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Full Length Research Paper

Evaluations of the methanol extract of *Ficus exasperata* stem bark, leaf and root for phytochemical analysis and antimicrobial activities

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The methanol extract of *Ficus exasperata* (stem bark, leaf and root) was investigated for activity against some human pathogenic organisms. Phytochemical screening revealed the presence of flavonoids, saponin, tannins, steroids and phlobatannins with no traces of alkaloids and anthraquinones. The results of *in vitro* antimicrobial screening of the methanol extract exhibited a wide range of activity on *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. The leaf methanol extract inhibited the growth of *P. aeruginosa, S. typhi, S. aureus*, and *E. coli* at concentrations of 5.0, 1.0, 1.5 and 1.25 mg/ml respectively, while the stem bark extract had minimum inhibitory concentration (MIC) of 50 mg/ml on *P. aeruginosa,* 1.0 mg/ml on *S. typhi* and 75 mg/ml on *S. aureus*. The extract from the root inhibited the growth of *P. aeruginosa* at a concentration of 75 mg/ml and inhibited *S. typhi* at concentration of 1.0 mg/ml, while *S. aureus* and *E. coli* were inhibited at a concentration of 1.25 mg/ml. The minimum bactericidal concentration (MBC) of the leaf extract were obtained against *P. aeruginosa, S. typhi S. aureus,* and *E. coli* at concentration of 75, 1.0, 5.0 and 1.25 mg/ml respectively, while the extract from stem bark had the MBC against *P. aeruginosa,* and *S. typhi* at concentration of 75 and 1.25 mg/ml respectively. The bactericidal effects from the root extract were 50, 5.0, 1.5, 1.25 and 1.25 mg/ml against *P. aeruginosa, S. typhi, S. aureus, E. coli* and *Vibrio cholerae* respectively. The above results show that *F. exasperata* leaf, stem bark and root contained bioactive substances with the highest inhibitory activities against some human pathogenic organisms.

Key word: *Ficus exasperata*, phytochemical, antimicrobial activity, minimum inhibitory concentration and minimum bactericidal concentration.

INTRODUCTION

Ficus exasperata belongs to the family Moraceae, with 800 species occurring in the warmer part of the world, chiefly in Indomalaya and Polynesia (Odunbaku et al., 2008). The Nigeria are replete with over 45 different species of *Ficus* (Keay and Onochie, 1964), such as *Ficus glomosa, Ficus lecardi, Ficus goliath, Ficus capensis, Ficus ingens* and *F. elastica,* which can be found in the Savannah, rainforest, besides rivers and streams.

F. exasperata is commonly known as sand paper tree ("Ewe ipin" in Yoruba) and is widely spread in West Africa in all kinds of vegetation and particularly in secondary forest re-growth. The leaf extract from *F. exasperata* reported to have diverse uses such as treating hypertensive patients (Buniyamin et al., 2007), heamostative opthalmia, coughs and heamorrhoid (Odunbaku et al., 2008).

In Nigeria, young leaves of *F. exasperata* are prescribed as a common anti-ulcer remedy. Various pharmacological actions such as anti-diabetic, lipid lowering and antifungal activities have been reported for *F. exasperata* (Sonibare et al., 2006).

Other industrial uses of sand paper leaves are for polishing woods (Cousins and Michael, 2002), stabilization of vegetable oils, suppression of foaming, supplement as food stock and antimicrobial (Odunbaku et al., 2008). The activities of leaf extract of *F. exasperata*

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against some pathogenic organisms have been extensively investigated (Buniyamin et al., 2007; Odunbaku et al., 2008). However, reports on bioactive activities of the extracts from root and stem bark of *F. exasperata* have not been widespread. This present study was intended to elucidate the chemical constituents' of methanolic extraction of leaf, stem bark and root with a view of authenticating the plant's antimicrobial potentials.

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh samples of *F. exasperata* leaf, stem bark and root were collected from teaching and research farm of Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

The plants were identified and authenticated at the Herbarium of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. A voucher specimen was deposited at the herbarium for reference purpose. The fresh leaf, stem bark and root of *F. exasperata* were dried for 5 min in an oven at 60°C to stop enzyme activity (Effraim et al., 2000), then air dried at ambient temperature (30 ± 2°C) for 10 days, pulverized using Binatone blender (Model BLG-401) and obtained powdery form were store until further uses.

