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Full Length Research Paper

Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Bridelia ferruginea*

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The root, stem bark and leaves of *Bridelia ferruginea*, which have some ethnomedicinal applications were investigated for their action against some disease causing microorganisms. Phytochemical screening revealed the presence of alkaloids, tannins, saponins, steroids, flavonoids, anthraquinones and phlobatannins (mostly in root and stem bark). The antimicrobial screening of the crude methanol extract carried out *in vitro* had wide range of activity on *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus, Proteus mirabilis* and *Candida albicans.* The crude root extracts inhibited the growth of *E. coli*, *S. aureus*, *S. typhi, P. mirabilis* and *C. albicans* at concentrations of 40 , 100, 60, 60, and 80 mg/ml respectively, while the stem bark had minimum inhibitory concentration (MIC) of 60 mg/ml on *S. typhi* and 10 mg/ml on *C. albican.* The extract from leaf had no antimicrobial activity against any of the clinical isolates used for this work. The findings indicate that the extracts from root contain the most active components.

Key word: *Bridelia ferruginea*, phytochemical, antimicrobial agent, crude extract, minimum inhibitory c**o**ncentration.

INTRODUCTION

Bridelia ferruginea belongs to the family Euphorbiaceae which is commonly found in Savannah regions (Ekanem et al., 2008). It is usually a gnarled shrub which sometimes reaches the size of a tree in suitable condition. Its common names are Kizni (Hausa), Marehi (Fulani), Iralodan (Yoruba), Ola (Igbo); and Kensange Abia (Boki). Its habitat is the Savannah, especially in the moister regions extending from Guinea to Zaire and Angola. The tree is 6 - 15 m high, up to 1.5 m in girth and bole crooked branching low down. The bark is dark grey, rough and often marked scaly (Rashid et al., 2000).

B. ferruginea has diverse uses. A decoction of the leaves has been used to treat diabetes. It is also used as purgative and a vermifuge (Cimanga et al., 1999). The bark extract has been used for the coagulation of milk and also lime juice for the formulation of a traditional gargle “egun efu” (Orafidiya et al., 1990). It is also reported of having potential for water treatment (Kolawole and Olayemi, 2003).

In Togo, the roots of the plant are used as chewing sticks and the root bark is used for intestinal and bladder disorder remedies as well as skin diseases (De-Bruyne et al., 1997). Other reported activities of the bark extract include typanocidal (Ekanem et al., 2008), molluscidal (Iwu, 1984), antimicrobial (Adeoye et al., 1988) and anti-inflammatory (Olajide et al., 1999). Antimicrobial properties of stem bark of *B. ferruginea* against facultative Gram negative rods have been reported by Ndukwe et al. (2005). The activities of the methanol, petroleum ether and chloroform bark extracts of the *B. ferruginea* against some potential pathogenic organisms have been extensively investigated (Iwu, 1984; Adeoye et al., 1988; Olajide et al., 1999; Akiwodi, 2005). However, reports on bioactive activities of the extracts from root and leaf of *B. ferruginea* have not been widespread. This present study

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was intended to elucidate the chemical constituents of root, stem bark and leaves with a view of authenticating the plant's antimicrobial potentials.

MATERIALS AND METHODS

Plant materials and preparation

Fresh root, stem bark and leaves of *B. ferruginea* were collected from Teaching and Research Farm of Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The samples were air-dried in the laboratory at ambient temperature (30 ± 2°C) for 10 days, pulverized using a mechanical grinder and the powders obtained were stored until further use. 100 g of each sample (root, stem bark and leave) was packed in soxhlet extractor extracted with methanol and evaporated to dryness using a rotary evaporator (Stuart, Barloworld, Model RE 300) to obtain 22.3 g (root), 25.3 g (stem bark) and 15.7 g (leaves) of crude methanol extracts. The various crude extracts were later subjected to bioassay analyses.

Preparation of crude extract

The stock solutions of the extracts were prepared by dissolving 10 g of each extract (root, stem bark and leaves) in 10 ml of dimethylsulphoxide (DMSO) to obtain stock solutions of 1000 mg/ml concentration each. From the stock solution, concentrations of 250, 200, 150, 100, 50 and 10 were obtained by serial dilution. These were stored at 15°C until further use.

