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EFFECTS OF COLUMN FRACTIONS OF THE LEAVES EXTRACT OF *Bridelia ferruginea* ON BACTERIA

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ABSTRACT
Bridelia ferruginea is extensively used in Nigeria traditional and folk medicines to cure various human ailments. The preliminary phytochemical screening of the plant parts revealed the presence of tannins, saponins, flavonoids and alkaloids. The in vitro antibacterial studies were carried out on both crude extracts and fractions obtained after column chromatography against medically important bacterial strains including; Escherichia coli, Staphylococcus aureus, Micrococcus species and Pseudomonas aeruginosa for different concentrations of 30mg/ml, 60mg/ml, 90mg/ml, and 120mg/ml. The results of antibacterial assay revealed that both crude extracts and fractions showed good inhibitory activity against all the tested bacteria compared with standard antibiotics. The active fractions gave four spots on the Thin Layer Chromatogram. The detection of other metabolites coupled with antibacterial activities of the plant calls for further work on the plant and its active compound towards development of new drugs for the benefit of humanity.
Keywords: Bridelia ferruginea, phytochemical, crude extract, antimicrobial activity, zone of inhibition

INTRODUCTION

Natural products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicine in different parts of the world from ancient time, and an impressive number of modern drugs have been developed from them (Kharb *et al.*, 2012). Ethnopharmacological uses of the plants continue to play a prominent role in primary health care of 80% of the world's population (Owoseni *et al.*, 2010). The study investigates the fundamental scientific bases for the use of *Bridelia ferruginea* in the treatment of ailment such as diarrhea, dysentery, fever and skin irritation as practice traditionally.

MATERIALS AND METHODS

Sample Collection

The leaves, stem bark and root of the plant materials were obtained in Zuru Local Government area of Kebbi State, Nigeria. The plant was identified in Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria.

Extraction of Plant Materials

The plant parts were rinsed with distilled water, shade dried for one week and made into fine powder of 40mm mesh size from which 100g each of the powder was extracted in different solvents (water and methanol) and stand for 48hours. The extracts were filtered and concentrated to dryness using rotary evaporator under reduced pressure.

Test Organisms

The bacterial cultures used in this study were obtained from Microbiology Department of Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria. Bacterial cultures include; *Escherichia coli, Staphylococcus aureus, Micrococcus species, and Pseudomonas aeruginosa*. All cultures were grown in Muller – Hilton medium. The inoculum was used for antibacterial assay.

Phytochemical Screening

The phytochemical analysis of the extract was conducted by Trease and Evan (1989); Harborne (1998) and El – olemey *et al.* (1994) methods.

Antibacterial Assay

The four different concentrations of the extracts were tested for antibacterial activity using agar disc diffusion assay according to the method of (Pelezer *et al.*, 1993). Plates of nutrient agar were seeded with test bacteria, and four well were made in each of the plate with sterile 6.0mm diameter coke borer. Each of the four wells was filled with a given concentration of the extract mixed with plane sterile agar. The plates were then incubated at 37^0C for 24hours. The diameters of zone of inhibition were measured using ruler and the value for each organism was recorded.

A controlled experiment was set up with well containing standard antibiotic tetracycline at 0.2mg/ml. The plates were incubated at 37^0C for 24hours. The diameter of these zones were measured and recorded appropriately against the extract.

Column Chromatography

A piece of glass wool was inserted at the bottom of a 50cm^3 glass column, packed with 30g silica gel powder (100 – 200mesh) in to the column, and glass wool was inserted just above the silica gel surface. The column was washed with distilled water with care to avoid air bubble. The samples (5cm^3 of the extract) each of leaves and bark was transferred on column and subsequently eluted with ethanol (Patty, 2002). Flow rate was observed and ten fractions were collected at 5ml per fraction for both the leaves and stem bark, and it was labeled L_1 – L_{10} and B_1 – B_{10} respectively which antibacterial activity test was carried on each of the fractions.

Thin Layer Chromatography

TLC was carried out on the leaves extract using commercially prepared silica gel coated TLC plates (5 to 20cm^3). The extract was dissolved in a little ethyl acetate and the solution spotted on the line drawn 2cm near or from the bottom edge end of the plate using capillary tube. The chromatogram was developed with benzene – ethyl acetate (3:1) solvents. The dried chromatogram was visualized by spraying 5% H_2SO_4 solution. The R_f value of each band was calculated.

RESULTS and DISCUSSION

Results of the experiments carried out are given in Table 1 – 13.

Table 1: Phytochemical Screening of Aqueous and Methanol Extracts of *B. ferruginea*.

