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Investigation of Phytoconstituents, Antibacterial Activity and Cytotoxic Effect of *Ficus exasperata* Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMO designed the study and wrote the protocol. Author ARJ wrote all the drafts of the manuscript. Authors SOB and TOK managed the laboratory work and statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to subject *Ficus exasperata* to standard scientific scrutiny by investigating the phytochemical composition as well as the antibacterial activity and cytotoxic effect of its leaf extract.
Methodology: The plant leaf was air-dried under shade and thereafter ground mechanically, macerated in a solvent (ethanol) for 48 hr and dried using rotary evaporator. The presence of phytochemicals was assayed qualitatively; agar well diffusion method was used to assess the antibacterial activity while brine shrimp assay was used to determine the cytotoxic potential of the plant extract.
Results: The qualitative phytochemical screening of *Ficus exasperata* leaf extract revealed the presence of alkaloid, tannin, flavonoids and cardiac glycoside whereas saponin and steroids were absent. In the antibacterial activity assay, *Klebsiella pneumoniae* showed the highest susceptibility against the leaf extracts at all the test concentrations recording inhibition zone range of 7.33±0.58 mm to 18.33±0.58 mm. The least zone of inhibition (11.67±0.58 mm) was recorded against

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Streptococcus pneumonia at the highest concentration used (50 mg/ml). The lowest minimum inhibitory concentration (2.5 mg/ml) of the extract was found against *S. aureus, S. pneumoniae, E. faecalis* and *K. pneumonia* while the highest MIC value (25 mg/ml) was recorded against *S. typhi* and *Ps. aeruginosa*. The plant's leaf extract completely killed three of the pathogens within 10 minutes of exposure these were *S. aureus, E. faecalis* and *K. pneumoniae.* Higher mortality percentage of brine shrimp was observed with the increase in concentrations of the extracts with an LC_{50} value of 39.76 ppm was obtained for the extract.

Conclusion: From the foregoing, there is a great amount of evidence suggesting that the ethanol extract of *Ficus exasperata* leaf contained bio-active substances which confer on it antibacterial activity and cytotoxic effect. Therefore, it may be expediently exploited in the development of new antibiotics especially against susceptible bacteria.

Keywords: Ficus exasperata; phytochemical; antibacterial; cytotoxic; leaf extract.

1. INTRODUCTION

Medicinal plants have enormous therapeutic potentials to heal many infectious diseases and at the same time avoiding many side effects. The plant kingdom has been the major source of remedies for curing a variety of diseases in Africa and medicinal plants have played a key role in Nigeria for the maintenance of health [1]. Natural products of higher plants are an important source of therapeutic agents; therefore many research groups are currently screening the different activities of plants. The use of plant-based products for the management of health has been on the increase since the turn of the millennium. It is now popular among different groups of people all over the world [2].

Medicinal plants contain many bioactive secondary metabolites like saponins, tannins, flavonoids, alkaloids, steroids, phenolics and a host of others with the ability to ameliorate disease conditions; some have been reported to possess curative potentials [3]. These phytochemicals are produced by plants to improve their chances of survival especially in their defence mechanism against infectious agents. Nevertheless, they have been found useful for the treatment of animal diseases including that of humans [4]. The increase in the multiple drug resistance among various pathogenic microorganisms especially bacteria in this millennium has led to a search for potent alternatives from natural sources [5].

Ficus exasperata commonly called sand paper plant belongs to the family Moraceae, and is found in all parts of Nigeria. In folklore medicinal practices, the plant is employed in the management of various types of diseases ranging from hypertension, ringworms and boils. Also, the young leaves are used as ulcer remedy and as anti-fungal agent [6]. This study was therefore designed to investigate the claims of folklore medicinal efficacy of *F. exasperata* by subjecting it to scientific scrutiny based on its phytochemical composition, antibacterial activity and cytotoxic effect.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Leaves

Fresh leaves of *Ficus exasperata* were harvested from parent plants within the Rufus Giwa Polytechnic (7.2289º N, 5.5539º E), Owo, Ondo State, Nigeria in September, 2017. The plant was then authenticated at the Herbarium section of the Department of Forest Resources Technology, Rufus Giwa Polytechnic, Owo and voucher specimen (X-FE8617L) was deposited in the same Herbarium. The authenticated leaves were washed under tap and then air-dried under shade for 4 weeks. Afterwards, the dried leaves were milled into powder with the use of a mechanical grinder and were stored in clean air-tight containers, and kept in a cool, dry place until required for use.

