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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 (Kharb from them 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° C 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^{0} C 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 (5cm³ 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% H₂SO₄ 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	Extrac	t	Methanol	ic Ext	ract	
	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 Inh	nibition	(mm) Co	ncentration of	
		Extract	in mg/	ml		
		30	60	90	120	
	Micrococcus Spp	14	16	16	20	
Leaves	Staphylococcus aureus	12	12	14	16	
	Escherichia coli	10	12	16	18	
	Micrococcus Spp	08	08	08	10	
Bark	Staphylococcus aureus	06	06	06	08	
	Escherichia coli	08	10	10	12	
	Micrococcus Spp	06	06	06	06	
Root	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

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Plant parts	Bacteria species		Bacte Zone		hibiti	on(mr	n) Ca	oncer	ntrat	ion	of	
		-			in m	•	,				2.	
			L/	30	60		90	1	20			
	Micrococcus Spp			12	14		4		8			
Leaves	Staphylococcus au	PUIS		10	10	-	2	-	14			
Leaves	Escherichia coli	eus		08	10		4		6			
	LSCHEITCHIU COU			00	10	1	4		0			
	Micrococcus Spp		(06	06	0	6	08	R			
Bark	Staphylococcus au	roug		00	00	0	-	00				
Dark	Escherichia coli	reus		08	08	-	0	1				
	Escherichia cou			00	00	I	0	1	0			
	Micrococcus Spp			06	06		06		08			
Root	Micrococcus Spp	rous		06	00		06		06			
ROOL	Staphylococcus au	reus		06 06								
Notas Diamatar of	Escherichia coli	thora			06	ontor	06		06 m.ch	0.11	o ctivi	F 1 /
Note: Diameter of	the agar well is 6mm	, therei	fore a	any w	eu gr	eater	thar	1 6mr	n sn	ow	activi	cy .
Table 4: Effect of	Column Fractions of t	he Lea	ves o	n the	e Test	Bacte	eria ((Meth	ano	l Fra	action)
Bacteria Isolates	Zon	e of In	hibiti	on(m	m) fo	r L ₁ -	L ₁₀ (0.5m	l) ea	ιch		
	L_1	L ₂	L_3	L_4	Ĺ ₅	L_6	L ₇	L_8	L9	L_{10})	
Escherichia coli	6	8	8	8	10	8	10	12	14	12		
Pseudomonas aure	eginosa 6	8	8	8	8	8	8	10	10	12		
Staphylococcus au		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		
	the agar well is 6mm	-	-	-	-	-	-	-	•	-	activi	tv
												cy
	Column Fractions of t										ion)	
Bacteria Isolates	Zon	e of Inl	hibiti	on(m	m) fo	r B ₁ -	B ₁₀ (0.5m	ıl) ea	ach		
	B ₁	B ₂	B_3	B4	B_5	B ₆	B ₇	B ₈	B9	1	B ₁₀	
Escherichia coli	6	6	6	8	8	8	8	8	8		8	
Escherichia coli Pseudomonas aure		6 6	6 6	8 8	8 8	8 8	8 6	8 8	8 8		8 8	
Pseudomonas aure	eginosa 6		-									
Pseudomonas aure Staphylococcus au	eginosa 6	6	6	8	8	8	6	8	8		8	
Pseudomonas aure Staphylococcus au Micrococcus Spp	eginosa 6 ireus 8 8	6 8	6 6	8 8	8 8	8 8	6 8	8 6	8 6		8 8 6	
Pseudomonas aure Staphylococcus au Micrococcus Spp Positive control	eginosa 6 Ireus 8	6 8 8	6 6 8	8 8 8	8 8 6	8 8 6	6 8 6	8 6 6	8 6 6		8 8	
Pseudomonas aure Staphylococcus au Micrococcus Spp Positive control Negative control	eginosa 6 Ireus 8 8 Tetracycline Water	6 8 14 6	6 6 8 14 6	8 8 14 6	8 8 6 14 6	8 6 18 6	6 8 6 20 6	8 6 18 6	8 6 18 6		8 8 6 18 6	ty
Pseudomonas aure Staphylococcus au Micrococcus Spp Positive control Negative control Note: Diameter of	eginosa 6 Ireus 8 8 Tetracycline Water the agar well is 6mm	6 8 14 6 , theref	6 6 8 14 6	8 8 14 6	8 8 6 14 6	8 6 18 6	6 8 6 20 6	8 6 18 6	8 6 18 6		8 8 6 18 6	ty
Pseudomonas aure Staphylococcus au Micrococcus Spp Positive control Negative control Note: Diameter of Table 6: TLC Resu	eginosa 6 nreus 8 8 Tetracycline Water the agar well is 6mm ilts on the Crude Comp	6 8 14 6 , theref	6 6 8 14 6	8 8 14 6 any w	8 6 14 6 vell gr	8 6 18 6 eater	6 8 6 20 6	8 6 18 6 n 6mr	8 6 18 <u>6</u> n sh	IOW 1	8 8 6 18 6	ty
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Table 3: Effect of Methanolic Extracts on Test Bacteria

