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Exploration of the Antimicrobial Properties of *Ficus exasperata* Leaves from Akure Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SIA and OEA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors OAA, OA and OTO managed the literature searches, analyses of the study and performed the spectroscopy analysis. All authors managed the experimental process and author SIA identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study was conducted to explore the antibacterial and antifungal properties of the leaf extracts of *Ficus exasperata* (Sand paper tree) *in vitro*.
Study Design: Extracts from *Ficus exasperata* leaves were qualitatively screened for the phytochemical constituents, and their *in vitro* antimicrobial potency was evaluated against fourteen (14) fungal and bacterial isolates.
Results: The tested extracts contained tannins, flavonoids, terpenoids, alkaloids and cardiac glycosides whereas, saponin, steroids, phlobatannin and anthraquinone were absent. The acetone extract of the leaf demonstrated better antimicrobial activity against 10 of the test organisms. However, the highest antimicrobial activity (31.27 mm) was exhibited by the methanol extract against referenced culture of *Staphylococcus aureus*. In addition, the extracts also displayed better antibacterial than antifungal activity. The minimum inhibitory concentration (MIC) of the extracts ranged between 0.391-1.563 mg/mL, with the acetone extract displaying lower MIC values.
Conclusion: The occurrence of the observed phytochemicals in the extracts of *Ficus exasperata*

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(Sand paper tree) could be involved in the antimicrobial efficacy of the plant. The results from this study thus supports the folkloric use of the plants. Additionally, the plant could also be exploited for the production of drugs especially for staphylococci infections.

Keywords: Medicinal plants; antimicrobial; phytochemicals; extracts; Ficus exasperata.

1. INTRODUCTION

For ages, mankind has faced a constant battle with infectious diseases. This has led to increased morbidity and mortality especially among population from developing countries. Many populations have adopted the traditional healthcare system as a way of preventing and treating diseases of microbial origin [1]. Traditional medicine remains the most sort after, as it is considered safer, affordable, and readily available [1].

Due to the upsurge in resistance to conventional drugs by microbial agents, novel antimicrobial agents from different biological sources have been sort after and reported to be effective in combating pathogenic organisms. The use of herbal remedies containing plants or part of plants has in recent years gained ground in developed countries [2]. Pharmaceutical companies have thus developed new antimicrobial drugs and also improved on the existing ones through the modification of their structures with a view to increasing their efficacy [3].

Ficus exasperata otherwise known as the sandpaper tree is native to tropical Africa [4]. The leaves of *F. exasperata* have been employed in folkloric medicine for the treatment of various diseases such as ophthalmic and oral infections, venereal diseases, parasitic infection (cutaneous, subcutaneous), leprosy, and malaria [5,6]. The study therefore investigates the claim of the antimicrobial potential of *F. exasperata*, in a bid to develop novel antimicrobials.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Extracts from Leaves Samples

The leaves of *F. exasperata* were collected from its tree at a building near Life Spring College, Apatapiti layout, Federal University of Technology, Akure, Ondo State (Latitude: 7.289N, Latitude: 5.150E) Nigeria in the month of April, 2015. Samples of the leaves were taken to the Department of Crop, Soil and Pest, FUTA for authentication. Afterwards the leaves were cleansed with water, shade dried, grinded and stored in airtight container. Thereafter, the powdered leaves (100 g) of *F. exasperata* was weighed separately into different plastic containers and 1000 mL of 100% acetone and methanol added to the containers for extraction. Aluminium foil was placed on each container before covering. Each solution was allowed to stand for 3 days with continuous stirring. The extracts were thereafter obtained by filtering the solutions through a funnel fitted with a filter paper. The filtrates were thereafter evaporated to dryness at 50℃ in a rotary evaporator (RE-52A; Union Laboratory, England) with 90 rpm under reduced pressure. The obtained concentrated extracts were stored in dark at 4℃ until further analysis.

2.2 Phytochemical Screening of Leaf Extract of *Ficus exasperata*

The plant extracts were subjected to qualitative phytochemical screening using standard protocols described by Odebiyi and Sofowora [7], Trease and Evans [8], and Harborne [9].