2.2 Extraction of the Samples

Two hundred gram (200 g) of the powdered sample was soaked in 500 ml of ethanol for 48 hr with intermittent stirring using sterile spatula. The plant extracts were then filtered through muslin cloth into sterile McCartney bottles and then dried invacuo using rotary evaporator at a temperature of 50ºC to yield crude extracts [7]. From the crude extract five concentrations were prepared by diluting 0.10 g, 0.20 g. 0.3 g, 0.40 g and 50 g of the extracts in 10 ml of 0.01% DMSO

to obtain concentrations of 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml and 50 mg/ml respectively [8].

2.3 Test Microorganisms

The bacteria used in this study include *Staphylococcus aureus, Bacillus subtilis, Streptococcus pneumoniae, Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhi.* They were obtained from the Microbiology and Pathology Laboratory of Federal Medical Center, Owo, Nigeria.

2.4 Phytochemical Screening of Plant Material

The leaf extracts of the plant were subjected to qualitative phytochemical screening for the presence of tannins, saponin, flavonoids, alkaloids, steroids and cardiac glycosides using standard procedures as described by Odebiyi and Sofowora [9].

2.5 Antibacterial Activity

The extracts obtained from the plants leaf were screened against the bacteria by agar well diffusion method [10]. A portion of 0.1 ml of bacterial inoculum was inoculated in 5 ml of nutrient broth and then incubated at 37ºC for a few hours for it to reach a sufficient turbidity equal to 0.5 McFarland solution measured at 600 nm (1×10^6 CFU/ml) to prepare the inoculum. A 25 ml of Nutrient agar was poured into each Petri dish and after the agar solidified, the pathogenic test organisms were inoculated on the surface the plates using a sterile glass spreader and allowed to sink properly. Subsequently, the surface of the agar was punched with 6 mm diameter cork borer into wells and a portion of 50 µl of each of the extract concentrations was filled into the wells. Control wells containing the same volume of Dimethyl sulphoxide (DMSO) served as negative control, while Chloramphenicol (50 µg) was used as positive control for the plates respectively and the plates were incubated at 37ºC for 24 hr. The diameter of the zones of inhibition was then measured in millimeters using a meter rule.

2.5.1 Determination of minimum inhibitory concentration (MIC)

The MIC of the plants extracts were determined by double dilution broth methods of Ghosh et al. [11]. Twofold serial dilutions of the extracts were prepared in Nutrient broth to achieve a decreasing concentrations ranging from the least concentration that produced clear zone of inhibition (10 mg/ml to 0.156 mg/ml). All tubes with the controls were labeled accordingly. Each dilution was seeded with 1 ml of standardized inoculums (1.0×10^6 CFU/ml) and incubated at 37ºC for 24 hr. A tube containing only seeded broth (i.e. without plant extract) was used as the positive control while the un-inoculated tube was used as negative control. The lowest concentration of each extract that showed a clear of inhibition when compared with the controls was considered as the MIC.

2.5.2 Determination of the killing time of plant extracts

The MIC of each test organism was used for this assay. Each organism was exposed to the respective concentration for different time. A 0.1 ml of each concentration was added to test tube containing 10 ml of standardized inoculum, then it was centrifuged at 1000 rpm for 2 hr. At 5 min interval, an aliquot of 1ml from the test tube is cultured on fresh Nutrient agar and incubated for 24 hr at 37ºC, the time at which there was no visible colony formation on agar plate was taken as the killing time of the extract against the organisms [12].

