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Phytochemical screening and antibacterial activities of aqueous and ethanolic extracts of the stem bark and leaves of *Bauhinia rufescens*

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ABSTRACT: The *Bauhinia rufescens* stem-bark and leaves were extracted using water and ethanol. The ethanol extracts were fractionated using petroleum ether, chloroform, ethyl acetate and methanol. The aqueous extracts, the fractions and the fractionation residues of the ethanol extracts were subjected into phytochemical screening and antibacterial activity testing using standard methods. The phytochemical screening revealed the presence of alkaloids, carbohydrates, resins, saponins, sterols and tannins in the extracts and fractions of the plant materials. *In-vitro* antibacterial activities of the extracts and fractions were investigated against *Staphylococcus aureus, Proteus vulgaris, Streptococcus pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Streptococcus pyogenes, Salmonella typhi* and *Shigella dysenteriae* using agar-disc diffusion method. waThe aqueous extracts, the fractions of ethanol extracts and the fractionation residues of the *Bauhinia rufescens* stem-bark and leaves showed antibacterial activities against the test bacterial isolates. The chloroform and methanol fractions of the stem bark as well as the methanol fraction and the aqueous extract of the leaves showed MIC and MBC against some test bacterial isolates within the range of 10mg/ml to 40mg/ml. The overall results of the study suggested that the stem-bark and leaves of *Bauhinia rufescens* could be a good source of antibacterial compounds.

Keywords: *Bauhinia rufescens*, phytochemical, screening, antibacterial, activity

Introduction

The frequent emergence of antibiotic resistance strains of pathogenic bacteria has led to the need of finding alternative treatment using among others, plant extracts singly or in combinations. Plants have served as the basis of traditional medicine systems for thousands of years in countries such as china, India and in Africa (Mukhtar and Okafor, 2002). The need for new antimicrobial agents is closely associated with the problems of the emergence of strains that are resistant to most conventional antibiotics (Finland *et al*.,1966).

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Bauhinia rufescens (Orchid bush) was traditionally believe to have therapeutic values and is a member of family *Fabaceae* (*Caesalpiniaceae).* It is a shrub or small tree up to 8m high. Bark grey, smooth, very fibrous and scaly when old. Slash pink twigs arranged in one plane like a fish bone, with 10cm long thorn-like, lignified. Lateral shots leaves small, up to 2.5cm long, glabrous, grey green. Bilobed almost to the base. lobes semicircular to ovate. In more humid regions *Bauhinia* is ever green. Flowers greenish-yellow to white and pale pink few-flowered racemes. Petals (5) spatulate, 15-20mm long, 10 stamens, filaments hairy at the base. Fruit aggregated, long, narrow pods, twisted up to 10cm long, glabrous, obliquely constricted, showing dark red-brown with 4-10 seed each. The pods remain on the shrub for a long time (Aliyu, 2006). *Bauhinia rufescens* stem-bark was found to be used traditionally in northern Nigeria as a remedy against diarrhea, dysentery and other related diseases, which are caused, by *Corynaebacterium spp., Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa and Shigella dysentery* (Usman *et al*. 2009).

The use of herbal drugs in traditional medicine needs to be evaluated by using current scientific approaches with the view to giving the patient an appropriate dosage of the medication as against the most practiced unquantifiable approach by the native healers (WHO, 1991). The present study was therefore aimed at investigating the phytochemical constituents and antimicrobial activities of the stem-bark and leaf extracts and fraction of *Bauhinia rufescens*.

Materials and Methods

Collection and identification of the research plants

The plant material was collected from Dawakin Tofa Local Government Area of Kano State. When collected the plants was identified in the Botany section of the Department of Biological Sciences, Bayero University, Kano with the aid of botanical keys (Arber, 1972).

