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Antimicrobial Properties of Crude Ethanolic Extract of *Ficus exasperata* Root

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ABSTRACT

Pathogenic microorganisms are becoming increasingly resistant to existing antibiotics at alarming rates, creating problems in health care delivery in man and animals, thus shifting attention towards the use of herbs by traditional healers. Also several important drugs have been discovered from plants which are now synthesized chemically for commercial purposes. In this study, the ethanolic extract of *F. exasperata* was screened against *Salmonella typhi*, *Shigella dysenteriae* and *Pseudomonas aeruginosa.* Fresh roots of *F. exasperata* were collected from its natural habitat and extraction carried out using Soxhlet extractor. Agar well diffusion method was used to determine the inhibitory property and the minimum inhibitory concentration (MIC) of the extract at 300 mg/ml, 400 mg/ml and 500 mg/ml. The extract did exhibit antimicrobial property on *S. typhi*; antimicrobial effect was observed on *S. dysenteriae* at 500 mg/ml which is the MIC while *P. aeruginosa* was susceptible at 300 mg/ml thus having an MIC of < 300 mg/ml. The extract had activity on two of the organisms studied and could be a source of novel compound for the control of infections caused by these organisms. The active components responsible for the antimicrobial activity should be determined, separated and studied.

Keywords: Extract, Pathogenic Microorganisms, MIC, Novel Compound, Resistance.

INTRODUCTION

Herbal medicines have been shown to be effective and about 60% of rural populations depend on it for their primary health care due to affordability, accessibility, uneven distribution of health facility and personnel between rural and urban areas [6]. A variety of herbal preparations are used to treat different kinds of ailments such as typhoid and paratyphoid fevers, dysentery, malaria, diarrhoea, etc in Nigeria. [4] reported the activity of *Terminalia avicennoides* against *Vibrio cholerae* and *Salmonella typhi*. [13] reported the antimicrobial activity of extract of *Anacardium occidentale* and *Gossypium hirsutum* against *Escherichia coli, Shigella dysenteriae, Salmonella typhimurium*, *Staphylococcus aureus* and

P*seudomonas aeruginosa.* Antimicrobial activity of extracts of *Silene montbretiana* Boiss has also been reported against *E. coli, S. aureus, K. pneumoniae, P. aeruginosa, C. albicans* and five other microorganisms [14].

Ficus exasperata Vahl belongs to the family Moraceae and is native to Sub-Saharan Africa. It is about 20m in height [7, 12]. The plant is widely distributed in Nigeria and is known as Forest sandpaper fig, forest sandpaper tree, sandpaper tree, sandpaper leaf tree, white fig tree (En). Papier de verre (French), Msasa, mkuyu (Sw), Msasa, mkuyu (Sw), Ewe ipin (Yoruba), Opoto (Calabar), Anwulinwa (Igbo) [5] and *Ijikpi* in Igala, Nigeria.

Extracts of *F. exasperata* have been studied on *Staphylococcus aureus*, *Eschericha coli*, *Bacilius subtitis*, *Aspergillus niger*, *Candida albican* [9, 11]. Other pharmacological activity reported for *F. exasperata* includes antiulcer, antidiabetetic, lipids and high blood pressure lowering and the treatment of diarrhea, arthritis, cough, boils, epilepsy, haemostative ophthalmia and intestinal pain and colics [1, 2].

Resistance development by pathogenic microorganisms to existing antibiotics is increasing at an alarming rate [6, 14], and this creates problems in health care delivery in relation to microbial infections in man and animals. Adulteration and faking of drugs have also become major problems in recent times which Government agencies are contending with without success, thus obtaining original drugs for common ailments has become impossible. The high cost of drugs has also led people to opt for herbal treatment which have no scientific certification. Attention has thus shifted towards medicinal research to substantiate the claims of cure made by traditional healers to provide scientific basis for their efficacy. Several important drugs have also been discovered in plants and are now synthesized chemically for commercial purposes. Therefore, the search for new ones continues especially in the tropical plants [6, 9]. Thus, this study evaluated the antimicrobial effect of the ethanolic extract of *F. exasperata* root on some selected pathogens.