Phytochemical screening

The phytochemical analyses of the plants extracts were carried out following the methods of Sofowora (1986), Trease and Evans (1983), Wallis (1967), Rai and Obayemi (1973) and Elujoba et al. (1989). They are briefly described below.

Evaluation of antimicrobial activity

Pure clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus anthracis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Candida albicans* were obtained from the Department of Medical Microbiology, Teaching Hospital, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria. The organisms were subculture on Nutrient agar slant in bijou bottles and incubated at 37°C for 24 h. The organisms were then stored at 4°C until needed.

The agar diffusion method as described by Osadebe and Ukwueze (2004) was adopted for the study. Broth cultures of the test isolates (0.1 ml) containing 1.0 X 10^5 cfu/ml of organism was introduced into a sterile Petri dish and 15 ml of molten Mueller Hinton agar were added. The content was thoroughly mixed and then allowed to solidify. Holes were bored in the plates, using a standard sterile cork borer of 5 mm diameters and equal volumes of the plant extracts (1000 µL) were transferred into the wells with the aid of micropipette. The experiments were carried out in duplicate. The plates were allowed to stand for 1 h for prediffusion of the extracts to occur (Esimone et al., 1998) and incubated at 37°C for 24 h.

At the end of incubation, the plates were observed and zones of inhibition were measured. The average of the zones of inhibition was recorded.

Table 1. Phytochemical characteristics of root, stem bark and leaves of *B. ferruginea*.

Phytochemical parameter	Root	Stem bark	Leaves
Alkaloids	+	+	-
Flavonoids	+	-	+
Authraquinones	+	-	-
Cardiac glycosides	-	+	+
Steroids	+	+	-
Tannins	+	+	+
Saponins	+	+	-
phlobatannius	+	-	+

+, present; -, absent.

Determination of minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration (MIC) was carried out according to the methods of Ndukwe et al. (2007). The medium used is nutrient agar solution which was prepared according to the manufacturers' standard of 28 g/1000 ml. In this case double strength was prepared by dissolving 28 g in 500 ml of distilled water which was swirled and mixed thoroughly by heating to allow uniform dissolution after which 5 ml of it was dispensed into 30 sets of universal bottles and sterilized in an autoclave at 121°C for 15 min. The agar was allowed to cool to 45°C and each graded solution was then mixed gently with molten double strength nutrient agar in a Petri-dish and allowed to solidify for 1 h. Extracts concentrations of (100, 80, 60, 50, 40, 30, 10, 5 and 0.5) mg/ml were prepared by serial dilution. Each plate was divided into eight equal sections and labeled appropriately. The 5 mm diameter filter paper discs (Whatman No. 1) were placed aseptically in the labeled section of the plate using sterile forcepts. With a micropipette, 0.1 ml of each bacterial suspension was taken and transferred aseptically with care into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24 h at 37°C after which they were observed for growth or death of the test organisms. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

The phytochemical screening of the root, stem bark and leaves of *B. ferruginea* revealed the presence of tannins (root, stem bark and leaves), alkaloids, steroids and saponins (root and stem bark), phlobatannins and flavonoids (root and leaves) and cardiac glycosides (stem bark and leaves) as shown in Table 1. The presence of saponins, tannins alkaloids and steroids in the root and stem bark of the plant, shows that the extracts were of pharmacological importance. Table 2 shows the antimicrobial activities of the stem bark against *Salmonella typhi* and *C. albican* and extract from root was more active, it inhibited the growth of *E. coli, S. aureus, S. typhi P. mirabilis* and *C. albicans* (Table 3). Many triterpene saponins and their aglycones have been reported by Hostettmann and Martson (1995) to have varied uses as antiulcerogenic, anti-inflamatory, fibrinolytic, antipyretic,

Table 2. Antimicrobial activities of different concentrations of the methanolic extract of *B. ferruginea* stem bark.