Extraction of the *Bauhinia rufescens* (bark and leaves)

The Stem bark and Leaves of *Bauhinia rufescens* were extracted in accordance with the procedure used by Fatope *et al* (1993), using distilled water and ethanol.

i) Aqueous extracts

Hundred grams (100g) of the powdered air dried plant parts (stem-bark and leaf) were percolated in one litre of distilled water for one week with occasional shaking. At the end of one week, the extract was filtered using a Whatmans No.1 filter paper and the crude extract was evaporated to dryness using a water bath at 40^0C. The dried extracts were weighed and kept in a freezer until required for further analysis (Fatope *et al*, 1993).

ii) Ethanol extracts

A hundred grams (100g) each of powdered, air-dried plant parts (stem-bark and leaf) were percolated in one litre of ethanol (BDH 99.7 – 100%) for two weeks, after which the extract was filtered using a Whatmans No.1 filter paper. The crude extract was concentrated to dryness using a rotary evaporator at 40^0C (Fatope *et al*, 1993).

Fractionation of the crude ethanol extracts

The crude ethanol extract of the stem bark and leaves were fractionated by maceration procedure using petroleum ether, chloroform, ethyl acetate and methanol. The extract was macerated several times with the individual solvent using a volume ranging between 20 and 40ml until the initial colouration observed when the solvent was first added becomes very faint and negligible. The fraction recovered was filtered with filter paper, and labeled as the fraction of the particular solvent used.

Fractionation residue

The left over extract after final maceration with the last solvent was dried and labeled as the residue fractions. The other four fractions were evaporated using rotary evaporator at 40 ^{0}C. The dried fractions and the fractionation residues were weighed and kept in a freezer until required for further use.

Phytochemical Screening of the aqueous extracts, fractions of ethanol extracts and fractionation residues

Phytochemical analysis was carried out to determine the active ingredients content of the aqueous extracts, fraction of ethanolic extracts and fractionation residues of the *Bauhinia rufescens* stem bark and leaves. A procedures described by Sofowora (1993) was adopted for detection of the presence of alkaloids, carbohydrates, flavonoids, glycosides, resins, saponins, sterols and tannins.

Bioassay studies

The test microorganisms

The test organisms were biochemically identified clinical isolates of *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgaris, Streptococcus pneumonia, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Shigella dyesnteriae*. These were obtained from Aminu Kano Teaching Hospital (AKTH) and some biochemical tests were carried out in the Microbiology laboratory of Bayero University Kano to confirm the authenticity of their identity.

Standardization of bacterial Inoculum

The bacterial isolates were sub cultured in nutrient broth for 24 hours. A loopful of the overnight nutrient broth was diluted in normal saline (0.85% Nacl w/v) until their turbidity matched with 0.5 McFarland standard thought to contain a mean of 3.33 x 10^6 cfu/ml, which matches with the standard turbidity of 1% (w/v) barium sulphate solution (Mukhtar and Tukur, 2000).

Preparation of extract Concentrations

The extract concentrations were prepared in accordance with the dilution method described by Baker *et al.* (1993). A 400,000µg/ml, 200,000µg/ml, 100,000µg/ml and 50,000µg/ml were prepared using sterile distilled water for the aqueous extracts and DMSO for the fractions and the fractionation residues. Stock solutions were prepared by dissolving 0.8g (800mg) of the aqueous extract in 2ml of sterilized distilled water and the fractions and the fractionation residue each in 2ml of DMSO. Thus, each stock solution has a concentration of 400mg/ml (400,000µg per ml). Subsequent test concentrations were prepared from the stock solutions using the formula demonstrated by Baker *et al.* (1993) i.e. (R x V)/O, which give the volume of the stock solution that was diluted to the final volume required with the distilled water. 'R' is the required concentration, 'V' the total volume of solution required and 'O' is the original concentration of the stock solution.