Preparation of Methanol extract

The Adebayo and Ishola (2009a) method of extraction was used. 250 g of each plant part (Leaf, stem bark and root) were packed in a soxhlet extractor and extracted with methanol. The methanol extracts were evaporated to dryness using a rotary evaporator (Stuart, Barloworld and Model RE 300). The obtained crude extracts were 27.3 g (68.8%) leaf, 31.2 g (78%) stem bark and 36.3(90.8%) root. 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 (leaf, stem bark and root) in 10 ml of dimethylsulphoxide (DMSO) to obtain stock solutions of 1000 mg/ml concentration each. From the stock solution, concentrations (mg/ml) of 250, 200, 150, 100, 50 and 10 were obtained by serial dilution. These were store at 15°C until further uses.

Phytochemical analyses

The phytochemical analyses of the plants extracts were carried out following the methods of Sofowora (1986), Rai and Obayemi (1973), Wallis (1967) and Elujoba et al. (1989).

Test organisms

Clinical isolates of *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Vibro cholerae*, *Candida albicans* and *Klebsiella pneumoniae* were obtained from University of Lagos Teaching hospital. The isolates were tested for viability by resuscitating them in buffered peptone water after which they were subculture into nutrient agar medium and incubated at 37°C for 24 h. The organisms were then stored at4°C until needed.

Evaluation of antimicrobial activity

Agar well diffusion techniques as described by Adeniyi et al. (1996) was adopted for the study. 18 ml of Mueller Hinton agar plates (MHA oxoid) England, were inoculated with 0.1 ml of an overnight broth culture of each bacteria isolate (Equivalent to 3 x 10^7cfu/ml) MF (Mcfarland standard) (Cheesbrough, 2000) in sterile Petri-dish. The seeded plates were rocked for uniform distribution of isolates and allowed to set.

Holes were bored on the plates by using standard sterile cork borer of 6 mm diameters and equal volumes of the plants extracts (1000 µl) were transferred into the well with the aid of micropipette. The experiments were carried out in duplicate.

The control experiments were setup with 1000 µl of 70% methanol in separate welled. The plates were allowed to stand for one hour at room temperature to allow proper diffusion of the extract (Esimone et al., 1998). The plates were incubated at 37°C for 24 h until marked decline in the potency of the extracts to inhibit the growth of the test isolates was observed. Zone of inhibitions were measured in millimeter (mm) and the average values were calculated and recorded.

Determination of the minimum inhibitory concentration

The determination of the minimum inhibitory concentration (MIC) was carried out on the extract because it showed sensitivity against the growth of the test organisms. The medium used was Mueller Hinton Agar solution which was prepared according to the manufacturer's standard of 38 g/1000 ml. In this case double strength was prepared by dissolving 38 g in 500 ml of distilled water, homogenized and 5 ml was dispensed into 40 sets of McCartney 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 poured into Petri-dishes and allow to solidity for one hour.

Extracts' concentration of 100, 75, 50, 25, 5, 1.5, 1.25, 1.0, 0.75 and 0.50 mg/ml were prepared by serial dilution. Each plate was divided into 4 (four) equal section and labeled accordingly. The 5 mm diameter paper discs were placed aseptically into each labeled section of the plate using sterilized forceps. With an automatic micropipette, 0.1 ml of each bacterial suspension was taken and transferred aseptically 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 growths or death of the test organisms. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC).

Determination of the minimum bactericidal concentration (MBC)

The determination of MBC was carried out by preparing 40 sets of plates of Mueller Hinton agar and sterilized. The paper discs in all the plates from MIC tests were reactivated, using a mixture of 0.5% egg lecithin and 3% Tween 80 solution.

The reactivated organisms were subcultured into appropriately labeled quadrants of the sterilized Mueller Hinton agar plates. The organisms were uniformly streaked on labeled quadrants using wire loop. The organisms were incubated at 37°C for 24 h, after which growth were observed and recorded. The MBC was the quadrant with lowest concentration of the extract without growth.

RESULTS AND DISCUSSION

The preliminary phytochemical tests carried out on the powdered leaf, stem bark and root showed the presence of tannins, flavonoid, saponins, Phlobatannins, glycolsides

Table 1. Phytochemical analyses of leaf, stem bark and root of *F. exasperate.*

Phytochemicals	Leaves	Stem bark	Root
Tannins	+	+	+
Flavonoid	+	+	+
Saponins	+	+	+
Phlobatannins	+	+	+
Anthraquinones	-	-	-
Glycosides	+	+	+
Steroids	+	+	+
Alkaloids	-	-	-

+ = present, - = absent.