2.3 Measurement of Antibacterial and Antifungal Activities of Leaf of *Ficus exasperata*

Varying concentrations of the leaf extracts of *F. exasperata* (3.125-100 mg/mL) were prepared by dissolving different amount of the extracts in 5 mL of 30% tween 20. For example, concentrations of 100, 50, and 25 mg/mL were prepared by dissolving 500, 250 and 125 mg of the extracts into 5 ml of 30% tween 20 respectively. Afterwards the prepared extracts were sterilized by passing them through a 0.22 µm millipore membrane filter. The agar well diffusion method as described by Schinor et al. [10] was employed in assessing the antimicrobial activity of *F. exasperata* leaf extracts. A total of 14 clinical and referenced microbial strains were used for the experiment. The test organisms were obtained from the Pathology and Clinical Laboratory (PATHCARE), Lagos State University

Teaching Hospital, Lagos State, Nigeria and the Department of Microbiology, FUTA. Active broth cultures of the test organisms were prepared from stock cultures. To 5 ml of nutrient broth 0.2 ml of bacterial culture was inoculated and incubated till it reached the turbidity equal to that of the standard 0.5 McFarland solution measured at 600 nm which is equivalent to 10^6– 10^8 CFU/ml. Suspensions of fungal spores were prepared from fresh mature (5 days) cultures that grew at 26 ± 1ºC on a Sabouraud dextrose agar. Spores were rinsed with sterile distilled water. The suspensions were then adjusted to 10^6 spores per/ml by microscopic enumeration with a cell counting hematocytometer. An aliquot of 100 µL of bacterial and fungal solution was evenly spread on already solidified Mueller Hinton agar plates. Afterwards, wells of 7mm diameter were bored in the solidified Mueller Hinton agar plates using a sterile cork-borer. Thereafter, an aliquot of 100 uL of the sterilized extract was added into the bored agar wells. The plates were thereafter incubated at 37ºC for 24 hour for bacteria and at 26 ± 1ºC for 48 to 72 hours for fungi. The plates were observed for clear zones of inhibition and the measurements were taken using a ruler calibrated in millimetres. Commercial antifungal drugs (clotrimazole, nystatin and gluseofluvin) and commercial antibacterial drug (ciprofloxacin (10 µg), rocephin (25 µg)) were used as the positive control, while 30% tween 20 was used as the negative control. To determine the minimum inhibitory concentrations (MIC), the agar diffusion method described above was used to screen the antimicrobial effect of the different concentrations of extracts (0.391-100 mg/mL). The MIC value was determined by establishing visible growth of microorganisms. The boundary dilution without any visible growth was defined as the MIC for the tested microorganism at the given concentration.

2.4 Statistical Analysis

Experiments were carried out in triplicates were applicable. The results were expressed as mean ± standard error of three values. Data analysis was carried out using the One Way Analysis of Variance (ANOVA) and treatment means were compared using New Duncan's Multiple Range Test (SPSS version 16). Differences were considered significant at $P<0.05$.

3. RESULTS

Table 1 shows the presence of tannin, flavonoid, terpenoids, alkaloids and cardiac glycosides in *F. exasperata* leaf extracts, and the absence of saponin, steroids, phlobatannin and anthraquinone.

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

Phytochemical	Extracts	
	FEM	FEA
Saponin	-	-
Tannin	+	+
Flavonoid	+	+
Steroids	-	-
Terpenoids	+	+
Alkaloids	+	+
Phlobatannin	-	-
Anthraquinone	-	-
Cardiac glycosides		
Legal test	+	+
Keller kiliani	+	+
Salkowski	+	+
Liberman test	+	+

Keys: FEA: Acetone leaf extract of F. exasperata; FEM: Methanol leaf extract of F. exasperata

The antimicrobial activity of the leaf extracts of *F. exasperata* showed that the acetone leaf extract exhibited better activity against most of the test organisms used for the study (Table 2). However, the highest antimicrobial activity (31.27 mm) was exhibited by the methanol extract of *F. exasperata* against referenced *Staphylococcus aureus* and this was found to be slightly higher that observed in the acetone extract (29.40 mm) against the same organism. In like manner, the leaves extracts displayed better antibacterial than antifungal activity. The antifungal activity of the acetone extract of the plant was however a better than that of the methanol extract.