Preparation of sensitivity discs

The sensitivity discs were prepared by punching a Whatman's No. 1 filter paper using a perforator (6mm diameter). The discs were sterilized by autoclaving at 121^{0}C for 15 minutes and a 1ml of each (400,000µg/ml, 200,000µg/ml, 100,000µg/ml and 50,000µg/ml) for the aqueous extracts, fractions and the fractionation residues was used to impregnate 100 filter paper discs. Thus, the disc potencies of 4000 µg/ml 2000 µg/ml, 1000µg/ml and 500µg/disc were obtained respectively. The impregnated discs were then dried in an oven at 37^{0}C for sixty minutes (Stokes and Ridgesway, 1980).

Sensitivity testing

The sensitivity testing was carried out using disc diffusion method described by Kirby Bauer (1966). Appropriate sterile agar (nutrient, blood or chocolate) media were prepared depending on the test organism in use

and carefully transferred in to sterile Petri dishes. The media were allowed to solidify and the plates were placed in a drier to remove excess moisture. The plates were marked to indicate the organism and the position of four discs of different test concentrations (50,000, 100,000, 200.000 and 400,000ug/ml). From the standard inoculum of each isolate, a loopful of a test bacterial inoculum was taken and streaked over the entire surface of the dried agar. Four discs of different concentrations were placed at the marked positions while one antibiotic disc was placed in the center to act as positive control. The plates were inverted and incubated for 24 hours at 37^{o}C. At the end of this incubation period, the plates were observed for the presence of zones of inhibition as evidence of antibacterial activity. The degree of sensitivity was determined by measuring the diameter of visible zones of inhibition to the nearest millimeters with respect to each isolate and extract concentration.

Determination of MIC

The minimum inhibitory concentrations of the aqueous extract, fractions of ethanolic extract and fractionation residues were determined using tube dilution technique. Solutions of two fold dilutions were prepared using sterilized distilled water to obtain concentrations of 5mg/ml, 10mg/ml, 20mg/ml and 40mg/ml. Equal volume of the above concentrations were incorporated in nutrient broth in 1:1 ratio and 0.1ml of standard suspension of the test organisms (3.33×10^{6} cfu/ml) was added to each of the test tube. The tubes were then incubated aerobically at 37^{0}C for 24 hours. Tubes containing broth and extract without inocula were included to serve as positive control while a tube containing broth and inocula serves as negative control for comparison. The presence of growth (Turbid Solution) or absence of growth (clear Solution) at the end of incubation period was recorded. The highest dilution (least concentration) of the extract showing no detectible growth was regarded as the minimal inhibitory concentration. (Baker *et al.*, 1993, NCCLS 1999).

Determination of MBC

The Minimum Bactericidal Concentration (MBC) of the aqueous extracts, fractions of ethanol extracts and the fractionation residues were determined by sub culturing 0.1ml from the last MIC test dilution that show visible growth (Turbidity) and all others in which there is no detectable growth on a fresh extract free solid medium and incubated at 37^{o}C for further 24 hours. The highest dilution that shows no single bacterial colony was considered as the minimum bactericidal concentration (Baker *et al.*, 1993, NCCLS 1999).

Results and Discussion

Table 1 and 2 showed the results phytochemical screening of the aqueous extracts, fractions of ethanol extracts and the fractionation residues of the stem bark and leaf of *Bauhinia rufescens*. Tables 3 to 8 presents the results of the antibacterial activity of the aqueous extracts, fractions of ethanolic extracts and fractionation residues of the *Bauhinia rufescens* stem-bark and leaf. Tables 9 to 14 showed the results of the MIC and MBC testing of the aqueous extracts, fractions of ethanolic extracts and fractionation residues of the *Bauhinia rufescens* stem-bark and leaf.

Table 1: Phytochemical constituents of the aqueous extract, fractions of ethanol extract and fractionation residue of *Bauhinia rufescens* stem-bark

	Aqueous extract	Petroleum ether fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction	Fractionation residue
Alkaloids	-	+	+	-	-	-
Carbohydrates	+	-	-	+	+	+
Flavonoids	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-
Resins	+	+	+	+	+	+
Saponins	-	-	+	-	-	+
Sterols	+	-	+	+	+	+
Tannins	+	-	-	+	+	-

Key: + = present, - = absent.