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)

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	Separate				
Bacteria species	Zone of I		n(mm) Co	oncentrat	ion 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	Separate	d Fracti	on B on tl	he Test B	acteria
Bacteria species	Zone of	nhibitio	n(mm) Co	oncentrat	ion 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	
F 1	6	6	6	6	
Escherichia coli Table 12: Effect of the	Separate	d Fracti	on C on th	he Test B	
	Separate Zone of	d Fracti Inhibitio	on C on th n(mm) Co	he Test B	acteria ion of Extracts in mg/ml
Table 12: Effect of the Bacteria species	Separate Zone of 0.5	d Fracti nhibitio 1.0	on C on th n(mm) Co 1.5	he Test B	
Table 12: Effect of the Bacteria species Micrococcus Spp	Separate Zone of I 0.5 6	d Fracti Inhibitio 1.0 6	on C on th n(mm) Co	he Test B	
Table 12: Effect of the Bacteria species Micrococcus Spp Staphylococcus aureus	Separate Zone of 0.5	d Fracti nhibitio 1.0	on C on th n(mm) Co 1.5	he Test B oncentrat 2.0	
Table 12: Effect of the	Separate Zone of I 0.5 6	d Fracti Inhibitio 1.0 6	on C on th n(mm) Co 1.5 6	he Test B pncentrat 2.0 6	
Table 12: Effect of theBacteria speciesMicrococcus SppStaphylococcus aureusEscherichia coli	Separate Zone of 1 0.5 6 6 6	d Fracti Inhibitio 1.0 6 6 6	on C on th n(mm) Co 1.5 6 6 6	he Test B pricentrat 2.0 6 6 6 6	ion of Extracts in mg/ml
Table 12: Effect of theBacteria speciesMicrococcus SppStaphylococcus aureusEscherichia coliTable 13: Effect of the	Separate Zone of 1 0.5 6 6 6 6 Separate	d Fracti nhibitio 1.0 6 6 6 6 d Fracti	on C on th n(mm) Cc 1.5 6 6 6 6 0 0 D on th	he Test B oncentrat 2.0 6 6 6 6 he Test B	ion of Extracts in mg/ml
Table 12: Effect of theBacteria speciesMicrococcus SppStaphylococcus aureusEscherichia coliTable 13: Effect of the	Separate Zone of 1 0.5 6 6 6 6 Separate	d Fracti nhibitio 1.0 6 6 6 6 d Fracti	on C on th n(mm) Cc 1.5 6 6 6 6 0 0 D on th	he Test B oncentrat 2.0 6 6 6 6 he Test B	ion of Extracts in mg/ml acteria
Table 12: Effect of theBacteria speciesMicrococcus SppStaphylococcus aureusEscherichia coliTable 13: Effect of theBacteria species	Separate Zone of 1 0.5 6 6 6 6 5 8 Separate Zone of 1	d Fracti nhibitio 1.0 6 6 6 d Fracti nhibitio	on C on th n(mm) Cc 1.5 6 6 6 6 0 n D on th n(mm) Cc	he Test B oncentrat 2.0 6 6 6 6 he Test B oncentrat	ion of Extracts in mg/ml acteria
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_ _ _ ation A on the Test Dee

The phytochemical screening of the leaves and stem bark of Bridelia ferruginea revealed the presence of flavonoids, tannins, saponins and alkaloids, but absent of anthraguinones, 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 obtained of fractions 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 effect inhibitory 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,

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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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