2.6 Determination of Cytotoxic Effect of Plant Extracts

The brine shrimp (*Artemia salina*) lethality bioassay was carried out according to the method described by Haq et al. [13]. Brine shrimp eggs were hatched in artificial sea water prepared by dissolving 38 g of salt in 1 liter of distilled water, filtered and put in shallow rectangular dish. A plastic divider with several holes of 2 mm size was clamped in the dish to make two equal compartments. Brine shrimp eggs were placed in one side of the compartment while the other compartment was illuminated. After 48 hr of illumination, phototrophic nauplii (Brine shrimp larvae) were collected by using pipette from the lightened side. Samples were then prepared by dissolving 20 mg each of the extracts in 2 ml of DMSO from where further diluted concentrations of 1000, 100, 10 and 1 ppm were prepared. A 5 ml portion of the artificial sea water was added into each test tube and 20 shrimps were transferred into it. This was followed by the addition of 1 ml of each of the test extracts and of previously prepared concentrations and maintained under illumination

at room temperature. Survivors were counted with the aid of magnifying glass after 24 hr. The percentage mortality was calculated using Abbot's formula and the LC_{50} was also determined by graphical method [14].

2.7 Data Analysis

Data were presented as mean±standard error (SE). Significance difference between different groups was tested using one-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version17.0 software. The significance was determined at the level of $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Composition of *F. exasperata* Leaf Extract

The results of the qualitative phytochemical screening of *F. exasperata* leaf extract revealed the presence of alkaloid, tannin, flavonoids and cardiac glycoside whereas saponin and steroids were absent (Table 1). The presence of these secondary metabolites in *F. exasperata* was reported by Awala et al. [15] who worked on the leaf extracts while Lawal et al. [16] reported the presence of cardiac glycosides, anthraquinones and saponins in the root bark of the plant. These phytochemicals are reported to possess an array of medicinal properties which include antioxidant and antimicrobial activities [17] and this indicates that *F. exasperata* leaf may be explored for the development of possible pharmaceutical products.

Table 1. Phytochemical constituent of *F. exasperata* leaf

Phytochemical	Leaf
Alkaloid	+
Tannin	+
Saponin	-
Flavonoid	+
Steroids	-
Cardiac glycosides	+

Key: - = not detected, + = present

3.2 Antibacterial Activity of *F. exasperata* Leaf Extract

The results of the antibacterial activity screening of the plant are presented in Table 2. The table revealed a wide spectrum activity against both the test Gram positive and Gram-negative bacteria used in this study. *Klebsiella pneumoniae* showed the highest susceptibility against the leaf extracts at all the concentrations used recording a zone of inhibition range of 7.33±0.58 mm to 18.33±0.58 mm. The least zone of inhibition (11.67±0.58 mm) was recorded against *S. pneumonia* at the highest concentration used (50 mg/ml). The wide zones of inhibition recorded against the test bacteria were comparable to that of chloramphenicol used as positive control. The antibacterial activities of *Ficus exasperata* leaf observed in this study may be due to the presence of various secondary metabolites in the leaf [18]. This observation correlates with earlier reports of Akinpelu et al. [19] who reported high antibacterial activity of various parts of the plant. The observed susceptibility of these versatile bacterial pathogens to the plant extract indicates that the plant has some bioactive chemicals which may be used to develop new antibiotics against these organisms some of which are known to be prone to antibiotic resistance.

3.3 Minimum Inhibitory Concentration of *Ficus exasperata* Leaf Extract against Selected Pathogens

The minimum inhibitory concentration (MIC) is a useful tool in the determination of the best first-line drug for immediate treatment in cases of emergency before appropriate diagnoses could be made [20]. The lowest MIC (2.5 mg/ml) of the extract was found against *S. aureus, S. pneumoniae, E. faecalis* and *K. pneumonia* while the highest MIC value (25 mg/ml) was recorded against *S. typhi* and *Ps. aeruginosa*. These MIC values are higher than what is reported by Abaoaba and Efuwape [21] who reported 1.562 mg/ml as the highest MIC of *F. exasperata* leaf against similar organisms whereas the MIC obtained in this study is similar to that obtained by Lawal et al. [22] who reported a range of 1.56 mg/ml to 50 mg/ml for the plant extracts against test microorganisms. These results suggest that this plant may be handy in the treatment of infections that may be caused by these organisms.