Table 2: Phytochemical constituents of the aqueous extract, fractions of ethanol extract and fractionation residue of *Bauhinia rufescens* leaves

	Aqueous extract	Petroleum ether fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction	Fractionation residue
Alkaloids	-	-	+	+	-	+
Carbohydrates	+	+	-	+	+	-
Flavonoids	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-
Resins	+	+	+	+	+	+
Saponins	+	+	-	-	+	-
Sterols	+	-	-	+	+	+
Tannins	+	-	-	-	+	+

Key: + = present, - = absent.

Table 3: Antibacterial activities of aqueous extracts of the *Bauhinia rufescens* (stem bark and leaves)

	Strep.	**Diameter of zone of inhibition (mm)**				**Extract concentration (µg/disc)**			
	Control	*Bauhinia rufescens* stem bark				*Bauhinia rufescens* leaves			
Isolates	30µg	500	1000	2000	4000	500	1000	2000	4000
Staph. aureus	22	-	7	9	10	8	9	11	12
Proteus vulgaris	22	-	-	-	-	-	-	9	10
Strep. pneumoniae	25	-	-	-	-	-	-	-	-
Pseudo. aeruginosa	22	-	-	-	9	-	-	8	9
Escherichia coli	25	-	8	10	14	-	8	9	10
Kleb. pneumoniae	21	-	-	-	-	-	-	11	12
Salmonella typhi	24	-	-	8	9	-	-	9	11
Strep. pyogenes	23	-	-	-	-	-	-	-	-
Shigella dysenteriae	22	-	-	-	-	-	-	-	-

Key: - = Disc diameter (6mm). Strep. = Streptomycin.

Table 4: Antibacterial activities of petroleum ether fractions of ethanol extract of *Bauhinia rufescens* stem bark and leaves

	Strep.	**Diameter of zone of inhibition (mm)**				**Extract concentration (µg/disc)**			
	Control	*Bauhinia rufescens* stem bark				*Bauhinia rufescens* leaves			
Isolates	30µg	500	1000	2000	4000	500	1000	2000	4000
Staph. aureus	22	-	-	-	9	-	-	7	9
Proteus vulgaris	22	-	-	-	9	-	-	-	-
Strep. pneumoniae	25	-	-	-	-	-	-	-	-
Pseudo. aeruginosa	22	-	-	-	-	-	-	-	8
Escherichia coli	25	-	-	-	8	-	-	-	-
Kleb. pneumoniae	21	-	-	7	8	-	-	-	-
Salmonella typhi	24	-	-	8	10	-	-	-	-
Strep. pyogenes	23	-	-	-	-	-	-	9	12
Shigella dysenteriae	22	-	-	8	9	-	7	8	9

Key: - = Disc diameter (6mm). Strep. = Streptomycin.

Table 5: Antibacterial activities of chloroform fractions of ethanol extract of *Bauhinia rufescens* stem bark and leaves

	Strep.	**Diameter of zone of inhibition (mm)**				**Extract concentration (µg/disc)**			
	Control	*Bauhinia rufescens* stem bark				*Bauhinia rufescens* leaves			
Isolates	30µg	500	1000	2000	4000	500	1000	2000	4000
Staph. aureus	22	-	-	9	12	-	10	11	13
Proteus vulgaris	22	-	7	8	12	-	-	-	-
Strep. pneumoniae	25	-	-	-	-	-	-	-	-
Pseudo. aeruginosa	22	-	-	-	-	-	-	-	10
Escherichia coli	25	-	-	7	9	-	-	-	10
Kleb. pneumoniae	21	-	-	12	15	-	-	-	-
Salmonella typhi	24	7	9	12	15	-	-	-	-
Strep. pyogenes	23	-	-	9	10	-	-	-	-
Shigella dysenteriae	22	7	9	10	12	-	8	9	10

Key: - = Disc diameter (6mm). S= Streptomycin.