MATERIALS AND METHODS

Collection and Identification of Plant

The roots of the plant were collected from its natural habitat in the forest in Okpachala village in Igalamela/Odolu Local Government Area of Kogi State. Identification of the plant was carried out by Dr. P.C. Okeke, a plant taxonomist in the Department of Botany, Nnamdi Azikiwe University (NAU), Awka, Anambra State, Nigeria. The plant

voucher was prepared and kept in the herbarium of Federal Polytechnic Idah.

Preparation of Plant and Extraction
The plant's roots were air dried at room temperature in Biology Laboratory, Federal Polytechnic Idah (FPI) for three weeks. The dried leaves were pounded in a clean wooden mortar in the Laboratory to increase its surface area for extraction. About 30g of the root powder was wrapped in Whattman No. 24 filter paper and Sohxlet extraction was carried out using Methanol at 65°C.

Source of the Organisms
The test bacteria (*Pseudomonas aeruginosa*, *Salmonella typhi*, and *Shigella dysenteriae*) were obtained from Bacterial Research Unit of National Veterinary Research Institute (NVRI) VOM, Jos Plateau State.

Phytochemical Screening
Phytochemical screening was carried out using the methods described by Anowi *et al.* (2012) and Chandrashekar *et al*. (2013).

Test for Alkaloids
20mls of 5% sulphuric acid in 50% ethanol was added to about 2g of the methanolic extract and heated on a boiling water bath for 10minutes, cooled and filtered. 2ml of the filtrate was tested with a few drops of Mayer's, Dragendroff's, Wagner's reagent and 1% picric acid. The remaining filtrate was placed in 100ml separating funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with two 5ml portion of dilute sulphuric acid, the Mayer's, Dragendroff's, Wagner's and picric acid respectively. A milky, brick red, reddish brown and yellow precipitate with one drop each of the reagents shows the presence of alkaloid.

Test for Glycosides
Fehling's Test
About 5ml of a mixture of equal part of Fehling's solution of the extract, dissolved in water and then heated or a water bath for five minutes. A brick red precipitate shows the presence of glycosides.

Hydrolysis Test
About 5ml dilute sulphuric acid were added to about 0.1g of leaf extract in a test tube and boiled for 15 minutes in a water bath, then cooled and neutralized with 20% potassium hydroxide solution. 10ml of a mixture of equal parts of Fehling's solution 1 and 2 were added and boiled for 15minutes. A brick red

precipitate indicates the presence of glycosides.

Carbohydrates

500 mg plant material boiled in 30 ml distilled water, filtered; 1ml of Molisch's reagent was added to 1ml of the filtrate and then 1ml of concentrated H_2SO_4. A reddish ring indicates the presence of carbohydrate; 2ml of Fehling's solution was added to 1ml filtrate and then boiled for 5 minutes. A brick red precipitate indicates the presence of reducing sugars; 1ml Barfoed's reagent was added to 1ml of filtrate and heated. A red precipitate indicates the presence of monosaccharide.

Test for Saponin

About 20ml of water was added to 0.25g of the methanolic extract of the leaf in 100ml beaker and boiled gently on a water bath for two minutes. The mixture was filtered hot and allowed to cool and the filtrates used for the following tests.

Frothing Test

About 5ml of the filtrate was diluted with 20ml of water and shaken vigorously. A stable froth upon standing indicates the presence of saponins.

Emulsion Test

To the frothing solution was added two drops of olive oil and the content shaken vigorously. The formation of emulsion indicates the presence of saponins.

Fehling's Test

To 5ml of the filtrate was added 5ml of Fehling's solution (equal parts of 1 and 2) and the mixture heated. A reddish precipitate indicated the presence of saponins further heating with sulphuric acid produce a brick red precipitate.