3.4 Killing Time of *F. exasperata* Leaf Extract against Selected Pathogens

The minimum time of exposure needed for the test organisms against the extracts to completely neutralize them is presented in Fig. 1. The plant's

Table 2. Antimicrobial activity of *Ficus exasperata* ethanol leaf extract on selected human pathogens

Conc. (mg/ml) Org	10	20	30	40	50	Antibiotics
	Zones of inhibition (mm)					
Bacillus subtilis	3.67±0.58^{a}	7.33±0.58^{b}	9.67±0.58^{c}	11.00±0.00^{d}	13.00±0.00^{e}	27.33±0.58^{d}
Staphylococcus aureus	6.00±0.00^{a}	11.67±0.58^{b}	14.33±0.58^{c}	17.33±0.58^{d}	18.00±0.00^{d}	30.67±1.00^{c}
Staphylococcus epidermidis	5.67±0.58^{a}	9.00±0.00^{b}	11.67±0.58^{c}	14.33±0.58^{d}	15.67±0.58^{d}	28.67±0.33^{c}
Streptococcus pneumoniae	NI	NI	6.67±0.58^{a}	9.67±0.58^{b}	11.67±0.58^{c}	30.33±1.15^{b}
Escherichia coli	6.67±0.58^{a}	10.33±0.58^{b}	13.67±0.58^{d}	15.33±0.58^{e}	17.67±0.58^{f}	29.33±0.58^{c}
Enterococcus faecalis	6.33±0.58^{a}	7.00±0.00^{a}	9.33±0.58^{b}	11.67±0.58^{c}	12.00±0.00^{c}	27.00±0.00^{d}
Klebsiella pneumoniae	7.33±0.58^{a}	12.67±0.58^{b}	15.00±0.00^{c}	16.00±0.00^{c}	18.33±0.58^{d}	28.33±0.58^{b}
Salmonella typhi	NI	NI	5.67±0.58^{a}	8.33±0.58^{c}	15.67±0.58^{d}	27.67±0.33^{b}
Pseudomonas aeruginosa	3.67±0.58^{a}	6.67±0.58^{b}	10.33±0.58^{d}	13.67±0.58^{e}	15.33±0.58^{f}	29.67±1.00^{c}

Key: Values are Mean±S.E.M (mm), Values followed by different superscripts along the rows are significantly different at p≤0.05, NI= no inhibition

leaf extract completely killed three of the pathogens within 10 minutes of exposure these were *S. aureus, E. faecalis* and *K. pneumoniae* while it took 15 minutes for *E. coli* to be killed whereas *S. typhi* took 50 minutes before it was completely killed. All the test bacteria were completely neutralized within an hour of exposure to the plant extract suggesting that this plant may be expeditiously used to make first-line drugs in the treatment of infections caused by these pathogens [23].

3.5 Cytotoxic Effect of *F. exasperata* Leaf Extract against Brine Shrimps

The results of the cytotoxic activity the plant's leaf extracts against brine shrimp are presented in Table 3. The popular brine shrimp lethality test was used, this test is based on the ability of the extract to kill laboratory grown larva of brine shrimp (*Artemia salina*). This assay is a very useful step for assaying for toxicity level of substances since the brine shrimp is highly sensitive to a variety of chemical substances and it is a rapid and simple bioassay for testing plant extracts bioactivity and according to Swati and Diboyajyoc [24] which in most cases correlates reasonably well with cytotoxic and anti-cancer properties.

