THE ANTIMICROBIAL PROPERTIES OF METHANOL EXTRACTS OF FICUS EXASPERATE LEAVES FROM AFIKPO METROPOLIS

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ABSTRACT
Ficus exasperate (sand paper tree) has been used in folk medicine to treat different ailments, including wound infections. The Phytochemical and antimicrobial activity of methanol and acetone extracts of *Ficus exasperate* was evaluated. The following phytochemicals were contained in the extracts; Tannins, Flavinoids, Terpenoids, Alkaloids and cardicglucosides. Saponins, Steriods and anthraquinone were absent. The highest antimicrobial activity was exhibited by the methanol extract against *Staphylococcus aureus.* The minimum inhibitory concentration (MIC) of the extracts ranged between 0.410-1.654mg/mL, with the acetone extract having low MIC values. The antibiotic efficacy of organic solvent extracts of *Ficus exasperata* could likely be because of the phytochemicals contained in the extract. The results obtained from this study supports the folkloric use of the plant. The plant could be useful in production of antibiotics. Further advanced investigation is hereby suggested.
Keywords: Medicalplants, *Ficus exasperata,* Phytochemical, antimicrobial

Introduction

Certain plants with medicinal properties have been identified and used by man to treat many diseases. Due to the increasing rate of resistance to conventional drugs by microbial agents, novel antimicrobial agents from different biological sources have been sought after and reported to be effective in killing pathogenic organisms (Awala*et al., 2017).*

*Ficusexasperata*Vahl. (Moraceae) popularly referred to as "Sand paper leaf tree" owing to the rough surface of the leaves is increasingly being used for a number of ailments and hence studies validating the traditional claims are on the increase (Faiyaz*et al*., 2012).Available reports indicate that leaves of *F.exasperata*have antiulcer, hypotensive, hypoglycemic, antimicrobial, activities among others uses (Faiyaz*et al*; 2012). Thispresent study was carried out to evaluate the phytochemical and antimicrobial activity of methanolic and acetone extracts of F.*exasperata*leaves in an attempt to elucidate its chemical constituents with a view of authenticating the plant's antimicrobial properties.

MATERIALS AND METHODS

Collection and Preparation of plant material

Fresh leaves of F.*exasperata*were collected from farm lands within and around the AkanuIbiam Federal Polytechnic Unwana, Ebonyi State; Nigeria.

The plants were identified and authenticated at Biology unit of the Department of Science Laboratory Technology of AkanuIbiam Federal Polytechnic Unwana. Voucher Specimen was deposited at the herbarium. The fresh leaves were cleaned with distilled water and dried for about 5 minutes in an oven at 60^0C to stop enzyme activity (Effraim*et al;*2000), then air dried at room temperature (30± 2ºC) for 10 days, before being ground into a fine powder using an electric blender. It was stored air tight containers.

Organic Solvent Extraction

About 500g of the powered leaves of *F.exasperata*were weighed and extracted by maceration for 72h in absolute methanol (Pandey and Tripathi, 2014) as well as acetone. The extracts were then filtered and, evaporated to dryness and stored in capped bottles and kept in the refrigerator at 4ºC until required.

Preparation of Crude Extracts

The stock solutions of the extracts were prepared by dissolving 10g of the extract in 10ml of dimethylsuphoxide (DMSO) to obtain a stock concentrations (mg/ml) of 250, 200, 150,50 and 10 by serial dilution. These were stored at 15ºCuntil further use.

Phytochemical analysis

The methods described by Harbone (2005), Trease and Evans (2005); Odebiyi and Sofowora (1978) was used to determine the phytochemical constituents.

Evaluation of antimicrobial activity

Agar well diffusion assay as described by Adeniyi*et al.* (1996) was adopted for this study. 18ml of Mueller Hinton agar plates (MHA,Oxoid) England, were inoculated with 0.1ml of an overnight both culture of each bacteria isolate (3×10^7cfu/ml)Mcfarland standard (Cheesbrough, 2000) in sterile petri dish. The seeded plates were rocked for uniform distribution of isolates and allowed to gel.

Holes were bored on the plates using standard sterile cork bores of 6mm diameter and equal volume of plant extracts (1000µl) were transferred into the wells with the aid of micropipette. The experiments were carried out in duplicates.

The control experiments were setup with 1000µl of 70% methanol is separate wells. 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 37ºCfor 24hrs 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 value were calculated and recorded.

Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was carried out on the extract because it showed sensitivity against the growth of the test organisms. Mueller-Hinton (MH) agar solution was prepared according to the manufacture's instruction. Double strength of the media was prepared by dissolving 39g/500ml against 38g/1000ml. the solution is homogenized and dispensed into Mccartney bottles and sterilized in an autoclave at 121ºCfor 15mins. The Media after cooling to about 45ºC is poured into Petri-dishes and allowed to gel for about one hour.

Extracts were serially diluted at different concentration. Each plate was divided into 4 equal sections and are properly labeled. Paper dishes (5mm) were aseptically placed on each labeled section of the plate using sterilized forceps. 0.1ml of each bacterial suspension was taken and transferred aseptically into the appropriate paper disc on the plates; which were incubated for 24hrs at 37ºC. the lowest concentration inhibiting growth was taken as the MIC.