Conc. of extract (mg/ml)	Zone of inhibition (mm)							
	EC	KP	SA	BA	ST	PA	PM	CA
250	NL	NL	45	NL	NL	NL	NL	50
200	NL	NL	30.5	NL	NL	NL	NL	35
150	NL	NL	20	NL	NL	NL	NL	22
100	NL	NL	14.5	NL	NL	NL	NL	22
50	NL	NL	NL	NL	NL	NL	NL	22
10	NL	NL	NL	NL	NL	NL	NL	12

NL = - No inhibition, EC = *E. coli*, KP = *K. pneumoniae*, SA = *S. aureus*, BA = *B. anthracis*, ST = *S. typhi*, *P. aeruginosa*, PM = *P. mirabilis*, and CA = *C. albicans.*
Values are means of duplicate readings.

Table 3. Antimicrobial screening of different concentrations of the methanolic extract of *B. ferruginea* root.

Conc. of extract (mg/ml)	Zone of inhibition (mm)							
	EC	KP	SA	BA	ST	PA	PM	CA
250	30	NL	30	NL	28	NL	26	19
200	30	NL	15	NL	25	NL	26	16
150	30	NL	15	NL	25	NL	23	13
100	30	NL	NL	NL	15	NL	19	13
50	30	NL	NL	NL	15	NL	NL	NL
10	NL	NL	NL	NL	NL	NL	NL	NL

NL = - No inhibition, EC = *E. coli*, KP = *K. pneumoniae*, SA = *S. aureus*, BA = *B. anthracis*, ST = *S. typhi*, *P. aeruginosa*, PM = *P. mirabilis*, and CA = *C. albicans.*
Values are means of duplicate readings.

analgesic and anti-edematous in action. The presence of saponin, alkaloids and tannin enhanced the antimicrobial activity against the broad spectrum of organisms (Ndukwe et al., 2005). The observed antibacterial effects corroborate its traditional uses. The plants stem bark is used traditionally in treatment of typhoid fever and various stomach related problems (Adetunji, 1999): in this work the extracts of the plant's stem bark inhibited the growth of *E. coli* and *S. typhi* to a high degree. These two bacteria are responsible for various stomach related illnesses; *S. typhi* is the causative organism of typhoid fever, a systemic infection associated with the consumption of contaminated food while, *E. coli* is responsible for a number of food related illnesses that manifest themselves in the form of diarrhea (Adams and Moss, 1999). The relatively large zone of inhibition exhibited by the extract on *S. aureus*, *P. mirabilis* and *C. albicans* suggests that it could be used in the treatment of infections commonly associated with the microorganisms. *B. ferruginea* has been listed as one of the medicinal plants in common use in African (Ndukwe et al., 2005). The MIC results were presented in Table 4. The crude extract of the root inhibited and fully prevented the growth of *E. coli* (40 mg/ml), *S. aureus* (100 mg/ml), *S. typhi* (60 mg/ml), *P. mirabilis* (60 mg/ml) and *C. albicans* (80 mg/ml), while the extract from the stem bark is minimal at 60 mg/ml against *S. typhi* and 10 mg/ml against *C. albicans* and extract from the leaves did not show any activity against the organisms.

Table 4. Summary of MIC of methanol extract of *B. ferruginea*.

Test organism	Minimum inhibitory conc. (mg/ml) of *B. ferruginea*		
	Leaves	Stem bark	Root
E. coli	NIL	NIL	40
K. pneumoniae	NIL	NIL	NIL
S. aureus	NIL	NIL	NIL
B. anthracis	NIL	NIL	NIL
S. typhi	NIL	60	60
P. aeruginosa	NIL	NIL	NIL
P. mirabilis	NIL	NIL	60
C. albicans	NIL	10	80

NIL = no MIC values.

The findings in this work agree with the use of this plant in ethnomedicinal treatment of wounds, sprain cold etc

Ndukwe et al., 2007). The plant can also be used as antimalaria and against other diseases which are caused by some of these organisms used in this study. More work is recommended to ascertain some of the bioactive compounds and their uses in this plant.

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