Table 6: Antibacterial activities of ethyl acetate fractions of ethanol extract of *Bauhinia rufescens* stem bark and leaves

	Strep.	**Diameter of zone of inhibition (mm)**				**Extract concentration (µg/disc)**			
	Control	*Bauhinia rufescens* stem bark				*Bauhinia rufescens* leaves			
Isolates	30µg	500	1000	2000	4000	500	1000	2000	4000
Staph. aureus	22	-	-	8	12	-	-	-	-
Proteus vulgaris	22	-	-	8	9	-	-	-	-
Strep. pneumoniae	25	-	-	-	-	-	-	-	8
Pseudo. aeruginosa	22	-	7	8	9	-	-	8	11
Escherichia coli	25	-	-	8	10	-	-	-	7
Kleb. pneumoniae	21	-	-	9	10	-	-	-	10
Salmonella typhi	24	-	-	9	11	-	-	-	-
Strep. pyogenes	23	-	-	11	15	-	-	-	-
Shigella dysenteriae	22	-	-	-	9	-	-	-	-

Key: - = Disc diameter (6mm). S= Streptomycin.

Table 7: Antibacterial activities of methanol fractions of ethanol extract of *Bauhinia rufescens* stem bark and leaves

	Strep.	**Diameter of zone of inhibition (mm)**				**Extract concentration (µg/disc)**			
	Control	*Bauhinia rufescens* stem bark				*Bauhinia rufescens* leaves			
Isolates	30µg	500	1000	2000	4000	500	1000	2000	4000
Staph. aureus	22	-	-	-	9	-	-	8	10
Proteus vulgaris	22	-	8	10	11	-	-	8	10
Strep. pneumoniae	25	-	-	-	-	-	-	-	-
Pseudo. aeruginosa	22	-	-	9	11	-	-	7	9
Escherichia coli	25	-	-	8	13	-	-	8	10
Kleb. pneumoniae	21	-	-	-	-	-	-	-	9
Salmonella typhi	24	-	-	-	10	-	8	9	11
Strep. pyogenes	23	-	-	-	-	-	-	-	-
Shigella dysenteriae	22	-	-	-	-	-	9	10	11

Key: - = Disc diameter (6mm). S= Streptomycin

Table 8: Antibacterial activities of fractionation residues of ethanol extract of *Bauhinia rufescens* stem bark and leaves

	Strep. Control	**Diameter of zone of inhibition (mm)** *Bauhinia rufescens* stem bark				**Extract concentration (µg/disc)** *Bauhinia rufescens* leaves			
Isolates	30µg	500	1000	2000	4000	500	1000	2000	4000
Staph. aureus	22	-	-	-	-	-	7	8	10
Proteus vulgaris	22	-	-	-	-	-	-	-	11
Strep. pneumoniae	25	-	-	-	-	-	-	10	15
Pseudo. aeruginosa	22	-	-	-	8	-	-	-	-
Escherichia coli	25	-	-	-	-	-	-	-	-
Kleb. pneumoniae	21	-	-	-	-	-	-	-	-
Salmonella typhi	24	-	-	-	-	-	-	8	9
Strep. pyogenes	23	-	-	-	9	-	-	-	-
Shigella dysenteriae	22	-	8	9	11	9	10	12	14

Key: - = Disc diameter (6mm). S= Streptomycin

Table 9: MIC and MBC of the aqueous extracts of the *Bauhinia rufescens* stem bark and leaves

	Bauhinia rufescens stem bark		*Bauhinia rufescens* leaves	
Bacterial Isolates	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Staph. aureus	-	-	-	-
Proteus vulgaris	-	-	40	-
Strep. pneumoniae	-	-	-	-
Pseudo. aeruginosa	40	-	-	-
Escherichia coli	-	-	-	-
Kleb. pneumoniae	-	-	20	40
Salmonella typhi	40	-	-	-
Strep. pyogenes	-	-	-	-
Shigella dysenteriae	-	-	-	-

Key: - = >40mg/ml.

