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Short Communication

Evaluation of anti-bacterial properties of ethanol extract of *Ficus exasperata* leaf

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This study evaluated the antibacterial effect of ethanolic extract of *Ficus exasperate* leaf. Clinical isolates of *Escherichia coli, Pseudomonas aeruginosa, Staphyllococcus aureus* and *Klebsiella pneumoniae* were incubated in agar plates after serial dilutions of the extract were added in wells made in the agar and incubated for 24 h at 37°C. Thereafter, zones of inhibition (in mm) around the wells were measured. The same organisms were tested for sensitivity against standard antibiotics using disk diffusion. The extract demonstrated significant antibacterial activity. It was concluded that the extract had significant antibacterial activity against test organisms with minimum inhibitory concentration (MIC) of 250 µg/ml. Its use for treatment of infections by traditional healers is justified.

Key words: *Ficus exasperata*, minimum inhibitory concentration, antibacterial activity, agar dilution.

INTRODUCTION

Ficus exasperata belongs to the family Moraceae. It is a shrub found mainly in the tropics and subtropics. It is generally known as sand paper tree because its leaves have rough surfaces. It is also called *inwalinwa* (in Igbo) and *eweipin* (in Yoruba).

Its medicinal uses include treatment of skin infections (Chinsembu and Hedimbu, 2010), treatment of cough and hemorrhoids (Cousins and Michael, 2002) and treatment of peptic ulcers (Akah et al., 1998; Sonibare et al., 2006). It has anti-inflammatory, antipyretic and antinociceptive properties (Woode et al., 2009) as well as stimulatory effect on uterine smooth muscles (Bafor et al., 2010) and hypertensive effect (Ayinde et al., 2007). In Idemili North Local Government Area, Anambra State, Nigeria, decoction of its leaves, taken orally is used in the treatment of acute urethritis. Several other herbs recorded for the treatment of urethritis/STI include *Cocos nucifera, Acacia sieberiana* (Okoli et al., 2007), *Imperata cylindrica, Phyllatus muellerianus, Pseudondia microcarpa* and *Tapinanthus bangwensis* (Jiofack et al., 2010).

This study aimed at evaluating the claims of efficacy of this plant extract in the treatment of infectious disease by traditional medicine practitioners. Specifically, it aimed at demonstrating the activity of this extract against *Escherichia coli, Pseudomonas aeruginosa, Staphyllococcus aureus* and *Klebsiella pneumoniae.*

MATERIALS AND METHODS

Plant source

Fresh leaves of *Ficus exasperuta* were collected at Umuoji, Idemili North Local Government, Anambra State, Nigeria in the month of February 2010. The leaves were identified by Prof. C. U. Okeke of Botany Department, Nnamdi Azikiwe University, Awka.

Preparation of ethanolic extract

Fresh leaves of *F. exasperata* were air-dried at room temperature. 200 g of dry leaves were soaked in 2 L of absolute alcohol for 48 h

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Table 1. Inhibitory effect of increasing concentrations of extracts and control on test organisms.

Test organism	Concentration of extract (mg/ml)						
	3.1	6.25	12.5	25	50	100	Control
E. coli	-	-	-	2	6	8	2
P. aeruginosa	-	-	-	2	3	7	2
S. aureus	-	-	-	0.5	3	5	4
K. pneumoniae	-	-	-	-	-	5	3

Table 2. Inhibition of test organisms by standard antibiotics using disk diffusion test.

Test organism	Concentration of antibiotic (µg)	Ofloxacin	Gentamicin	Ceftriaxone	Ciproxin	Lincocin
		5	10	10	5	10
E. coli	zone of inhibition	25	10	30	30	8
P. aeruginosa	zone of inhibition	10	6	-	-	-
S. aureus	zone of inhibition	-	12	12	-	-
K. pneumoniae	zone of inhibition	-	5	10	6	-

and filtered. The filtrate was evaporated to dryness using Soxhlet extractor. It yielded 9.5 g of extract giving extractive value of 4.75%. This was stored in a refrigerator until use.