Chemical Composition	Aqueous Extract			Methanolic Extract		
	Leaves	Bark	Root	Leaves	Bark	Root
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Steroids						
(a) Liebermann Test	-	-	-	-	-	-
(b) Salkowaski Test	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Cardiac glycoside	-	-	-	-	-	-
Flavonoids						
(a) Sodium hydroxide test	+	+	+	+	+	-
(b) Lead acetate test	+	+	+	+	+	-
Alkaloids						
(a) Meyer's reagents	+	+	-	+	+	-
(b) Wagner's reagents	+	+	-	+	+	-
(c) Dragendoff's reagents	+	+	-	+	+	-

Key: + = Present - = Absent

Table 2: Effect of Aqueous Extract on the Test Bacteria.

Plant parts	Bacteria species	Zone of Inhibition(mm) Concentration of Extract in mg/ml			
		30	60	90	120
Leaves	*Micrococcus Spp*	14	16	16	20
	Staphylococcus aureus	12	12	14	16
	Escherichia coli	10	12	16	18
Bark	*Micrococcus Spp*	08	08	08	10
	Staphylococcus aureus	06	06	06	08
	Escherichia coli	08	10	10	12
Root	*Micrococcus Spp*	06	06	06	06
	Staphylococcus aureus	06	06	06	06
	Escherichia coli	06	06	06	06

Note: Note: Diameter of the agar well is 6mm, therefore any well greater than 6mm show activity

Table 3: Effect of Methanolic Extracts on Test Bacteria

Plant parts	Bacteria species	Zone of Inhibition(mm) Concentration of Extract in mg/ml			
		30	60	90	120
Leaves	*Micrococcus Spp*	12	14	14	18
	Staphylococcus aureus	10	10	12	14
	Escherichia coli	08	10	14	16
Bark	*Micrococcus Spp*	06	06	06	08
	Staphylococcus aureus	06	06	06	08
	Escherichia coli	08	08	10	10
Root	*Micrococcus Spp*	06	06	06	08
	Staphylococcus aureus	06	06	06	06
	Escherichia coli	06	06	06	06

Note: Diameter of the agar well is 6mm, therefore any well greater than 6mm show activity

Table 4: Effect of Column Fractions of the Leaves on the Test Bacteria (Methanol Fraction)

Bacteria Isolates	Zone of Inhibition(mm) for L_1 – L_{10} (0.5ml) each									
	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}
Escherichia coli	6	8	8	8	10	8	10	12	14	12
Pseudomonas aureginosa	6	8	8	8	8	8	8	10	10	12
Staphylococcus aureus	8	6	8	10	12	14	16	10	12	10
Micrococcus Spp	6	6	8	8	10	12	18	16	10	10
Positive control	Tetracycline	14	14	14	14	18	20	18	18	18
Negative control	Water	6	6	6	6	6	6	6	6	6

Note: Diameter of the agar well is 6mm, therefore any well greater than 6mm show activity

Table 5: Effect of Column Fractions of the Bark on the Test Bacteria (Methanol Fraction)

Bacteria Isolates	Zone of Inhibition(mm) for B_1 – B_{10} (0.5ml) each									
	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}
Escherichia coli	6	6	6	8	8	8	8	8	8	8
Pseudomonas aureginosa	6	6	6	8	8	8	6	8	8	8
Staphylococcus aureus	8	8	6	8	8	8	8	6	6	8
Micrococcus Spp	8	8	8	8	6	6	6	6	6	6
Positive control	Tetracycline	14	14	14	14	18	20	18	18	18
Negative control	Water	6	6	6	6	6	6	6	6	6

Note: Diameter of the agar well is 6mm, therefore any well greater than 6mm show activity

Table 6: TLC Results on the Crude Compounds

Fraction Used	Solvent System	No. of Spot	R_f Values
Ethanol Extract of Leaves	n-hexane:Methanol (4:1)	No Spot	------

Table 7: TLC Results of the Ethanol Extract of Leaves Using Benzene: Ethyl acetate Solvent System

Fraction Used	Solvent System	No. of Spot	R_f Values
Ethanol Extract of Leaves	Benzene:Ethyl acetate (3:1)	4	0.13
			0.24
			0.29
			0.43

Table 8: TLC Results of Ethyl acetate Extracts Using n-hexane: Methanol Solvent System

Fraction Used	Solvent System	No. of Spot	R_f Values
Ethanol Extract of Leaves	n-hexane:Methanol (4:1)	No Spot	------

Table 9: TLC Results of Ethyl acetate Extract Using Benzene: Ethyl acetate Solvent System

Fraction Used	Solvent System	No. of Spot	R_f Values
Ethyl acetate Extract of Leaves	Benzene:Ethyl acetate (3:1)	4	0.14
			0.25
			0.30
			0.45

The Tables below show the effect of the extracted active fraction on the test bacteria after TLC (Four fractions labeled **A** – **D** and the yield of each band is 3mg)