Table10 : MIC and MBC of the petroleum ether fractions of the *Bauhinia rufescens* (stem bark and leaves)

	Bauhinia rufescens stem bark		*Bauhinia rufescens* leaves	
Bacterial Isolates	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Staph. aureus	-	-	-	-
Proteus vulgaris	-	-	-	-
Strep. pneumoniae	-	-	-	-
Pseudo. aeruginosa	-	-	-	-
Escherichia coli	-	-	-	-
Kleb. pneumoniae	-	-	-	-
Salmonella typhi	-	-	-	-
Strep. pyogenes	-	-	-	-
Shigella dysenteriae	-	-	-	-

Key: - = >40mg/ml.

Table 11: MIC and MBC of the chloroform fractions of ethanol extract of *Bauhinia rufescens* stem bark and leaves

	Bauhinia rufescens stem bark		*Bauhinia rufescens* leaves	
Bacterial Isolates	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Staph. aureus	-	-	-	-
Proteus vulgaris	20	40	-	-
Strep. pneumoniae	-	-	-	-
Pseudo. aeruginosa	-	-	-	-
Escherichia coli	-	-	-	-
Kleb. pneumoniae	20	40	-	-
Salmonella typhi	10	20	-	-
Strep. pyogenes	-	-	-	-
Shigella dysenteriae	10	20	-	-

Key: - = >40mg/ml.

Table 12: MIC and MBC of the ethyl acetate fractions of ethanol extract of *Bauhinia rufescens* stem bark and leaves

	Bauhinia rufescens stem bark		*Bauhinia rufescens* leaves	
Bacterial Isolates	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Staph. aureus	-	-	-	-
Proteus vulgaris	-	-	-	-
Strep. pneumoniae	-	-	-	-
Pseudo. aeruginosa	40	-	-	-
Escherichia coli	40	-	-	-
Kleb. pneumoniae	40	-	-	-
Salmonella typhi	-	-	-	-
Strep. pyogenes	40	-	-	-
Shigella dysenteriae	-	-	-	-

Key: - = >40mg/ml.

Table 13: MIC and MBC of the methanol fractions of ethanol extract of *Bauhinia rufescens* stem bark and leaves

	Bauhinia rufescens stem bark		*Bauhinia rufescens* leaves	
Bacterial Isolates	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Staph. aureus	-	-	10	20
Proteus vulgaris	20	40	20	40
Strep. pneumoniae	-	-	-	-
Pseudo. aeruginosa	-	-	40	-
Escherichia coli	40	-	20	40
Kleb. pneumoniae	-	-	-	-
Salmonella typhi	20	40	20	40
Strep. pyogenes	-	-	-	-
Shigella dysenteriae	-	-	40	-

Key: - = >40mg/ml.

Table 14: MIC and MBC of the fractionation residues of ethanol extract of *Bauhinia rufescens* stem bark and leaves

	Bauhinia rufescens stem bark		*Bauhinia rufescens* leaves	
Bacterial Isolates	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Staph. aureus	-	-	20	40
Proteus vulgaris	-	-	-	-
Strep. pneumoniae	-	-	10	20
Pseudo. aeruginosa	-	-	-	-
Escherichia coli	-	-	-	-
Kleb. pneumoniae	-	-	-	-
Salmonella typhi	-	-	-	-
Strep. pyogenes	-	-	-	-
Shigella dysenteriae	-	-	-	-

Key: - = >40mg/ml.

Discussion

The phytochemical screening indicate the distribution of alkaloids, carbohydrates, resins, sterols, saponins and tannins among the extracts, fractions and fractionation residues of *Bauhinia rufescens* stem bark and leaf. Usman *et al.* (2009) in the previous study, stated that the preliminary phytochemical studies of the partitioned portion of *Bauhinia rufescens* stem bark showed the presence of aloes, anthraquinones derivatives, cardenolides and cardiac glycosides, flavonoids, resins, Saponins and tannins.