The percentage mortality of brine shrimp larvae exposed to the plant leaf extract was concentration dependent, higher mortality percentage was observed with the increase in concentrations of the extracts. The LC_{50} value obtained was 39.76 ppm which was less than 100 ppm/ml which is considered as the threshold concentration for determination of cytotoxicity. LC_{50} values higher than 1000 ppm are not significant while those within the range of 0-100 ppm/ml are considered to be very toxic [25]. The available reports of brine shrimps assay indicate that plant extracts have a likelihood of yielding anticancer compounds. This suggests that *F. exasperata* leaf extracts may contain bioactive substances that may have potential anticancer activity.

Table 3. The MIC of *F. exasperata* leaf extract against selected pathogenic bacteria

Organism	MIC (mg/ml)
Bacillus subtilis	10.0
Staphylococcus aureus	2.5
Staphylococcus epidermidis	5.0
Streptococcus pneumoniae	2.5
Escherichia coli	5.0
Enterococcus faecalis	2.5
Klebsiella pneumoniae	2.5
Salmonella typhi	25.0
Pseudomonas aeruginosa	25.0

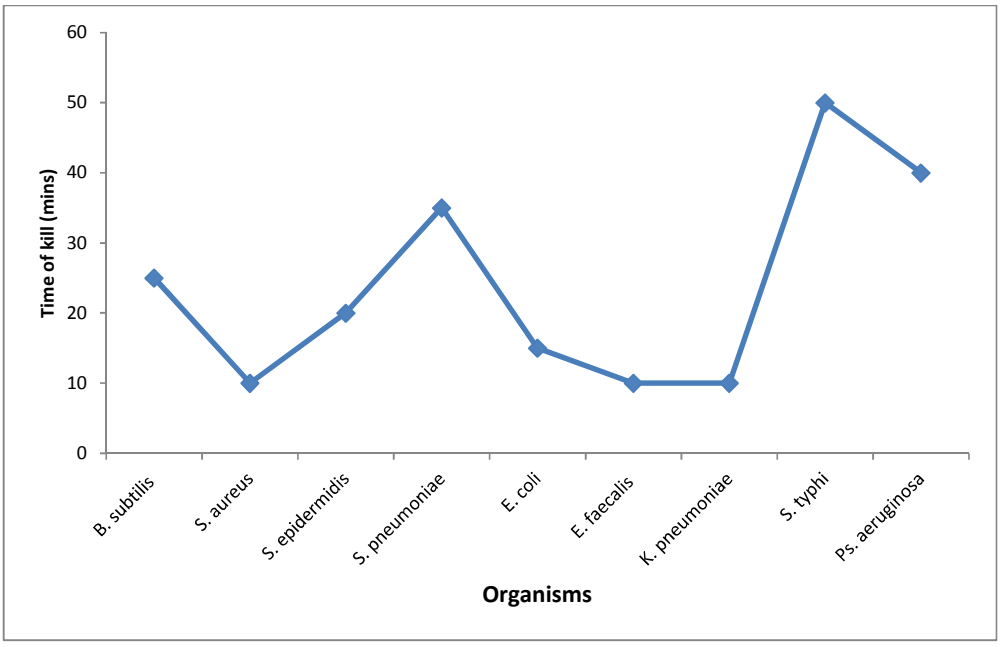

Fig. 1. The time of kill of *F. exasperata* leaf extract against selected pathogenic bacteria

Table 4. Percentage mortality of brine shrimps at different concentrations of *F. exasperata* leaf extract

Dosage (ppm)	Initial larvae	No. of survivors	No. of death	% mortality	Control (no of survivor)
1000	20	0	20	100	20
100	20	6	14	70	20
10	20	11	9	45	20
1	20	15	5	25	19
LC_{50}				**39.76 ppm**	

4. CONCLUSION

The leaf extracts of *F. exasperata* contain an alkaloid, tannin, flavonoids and cardiac glycosides. Further, the extracts possess wide spectrum antibacterial activity against the test bacteria with a low minimum inhibitory concentration and short killing time. Moreover, the extracts possess cytotoxic activity against brine shrimps. These support the folkloric claim of the plant's efficacy in the treatment of infectious diseases and may be explored for the manufacturing of useful drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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