Test for Tannins

About 0.5g of the extract was boiled with 25ml of water, filtered and used for the following test.

Ferric Chloride Test

To 3ml of the filtrate was added few drops of ferric chloride solution. A greenish black precipitate indicates the presence of tannins.

Phlobotannins Test

Aqueous extract of the test samples was boiled with 1% hydrochloric acid. Disposition of red precipitate shows the presence of phlobotannins (Akharaiyi *et al.*, 2012).

Test for Flavonoids

5ml of ethyl acetate were added to 0.1 g of the extract and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate used for the following test.

Ammonium Test

About 2ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layers were allowed to separate and the yellow colour in the ammoniacal layer indicates the presence of flavonoids.

Test for Resins

The plant extract was dissolved in 3ml acetone and 3ml concentrated hydrochloric acid was added. This mixture was heated in a water bath for 30 minutes. A pink colour which changes to red indicates the presence of resins.

Test for Steroids and Triterpenoids

About 9ml of ethanol was added to 1 g of the extract it was refluxed for a few minutes and filtered. The filtrate was concentrated on a boiling water bath. 5ml of hot distilled water was added to the concentrated solution, the mixture was allowed to stand for 1 hour and the waxy matter was filtered off. The filtrate was extracted with 2.5ml of chloroform using separating funnel. To 0.5 ml of the chloroform extract in a test tube was carefully added 1ml of concentrated H_2SO_4 to form a lower layer. A reddish brown interface shows the presence of steroids. 0.5ml of the chloroform was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey color indicates the presence of terpenoids.

Anthraquinones

Borntrager's Test

100mg of powdered plant in 5ml of chloroform, filtered. 2ml of 10% NH_4OH was added to 2ml filtrate. A bright pink colour indicates the presence of anthraquinones; Modified Borntrager's test: 200mg plant material boiled in 5ml 10% HCl, filtered. Filtrate extracted with 5ml benzene, and benzene layer shaken with 5ml 10% NH_4OH. A rose pink or cherry red colour indicates the presence of anthraquinone derivatives (Danmalam *et al.,* 2011).

Determination of Minimum Inhibitory Concentration Using the Agar Well Diffusion Method

McFarland standard concentration of each organism was inoculated by spread plating on Mueller Hinton agar and left to adsorb onto the medium on the bench for about 30 minutes. Then 6mm diameter well or holes were then bored in the agar using cork borer. The wells were then filled with appropriate concentration of the extract and left on the bench for about one hour for diffusion to take place. Drops of 20μm of Ciprofloxacin were put in the wells bored in the centre of the medium as standard control. The

plate was then incubated at 35-37°C for 24 hours. The zone of inhibition was then examined around each extract concentration, measured and compared with that of the control (Ciprofloxacin).

RESULT

Phytochemical Screening

Phytochemical analysis revealed the presence of alkaloid, steroids, tannins, flavonoids, saponins, and glycoside anthraquinones (Table 1).

Table 1: Phytochemicals Found in Ethanolic Roots' Extract of *Ficus exasperate*

Phytochemicals	Results
Alkaloid	+
Steroids	-
Tannins	-
Flavonoids	+
Saponins	+
Glycoside	+
Anthraquinones	+
Terpenes	+
Carbohydrates	+
Phlobotannins	+

Antimicrobial Effect of Ethanolic Extract of *Ficus exasperata* Root on *Salmonella typhi*, *Shigella dysenteriae* and *Pseudomonas aeruginosa*

At the concentration of 300mg/ml, the zone of inhibition produced was 6mm while at the concentration of 400mg/ml, the zone of inhibition produced was 6mm. At the concentration of 500mg/ml, the zone of inhibition produced was 6mm while the control (Ciprofloxacin); the zone of inhibition was 30mm (Table 2).