The aqueous extracts of *Bauhinia rufescens* stem bark and leaf showed antibacterial activities against some of the test bacterial isolates (Table 3). This may be due the presence of resins, sterols and tannins in the aqueous extracts of bark and leaf (Table 1and 2). The antibacterial activity of the aqueous extract of *Bauhinia rufescens* leaf against more bacterial isolates than the aqueous extract of the stem bark (Table 3) may be attributed to the presence of saponins in the leaf extract in addition to the resins, sterols and tannins (Table 2).

The antibacterial activity of petroleum ether fraction of ethanol extract of *Bauhinia rufescens* stem bark on more test bacterial isolates than the petroleum ether fraction of *Bauhinia rufescens* leaf (Table4) might be due to the presence of alkaloids in the petroleum ether fraction of the stem bark (Table1).

The chloroform fractions of the ethanol extracts of *Bauhinia rufescens* stem bark and leaf showed antibacterial activities against some of the test bacterial isolates (Table 5). The antibacterial activity of the chloroform fraction of the stem bark on more isolates than the leaf fraction might relate to the presence of saponins and sterols in the bark fraction in addition to the alkaloids and resins (Table 1)

The methanol fraction of ethanolic extract of *Bauhinia rufescens* stem bark and leaf both showed antibacterial activities against some of the test bacterial isolates (Table 7). The leaf fraction displayed antibacterial activities on greater number of bacterial isolates than the stem bark fraction and this might be due to the presence of saponins in the leaf fraction in addition to resins, sterols and tannins contained by both fractions (Table 1&2).

The fractionation residue of the *Bauhinia rufescens* leaf showed antibacterial activities on greater number of bacterial isolates than the stem bark residue (Table 8), this might be due to the presence of alkaloids and tannins in the leaf residue (Table 2). The antibacterial activities displayed by the aqueous extracts, fraction of ethanol extract and fractionation residue of the *Bauhinia rufescens* stem bark and leaf on various test bacterial isolates might be attributed to the presence of the various secondary metabolites detected in them in this study. Usman *et al.* (2009) reported that the presence of aloes, anthraquinones derivatives, cardenolides, cardiac glycosides, flavonoids, resins, Saponins and tannins in the partitioned portion of *Bauhinia rufescens* bark were responsible for the antibacterial

activity. Report of a study by Isaac and Chinwe (2001) revealed that alkaloids along with tannins and saponins are responsible for antibacterial activity of the extract of *Tetracapidium conophorum*. Onoruvwe and Olorunfemi (1998) also attributed the antibacterial effect of the root extract of *Dichrostachys cinerea* to alkaloids, saponins and flavonoids.

Conclusion

The results of the phytochemical screening showed that alkaloids, carbohydrates, resins, saponins, sterols and tannins were presence in different composition in the aqueous extracts, fractions and fractionation residues of ethanolic extracts of the *Bauhinia rufescens* stem bark and leaf. The extracts, fractions and fractionation residues also showed antibacterial activities on various test bacterial isolates.

Recommendations

Since the finding of this study revealed that the aqueous extracts, some fractions, and the fractionation residues of the crude ethanol extracts of *Bauhinia rufescens* stem bark and leaf showed antibacterial activity, It was therefore recommended that:

The chloroform fraction of *Bauhinia rufescens* stem bark may be preferred for the treatment of infections caused by *Proteus vulgaris, Klebsiella pneumoniae, Salmonella typhi* and *Shigella dysenteriae* and the methanol fraction of *Bauhinia rufescens* leaf for the treatment of infections caused by all the tested bacterial isolates with exception of *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Further studies were also recommended on them to:

a) Purify the bioactive compounds that has the antibacterial activity and
b) Ascertain the toxicity level of the extracts and fractions of parts of the plant under the study.

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