Phytochemical analysis

Preliminary phytochemical test revealed the presence of flavonoids, glycosides, phenolic acids, saponins, alkaloids, steroids.

Preparation of extract solutions

6 g of extract was dissolved in 60 ml of absolute alcohol giving a concentration of 0.1 g/ml (100 mg/ml). This was taken as stock solution. Serial dilutions of the stock solution were prepared by mixing 1ml of stock solution with 1, 2, 3, 4 and 5 ml of distilled water giving 50, 25, 12.5, 6.25 and 3.16 mg/ml respectively.

Laboratory procedures

Isolates of *S. aureus, E. coli, K. pneumomae* and *P. aeruginosa* used were obtained from Nnamdi Azikiwe University Teaching Hospital Clinics and stored in Oxford Nutrient Agar slants for one week before use. Twenty microliter Nutrient agar was prepared according to the manufacturer's (Oxford) instructions. The agar was poured into six Petri dishes and allowed to set. The agar in Petri dishes were sterilized and allowed to cool. The plates were inoculated with test organisms. Seven equal holes (6 mm) were made in each agar plate with sterile cork borer (one of the holes being central in position); two plates each for *E. coli, K. pneumomae S. aureus* and *P. aeruginosa*. The Petri dishes were labeled with their respective organisms and the wells marked with 100, 50, 25, 12.5, 6.25 and 3.1 mg/ml respectively for easy identification. The wells were filled with 5 drops of corresponding dilution of the extract. The central well was filled with absolute alcohol to act as the control. They were allowed to diffuse well into the agar medium. They were then incubated at 37°C for 24 h and zones of inhibition (in mm) recorded. For comparison, the organisms from the same clinical specimens were tested for sensitivity using standard antibiotic diffusion discs. The bench environment was sterilized while the procedure lasted by continuous burning of the Bunsen burner.

RESULTS AND DISCUSSION

The ethanolic extract exhibited significant inhibitory effects on *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae* as shown by increase in zones of inhibition as the concentration of extract increased. Table 1 shows the inhibitory effect of increasing doses of the extract and control on test organisms while Table 2 shows the effect of standard antibiotics on the test organisms for comparison.

The lowest concentration of the extract that inhibited growth of test organisms was 25 mg/ml. The inhibition of test organisms by the extract was dose dependent with the lowest concentration being 25 mg/ml. This was the MIC of the extract for *E. coli, S. aureus* and *P. aeruginosa*. For *K. pneumonia*, the MIC was 100 mg/ml. MICs are used by diagnostic laboratories to establish resistance or to determine *in vitro* activity of new antimicrobials (Andrews, 2002). The basic qualitative measures of the *in vitro* activity of antimicrobials are the minimum inhibitory concentrations (MIC) or minimum bacterial concentration (MBC).

Considering the MICs of standard antibiotics against the test organism (Table 2) this extract, being unrefined, can be said to have substantial antibacterial activity. *E. coli* exhibited the highest level of susceptibility to antibacterial effect of the extract as shown in Figure 1. This was also the pattern observed with the standard antibiotics. It is equally interesting that this extract exhibited antibacterial activity against *P. aeruginosa*, one of the most resistant organisms in clinical medicine. In an earlier work on this extract, Odunbaku et al. (2008) found

the MIC of this extract against *E. coli* to be 300 mg/ml. This high MIC (low activity) could be due to geographical location, age of plant at harvest, season of harvest and method of extraction, all of which affect the yield of active constituents of medicinal plants (Calixto, 2000; Evans, 2002). It could also be due to differences in laboratory procedures and reagents (Bonini et al., 2002; Wallack, 2007).

Conclusion

From the above findings, it could be concluded that ethanol extract of *F. exasperata* leaf has significant antibacterial activity. Its use for the treatment of infections by traditional healers is justified. It should be further explored for commercial production of novel antimicrobial agents.

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