RESULTS

Table 1 Shows the result of the qualitative phytochemical analysis of the leaves of *F.exasperata.* Tannins, flavonoids, Cardiac glycoside and alkaloids were present, while steroids, phlobatannin and anthraquinone were absent.

Table 1: Qualitative phytochemical screening of*F.exasperata leaf extracts.*

Extracts		
Phytochemical MLEALE		
Saponin	–	–
Flavonoid	+	+
Tannin	+	+
Steroids	–	–
Terpenoids	+	+
Alkaloids	–	+
Phlobatannin	–	–
Authraquinone	–	–
Cardic glycosides	+	+

KEY: MLE = Methanol Leaf extract of *F.exasperata*

ALE= Acetone Leaf extracts

Table 2 shows the result of the Antimicrobial activity of the leaf extracts and commercial drugs against selected organisms.

The acetone leaf extract of *F.exasperata*showed good antimicrobial activity against the test organisms. The highest antimicrobial activity (31:30mm) was shown by the methanol leaf extract

(30.30mm) when tested with the same organism. The Methanolic and Acetone leaf extracts of *F.exasperata*had better antibacterial activity than antifungal activity.

The antibacterial activity of Gram positive bacteria isolates was better than that of the Gram negative isolates. The extracts also showed better activity in some cases when compared with conventional antibiotics

Table 2: Antimicrobial activity of leaf extracts of *F.exasperata*compared with commercial drugs

	Zone of Inhibition (mm)						
Test organism	**ALE**	**MLE**	**CPX**	**R**	**CLOT**	**GRIS**	**NYST**
Salmonella typhi	18.43 ± 0.18^{c}	12.47 ± 0.15^{a}	12.40 ± 0.23^{a}	14.37 ± 0.20^{b}	NT	NT	NT
Staphylococcus aureus	30.30 ± 0.17^{c}	31.30 ± 0.15^{d}	14.40 ± 0.12^{a}	15.50 ± 0.17^{b}	NT	NT	NT
Escherichia coli	17.50 ± 0.26^{ab}	15.60 ± 0.17^{ab}	11.50 ± 0.23^{ab}	49.97 ± 36.67^{a}	NT	NT	NT
Pseudomonas aeruginosa	12.27 ± 0.15^{c}	15.40 ± 0.12^{b}	15.43 ± 0.15^{a}	16.33 ± 0.18^{b}	NT	NT	NT
Candida albicans	18.37 ± 0.23^{d}	10.27 ± 0.15^{b}	NT	NT	12.27 ± 0.15^{c}	20.50 ± 0.29^{e}	6.40 ± 0.21^{a}
Aspergilusniger	15.61 ± 0.18^{b}	3.46 ± 0.20^{b}	NT	NT	22.33 ± 0.33^{d}	20.50 ± 0.29^{e}	17.47 ± 0.31^{C}

Each value is expressed as mean ± standard error (n=3) Values with different superscript within a row are significantly different at (p=0.05).

Keys: ALE: Acetone Leaf extract of Ficus exasperate MLE: Methanol leaf extract of Ficus exasperate; CPX Ciprofloxacin (10µg); R: Rocephin (25µg); CLOT: Clotrimazole (1mg/ml); GRIS: Griseofluvin (1mg/ml); NYST:Nystatin (1mg/ml); NT: Not tested

Table 3: The Minimum inhibitory concentration (mg/ml) of values of values the leaf extracts of *F.exasperata*

MLC (mg/ml)

Test Organism	ALE	MLE
Salmonellatyphi	1.654	1.654
Staphylococcus aureus	0.40	0.771
Escherichia Coli	0.410	0.410
Pseudomonas aeruginosa	1.654	1.654
Candidaalbicans	0.410	0.410
Aspergillusniger	0.410	0.410

Key: ALE: Acetone Leaf extract of *F.exasperata*
MLE: Methanol leaf extract of *F.exasperata*

Discussion

The present study investigated the phytochemical analysis and antimicrobial activity of organic solvent leaf extracts of *Ficusexasperata*

The phytochemicals present in the leaf extracts is solely responsible for their medicinal properties as well as their antimicrobial activity as these compounds have been reported to be inhibitory against pathogenic microorganisms (Mangany*et al;* 2015)

The relatively large zone of inhibition shown by the extracts against *Salmonella typhi,Staphylococcus aureus*and *Pseudomonas acruginosa*suggests that, the extracts could be used to treat infections caused by the above mentioned organisms. *F. exasperata*is one of the Medicinal plants used in African countries to treat infections (Cousin and Michael, 2002).

The antibacterial activity demonstrated by the extracts is higher than antifungal activity, agreeing with the finding of several authors (Modarresi-Chahardehi*et al;*2012, Momooh*et al;* 2021).

Susceptibility of Fungi to antimicrobial agents is much lower than bacteria, due to the chitinous contents of the fungal cell wall (Madigan and Martinko, 2006). The findings from this work have justified the ethno- medical use of this plant leaves in the treatment of common diseases such as diarrhoea, stomach illness and ulcers (Sonibare*et al;*2006). There is a need for researchers to progress in the isolation, purification and identification of the bioactive compounds in this plant.

Conclusion

The extracts from the leaves of *F. exasperata*contained active compounds which inhibited the growth of tested organisms. These bioactive compounds could be extracted and used for the production of antibiotics with broad sprectrum activity

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