At the concentration of 300mg/ml, the zone of inhibition produced was 6mm while at the concentration 400mg/ml, the zone inhibition produced was 6mm. At the concentration of 500mg/ml, the zone of inhibition produced was 12mm while the control (Ciprofloxacin); the zone of inhibition was 300mm (Table 2).

At the concentration of 300mg/ml, the zone of inhibition produced was 12mm while at the concentration of 400mg/ml, the zone of inhibition produced was 17mm. At the concentration of 500mg/ml, the zone of inhibition produced was 20mm while the control (Ciprofloxacin); the zone of inhibition was 300mm (Table 2).

Table 2: Antimicrobial effect of Ethanolic Extract of *Ficus exasperata* Root on *Salmonella typhi*, *Shigella dysenteriae* and *Pseudomonas aeruginosa*

Microorganisms	Concentrations / Zone of Inhibitions			
	300	400	500	Ciprofloxacin
Salmonella typhi	6	6	6	30
Shigella dysenteriae	6	6	12	30
Pseudomonas aeruginosa	12	17	20	30

DISCUSSION

From table 1, the Phytochemicals identified in *F. exasperata* could confer antibacterial property on it. The observed antimicrobial properties of plants' products has been attributed to the presence of bioactive compounds present in them such as tannins, flavonoids, saponin, alkaloids and phenolic compounds [9, 12].

From Table 2, the zone diameter produced by ethanolic root extract of *F. exasperata* indicated that it had no inhibitory property on *Salmonella typhi* at the concentrations used. The zone diameter of 6mm obtained for the concentration 500 mg/ml is not comparable to a zone diameter of 9mm observed with MIC of 1.0mg/ml of methanolic extract of *F exasperata* reported in a previous study carried out by [2] on *Salmonella typhi*. Methanolic extracts of plants give rise to more phytocomponents than ethanolic extracts. The Minimum Inhibitory Concentration (MIC) for the organisms used in this study was 300mg/ml. The reason for the differences in the observation may be due to the age of the plants, the level of the active ingredient in the concentration of the extract, and the preparation of the extracts.

From Table 2, the ethanolic root extract of *F. exasperata* had no activity against *Shigella dysenteriae* at 300mg/ml. This result is in variance with the study carried out by [2] on *Shigella dysenteriae* in which an MIC of 128mg/ml was observed with methanolic extract of *F. exasperata*. Also, this result is not in agreement with the report of [10] in which *Ficus capensis* was used on *Shigella dysenteriae*, giving an MIC of 200mg/ml with a zone diameter of 7mm.

From Fig. 2, the results of the antimicrobial studies showed that the ethanolic root extract of *F. exasperata* had activity against *Pseudomonas aeruginosa* at the concentrations used. The Minimum Inhibitory Concentration (MIC) in this study was 300mg/ml with the

zone diameter of 12mm. This work does not agree with the study carried out by [9] in which the methanolic extract of *F. exasperata* root was tested on *Pseudomonas aeruginosa* and an MIC of 75mg/ml was obtained, with a zone diameter of 8.6mm. The large zone of inhibition exhibited by the extract on *P. aeruginosa* suggests that it contains substances which when extracted and purified could serve as a source of treatment of infections commonly associated with the microorganism. The study confirmed the observation by some authors of the antimicrobial action of the extract of the plant on some pathogens.

Further researches are needed on higher concentration of the extract on *Salmonella typhi*. Other extraction solvent should be used to extract *F. exasperata* and screened for antimicrobial activities on other pathogenic bacteria. There is need for drugs research in Nigeria and other parts of the world to look inwards into finding a remedy that will be cheap and accessible to the users. Government should give incentive to researchers considering the high cost of research. Studies should be carryout on other organisms.

CONCLUSION

The ethanolic extract of *F. exasperata* root contains phytochemicals known to have antimicrobial property. The extract showed activity against *Shigella dysenteriae*, *Pseudomonas aeruginosa* but no activity against *Salmonella typhi*. The plant may be effective in combating or treating infections associated with the tested organisms as claimed by the traditional healers

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