Table 10: Effect of the Separated Fraction **A** on the Test Bacteria

Bacteria species	Zone of Inhibition(mm) Concentration of Extracts in mg/ml			
	0.5	1.0	1.5	2.0
Micrococcus Spp	6	10	14	18
Staphylococcus aureus	8	6	10	14
Escherichia coli	6	8	14	16

Table 11: Effect of the Separated Fraction **B** on the Test Bacteria

Bacteria species	Zone of Inhibition(mm) Concentration of Extracts in mg/ml			
	0.5	1.0	1.5	2.0
Micrococcus Spp	6	6	6	6
Staphylococcus aureus	6	6	6	6
Escherichia coli	6	6	6	6

Table 12: Effect of the Separated Fraction **C** on the Test Bacteria

Bacteria species	Zone of Inhibition(mm) Concentration of Extracts in mg/ml			
	0.5	1.0	1.5	2.0
Micrococcus Spp	6	6	6	6
Staphylococcus aureus	6	6	6	6
Escherichia coli	6	6	6	6

Table 13: Effect of the Separated Fraction **D** on the Test Bacteria

Bacteria species	Zone of Inhibition(mm) Concentration of Extracts in mg/ml			
	0.5	1.0	1.5	2.0
Micrococcus Spp	6	6	6	6
Staphylococcus aureus	6	6	6	6
Escherichia coli	6	6	6	6

The phytochemical screening of the leaves and stem bark of *Bridelia ferruginea* revealed the presence of flavonoids, tannins, saponins and alkaloids, but absent of anthraquinones, steroids and cardiac glycosides in all the plant parts as shown in table 1. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery (Dharmananda, 2003). Delzel (1991) reported that presence of tannins is a proof as regards the use of the plant as an antidysentary and antidiarrhoea. The presence of saponnins in the plant could be responsible for its traditional use in relaxation of muscles and treatment of wound as practiced generally in Northern Nigeria (Adebayo and Ishola, 2009). Flavonoids were found to be present in the leaves and roots. This is expected because flavonoids are the colouring matter of plants that are commonly found in the leaves and flowers (Njoku and Ezeibis, 2007). They are used as antioxidant and other medicinal purposes like cancers and cardiovascular diseases (Ukwu and Ukanwa, 2010).

Many African medicinal plants have been investigated for their chemical components and pharmacological properties and quite a number of them have medicinal and pharmacological effects. The compounds present in *Bridelia ferruginea* are known to be biologically active and therefore aid antimicrobial activities of the plant. The presence of these chemical constituents underscores the important of these plants in medicine.

The crude extracts of different parts were tested for antimicrobial activity to ascertain the traditional claims. The results showed activity on *Staphylococcus aureus, Micrococcus species and Escherichia coli*. The water extract showed activity against the test bacterial, but the activity was high in leaves than roots and bark. The methanol extracts also showed activity against the test bacterial but there was little or no activity on the roots, as a result the root was not used for further work.

On the other hand, the antibacterial screening of fractions obtained from column chromatography of the leaves and bark as reported in Table 4 and 5 show inhibitory activities against the bacteria, but the leaves show more activity than the bark. Based on the above result, only the leaves were used for further work since it showed high activity against the test bacteria.

The results of the antibacterial activities reported above shows that the plant has antibacterial activity especially the leaves which are similar to that reported by (Adeoye *et al.,* 1998).

The leaves of *Bridelia ferruginea* have inhibitory effect on the growth of *Staphylococcus aureus, Pseudomonas aureginosa, Micrococcus species and Escherichia coli*. The leaves of the plant under study are therefore an effective antibacterial agent. The inhibitory activity promises the potentials application of the plant in the treatment of microbial induce ailment. The possession of antibacterial activity of *Bridelia ferruginea* provides scientific evidence to support the local use of the plant in the treatment of diarrhea, dysentery and fever (Okwu and Ukanwa, 2010).

The extracted crude extract after fractionation gave yellow solid product. The product was subjected to preparatory TLC in order to fractionate the extract. Appearance of four well separated spot on the TLC chromatogram indicated that the extract is a mixture of other compounds. The bands were scrapped off, dissolved and filtered to remove the silica gel then it was subjected to antibacterial activity again and similar results were also obtained. This result strongly suggested that the antibacterial activity of the crude extracts was due to the chemical constituents of the plant.

CONCLUSION

The present study on *Bridelia ferruginea* has confirmed the presence of saponins, tannins, flavonoids and alkaloids in the leaves. The extracts are active against *Staphylococcus aureus, Pseudomonas aureginosa, Micrococcus species and Escherichia coli*. This agreed with other studies (Talla *et al.,* 2002) that *Bridelia ferruginea* possess antimicrobial activity against some Gram – positive and Gram – negative micro – organisms. This is why the plant could be employed in the treatment of microbially induced ailments such as diarrhea, dysentery, fever and skin irritation.

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