Table 2. Antimicrobial activities of *F. exasperata* methanolic root extract.

Conc. of extract mg/ml	Zone of Inhibition (mm)							
	PM	PA	ST	SA	EC	VC	CA	KP
250	-	6±2.6	20±3.1	10±2.0	18±2.6	18±2.8	-	16±2.1
200	-	6. ±2.6	20±3.1	10.2.0	14±2.6	12±2.8	-	16±2.1
150	-	4±1.8	20±2.9	8±1.5	6±1.4	5±1.2	-	12±1.6
100	-	3±1.2	18±2.9	6±1.4	4±1.2	3±1.0	-	5±1.4
50	-	3±1.2	15±2.6	6±1.5	4±1.2	3±1.0	-	-
10	-	3±1.2	12±2.5	6±1.5	3±1.2	3±1.0	-	-

- = No inhibition
PM = *P .mirabilis,* PA = *P. aeruginosa*, ST = *S. typhi*, SA = *S. aureus,* EC = *E. coli,* VC = *V. cholerae*, CA= *C. albicans*, KP = *K. pneumoniae*, Values are means of duplicate readings

and steroids, with no traces of alkaloids and anthraquinones, which is shown in Table 1.

The presence of saponins, tannins, steroids and phlobatannins in the tested plant's parts is an indication that the plant is of pharmacological importance (Adebayo and Ishola, 2009b). Presence of flavonoid indicates the natural occurring phenolic compound, with beneficial effects in the human diet as antioxidants and neutralizing free radicals.

Table 2 shows the antimicrobial activities of the root methanolic extract against *P. aeruginosa, S. typhi, S. aureus, E. coli, V. cholerae* and *K. pneumoniae* and methanol extract from stem bark was active against *P. mirabilis, P. aeruginosa, S. typhi, S. aureus, E. coli, V. cholerae* and *K. pneumoniae* (Table 3), while methanolic extract from the leaf inhibited the growth of *P. aeruginosa, S. typhi, S. aureus, E. coli, V. cholerae* and *K. pneumoniae* (Table 4). Ndukwe et al. (2005) reported that saponins and flavonoid present in plant extracts have varied uses as antiulcerogenic, anti-inflamatory, fibrinolytic, antipyretic, analgesic and anti-edematous.

The activity of the extracts against *S. typhi, E. coli* and *V. cholerae* which are the potential causative agents of abdominal ailment agreed with previous work (Sonibare et al., 2006), reported that young leaves *F. exasperata* used as remedy for some abdominal ailments. The decoction of the leaf has been established to exhibit significant reduction in intestinal motility in addition to its anti-ulcer activity with no sign of toxicity (Gamaniel et al., 1997). The stem bark and leaf is used traditionally in treatment of typhoid fever and various stomach related problems (Odunbaku et al., 2008). In this work the extracts of the plant's parts (stem bark, leaf and root) inhibited the growth of *E. coli, S. typhi* and *V. cholerae* to a high degree. These bacteria are responsible for various stomach related illnesses; *S. typhi* is causative organism of typhoid fever, a systemic infection associated with the consumption of contaminated food (Adebayo and Ishola, 2009b), while *E. coli* and *V. cholerae* is responsible for a number of food and water related illnesses that manifest themselves in the form of diarrhea (Adams and Moss, 1999). The relatively large zone of inhibition exhibited by the extracts on *P. aeruginosa, S. aureus* and *K. pneumoniae* suggests that it could be used in the treatment of infections commonly associated with these microorganisms. *F. exasperata* has been listed as one of medicinal plants commonly used in Africa (Cousin and Micheal, 2002).

The MIC results were presented in Table 5. The extract of the leaf inhibited the growths of *P. aeruginosa* (50 mg/ml), *S. typhi* (1.0 mg/ml), *S. aureus* (1.5 mg/ml), *E. coli* (1.25 mg/ml) and *K. pneumoniae* (100 mg/ml), while the extract from the stem bark had minimal inhibition at 100 mg/ml (*P. mirabilis, E. coli* and *K. pneumoniae*), at

Table 3. Antimicrobial activities of *F. exasperata* methanolic stem bark extract.

