at this dose the activities across board was more confined to *Escherichia coli* with 23.00 ± 0.00 mm as diameter of inhibition zones expressed by the ethylacetate portion. There was no significant (P>0.05) difference between ERY and CF, GEN and EA, CF, NH against *S. typhi*. The activities against this dose between the NH, CF, NB and GEN were insignificant (P>0.05) against *S. pneumoniae* while CIP and NH, CF, EA, NB were not significantly (P>0.05) different among each other against *Klebsiella spp*. The effects by RA and GEN were not statistically different (P>0.05) against *E. coli*; similarly no significant (P>0.05) difference were noted on the activities of NH, CF, EA against *E. coli*. Other means were statistically different (P<0.05, 0.01, 0.001) compared to each other.

The data from Fig. 3 (5 mg/hole) revealed that, the extract portions had equally variable pattern of activities against the tested bacteria. The highest activity against the Gram positive bacteria was 19.00 ± 0.00 mm exhibited by the NH, EA and NB portions. The results from this figure against Gram negative organisms studied indicated 23.17 ± 0.44 mm as the highest diameters of inhibition zones (DIZ) against *E. coli* expressed by the chloroform portion. The statistical analysis revealed no significant (P>0.05) difference between NB and ERY against *E. coli*. The activities by NH, EA, NB were relatively insignificant (P>0.05) when compared against *Klebsiella spp*; the means of inhibition expressed by NH and CF were also insignificant (P>0.05) against *S. typhi*, similarly between NH and EA against *B. subtilis*. Other tests of means were found to be significant (P<0.01, 0.001).

Fig. 4 (2.5 mg/hole) showed DIZ ranging from 11.00 ± 0.00 to 17.00 ± 0.00 mm against Gram positive bacteria while the DIZ against Gram negative species was 10.33 ± 0.00 25.33 ± 0.33 mm. At this dose, the overall activity was more on the *E. coli* exhibited by the chloroform portion. The inhibition by RA compared to ERY was not significant (P>0.05) against *S. pneumoniae*, equally between ERY and NB, NH against *S. typhi*. The DIZ of NH, CF, NB were found to be insignificant (P>0.05) to each other against *B. subtilis* but other comparisons were variably significant (P<0.01, 0.001).

Table 3:
Sensitivity pattern of partitioned portions of the stem bark extract of *B. rufescens* against some pathogenic organisms

Portion	Percent activity			Spectral intensity index			Activity index (%)									
	%G+	%G-	%T	G+	G-	µ	BS		SP		EC		KB		ST	
NH	100	100	100	17.89	18.15	18.02	[a]73.03	[b]78.16	[a]68.80	[b]166.50	[a]78.74	[c]150.00	[a]85.42	[c]75.24	[a]56.84	[c]95.26
CF	100	100	100	17.17	18.99	18.08	[a]69.48	[b]74.19	[a]67.88	[b]164.28	[a]91.74	[c]174.00	[a]82.53	[c]72.68	[a]55.47	[c]93.09
EA	100	100	100	16.84	18.01	17.43	[a]68.81	[b]73.64	[a]67.20	[b]162.63	[a]77.74	[c]148.00	[a]88.63	[c]78.06	[a]54.11	[c]90.66
NB	100	100	100	18.29	17.72	18.01	[a]77.90	[b]83.35	[a]69.86	[b]169.12	[a]73.49	[c]140.00	[a]84.73	[c]74.65	[a]57.62	[c]96.57
RA	100	93.3	96.7	11.34	11.53	11.44	[a]44.48	[b]47.61	[a]45.60	[b]110.36	[a]55.01	[c]104.76	[a]57.61	[c]50.74	[a]30.34	[c]49.14

Key: BC=*Bacillus subtilis;* SP=*Streptococcus pneumoniae;* EC= *Escherichia coli;* KB= *Klebsiella spp.;* ST= *Salmonella typhi;* µ= mean, Computed with: a= Ciprofloxacin; b= Erythromycin; c= Gentamicin; NH= n-hexane; CF= Chloroform; EA= Ethylacetate; NB= n-butanol; RA= Residual aqueous, G^+=, Gram positive; G^- = Gram negative; T= total

The results of the dose of 1.25 mg/hole were presented in Fig. 5. The data revealed that the activities against Gram positive bacteria was 12.00 ± 0.00 to 18.67 ± 0.33 mm while activities against Gram negative species was 11.17 ± 0.17 to 21.67 ± 0.33 mm. The activities at the lowest concentration were insignificantly different (P>0.05) between ERY and NB, NH, EA against *S. typhi* likewise activities exhibited by NB and EA against *E. coli*. There were variably no significant difference (P>0.05) between the activities of all the portions studied against *S. typhi* and *Klebsiella spp*. The remaining comparisons of DIZ were found to be significantly different (P<0.01, 0.001).

Table 2 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract portions studied. The results revealed that NH, EA, CF and NB were more sensitive on *S. typhi* with highest MIC/MBC value of 0.78/1.56 mg/ml expressed by NH and EA and also 1.56/3.13 mg/ml by CF and NB. The activities of Gram positive organisms were highly susceptible to *B. subtilis* with MIC/MBC data of 1.56 mg/ml exhibited by EA and NB portions; also equal MIC/MBC value were recorded on NH against the same organism.

The sensitivity pattern of the extract portions shown on Table 3 revealed that the portions were variably more susceptible to Gram negative organisms as indicated by the spectral intensity index. The activity index (AI) which relates the activities of the test extracts against antibiotics showed that all the portions were more sensitive to *S. pneumoniae* and *E. coli* when compared respectively to ERY and GEN. NB had the highest AI against *B. subtilis* (77.90 %), *S. pneumoniae* (69.86 %) and *S. typhi* (57.62 %); CF recorded higher AI against *E. coli* (91.74 %), EA also had higher AI of 88.63 % against *Klebsiella spp*. when computed with broad-spectrum antibiotic CIP. Supportive to the data presented on Figures above and Table 2; it is confirmative to say that extractives from this plant are more sensitive to Gram negative organisms studied.

From the overall, the results showed a dose-dependent approach, with gross and broad-spectrum effects against the tested bacteria exhibited by the mid-polar portions (CF, EA, NB). These variabilities were in some cases consistent with earlier work on this genus by Parekh *et al.* (2006). It was observed that the activities of antibiotics against the tested microorganisms similarly fall in the range of 10.33 ± 0.67 to 36.00 ± 0.00 mm against Gram positive while activities of 14.00 ± 0.00 to 29.33 ± 0.67 mm against Gram negative specie studied. The results from this study indicates that *B. rufescens* had a better antibacterial substances compared to root of *B. racemosa* (Jain *et al.,* 2008); bark of *B. variegata* (Parekh *et al.,* 2006) but had similar activities with stem bark of *B. racemosa* (Kumar *et al.,* 2005).

In conclusion, the extracts were found to be effective against the studied pathogenic microorganisms at varying levels of inhibition. As a follow up of the bioassay directed activity studies, work is currently going on in our laboratory in order to isolate, purify and characterize the active compound(s) responsible for these interesting activities. From the results of this study, it is pertinent to say that organic solvents play a vital role in revealing bioactive extractives/compounds with related structural activity. Therefore, this study has supported the use of stem bark of *B. rufescens* traditionally in some parts of Northern Nigeria, as a remedy against diarrhoea, dysentery and other related diseases.

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