Conc. of extract mg/ml	Zone of Inhibition (mm)							
	PM	PA	ST	SA	EC	VC	CA	KP
250	12±1.4	8±2.7	18±2.7	15±2.4	16±2.6	6±1.6	-	16±2.1
200	10±1.4	8±2.1	18±2.7	15±2.4	6±1.6	6±1.6	-	16±2.1
150	10±1.4	6±1.6	16±2.7	12±2.3	6±1.6	-	-	4±1.2
100	4±1.0	6±1.6	16±1.6	4±1.7	4±1.2	-	-	-
50	-	4±1.2	14±1.2	-	-	-	-	-
10	-	-	12±1.2	-	-	-	-	-

- = No inhibition
PM = *P .mirabilis,* PA = *P. aeruginosa*, ST = *S. typhi*, SA = *S. aureus,* EC = *E. coli,*
VC = *V. cholerae*, CA= *C. albicans*, KP = *K. pneumoniae*
Values are means of duplicate readings

Table 4. Antimicrobial activities of *F. exasperata* methanolic leaf extract.

Conc. of extract mg/ml	Zone of Inhibition (mm)							
	PM	PA	ST	SA	EC	VC	CA	KP
250	10±1.7	10±1.7	17±2.1	12±1.5	10±1.7	8±1.4	-	19±2.1
200	-	5±1.4	16±2.1	12±1.5	4±1.1	-	-	+5±1.6
150	-	5±1.4	13±1.7	10±1.4	4±1.1	-	-	6±1.4
100	-	3±1.0	13±1.7	4±1.2	4±1.1	-	-	2±1.1
50	-	-	13±1.7	4±1.2	2±1.1	-	-	-
10	-	-	-	4±1.2	2±1.1	-	-	-

- = No inhibition
PM = *P .mirabilis,* PA = *P. aeruginosa*, ST = *S. typhi*, SA = *S. aureus,* EC = *E. coli.*
VC = *V. cholerae*, CA= *C. albicans*, KP = *K. pneumoniae*
Values are means of duplicate readings

Table 5. MIC values of methanolic and leaf extracts of *F. exasperate.*

Test orgs.	MIC at different conc. (mg/ml) of extracts		
	Leaf	Stem bark	Root
P. mirabilis	-	100	-
P. aeruginosa	50	50	75
S. typhi	1.0	1.0	1.0
S. aureus	1.5	75	1.25
E. coli	1.25	100	1.25
V. cholerae	-	-	-
C. albicans	-	-	-
K. pneumoniae	100	100	100

- = No MIC at coverage concentrations

50 mg/ml against *P. aeruginosa,* at (1.0 mg/ml) *S. typhi* and *S. aureus* at (75 mg/ml). The MICS for the root were 75 mg/ml against *P. aeruginosa*, 1.0 mg/ml against *S. typhi,* 1.25 mg/ml against *(S. aureus* and *E. coli*) and 100 mg/ml against *K. pneumoniae*.

The MBC results were shown in Table 6. The killing effect of the plant leaf extract were (75 mg/ml for *P. aeruginosa*, 1.0 mg/ml, 1.25 mg/ml for *E. coli* and 100 mg/ml for *K. pneumoniae*), while stem bark extract gave MBC of (100 mg/ml for *P. mirabilis, S. aureus, E. coli* and *K. pneumoniae*), 75 mg/ml for *P. aeruginosa* and 1.25 mg/ml for *S. typhi*. The MBCs values for root extract were 50 mg/ml against *P. aeruginosa,* 5.0 mg/ml against *S. typhi,* 1.5 mg/ml against *S. aureus*, 1.25 mg/ml against *E.*

Table 6. MBC values of methanolic root, stem bark and leaf extracts of *F. exasperate.*

Test orgs.	MIC at different conc. (mg/ml) of extracts		
	Leaf	Stem bark	Root
P. mirabilis	-	100	-
P. aeruginosa	75	75	50
S. typhi	1.0	1.25	5.0
S. aureus	5.0	100	1.5
E. coli	1.25	100	1.25
V. cholerae	-	-	1.25
C. albicans	-	-	-
K. pneumoniae	100	100	100

- = No MBC at coverage concentrations.

coli and *V. cholerae* and 100 mg/ml against *K. pneumoniae*. The results of MBC obtained shows that the extracts from the plants were very active at lower concentrations against the human pathogenic tested for in this research work. The findings in this work have justified the potent use of this plant in ethnomedicinal treatment of stomach illnesses, ulcer, diarrhea (Sonibare et al., 2006), which are caused by some of these organisms used in this study. Work is in progress in the isolation and characterization of the bioactive compounds of this plant.

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