Upon comparison of the activities of the leaf extracts against organism with Gram reaction positive and Gram negative bacterial isolates, the Gram positive organism were more susceptible than the Gram negative organism in most cases. The extracts antibacterial activity was comparatively better than that of the commercial antibacterial drugs in most of the tested organisms. Reverse was the case for the commercial antifungal drugs as they exhibited better activity than the extracts. The acetone extract of *F. exasperata* was found to exhibit lower minimum inhibitory concentration values than the methanol extracts. The results are displayed in Table 3.

Table 2. Antimicrobial activity of leaves extracts of *F. exasperata* and commercial drugs

Test organism	Zone of inhibition (mm)						
	FEA	**FEM**	**CPX**	**R**	**CLOT**	**GRIS**	**NYST**
Salmonella typhi (ATCC 33489)	15.20 ± 0.12^{c}	12.43 ± 0.18^{a}	12.27± 0.15^{a}	14.30±0.12^{b}	NT	NT	NT
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 (ATCC 43300)	29.40 ± 0.17^{c}	31.27 ± 0.15^{d}	14.40±0.12^{a}	15.50±0.17^{b}	NT	NT	NT
Staphylococcus aureus	27.33 ± 0.24^{b}	28.40 ± 0.12^{c}	15.43±0.20^{d}	12.50±0.12^{a}	NT	NT	NT
Escherichia coli (ATCC 35218)	22.20 ± 0.12^{b}	15.53 ± 0.15^{a}	11.20±0.12^{b}	14.27±0.15^{c}	NT	NT	NT
Escherichia coli	17.50 ± 0.26^{ab}	15.60 ± 0.17^{ab}	12.50±0.23^{ab}	49.97±36.67^{a}	NT	NT	NT
Pseudomonas aeruginosa (ATCC 27853)	12.27 ± 0.15^{c}	15.40 ± 0.12^{b}	15.43±0.15^{a}	16.33±0.18^{b}	NT	NT	NT
Shigella dysenteriae	17.40 ± 0.21^{a}	16.30 ± 0.12^{b}	14.40±0.12^{b}	14.27±0.15^{c}	NT	NT	NT
Bacillus cereus	15.60 ± 0.17^{c}	20.40 ± 0.12^{d}	12.33±0.18^{a}	14.53±0.20^{b}	NT	NT	NT
Bacillus subtilis	21.30 ± 0.12^{d}	12.43 ± 0.15^{a}	14.33±0.15^{b}	15.50±0.12^{c}	NT	NT	NT
Candida albicans	18.37 ± 0.23^{d}	10.27 ± 0.15^{b}	NT	NT	16.65 ± 0.68^{c}	20.50 ± 0.29^{e}	6.40 ± 0.21^{a}
Aspergillus niger	15.60 ± 0.17^{b}	3.47 ± 0.20^{a}	NT	NT	22.33 ± 0.33^{d}	21.67 ± 0.33^{d}	17.47 ± 0.32^{c}
Aspergillus flavus	12.40 ± 0.17^{c}	3.30 ± 0.12^{a}	NT	NT	25.00 ± 0.15^{e}	9.77 ± 0.15^{b}	18.73 ± 0.22^{d}
Aspergillus fumigatus	13.30 ± 0.17^{c}	5.30 ± 0.15^{a}	NT	NT	35.67 ± 0.44^{e}	9.33 ± 0.44^{b}	20.57 ± 0.30^{d}

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: FEA: Acetone leave extract of Ficus exasperata; FEM: Methanol leave extract of Ficus exasperata; CPX: Ciprofloxacin (10 µg);
R: Rocephin (25 µg); CLOT: Clotrimazole (1 mg/mL); GRIS: Griseofluvin (1 mg/mL); NYST: Nystatin (1 mg/mL); NT: Not tested

Table 3. Minimum inhibitory concentration (mg/ml) of leaf extracts of *Ficus exasperata*

Test organisms	MIC (mg/mL)	
	FEA	FEM
Salmonella typhi (ATC 33489)	0.781	0.781
Salmonella typhi	0.391	1.563
Staphylococcus aureus (ATC 43300)	0.391	0.781
Staphylococcus aureus	0.391	1.563
Escherichia coli (ATC 35218)	0.391	0.781
Escherichia coli	0.391	0.391
Pseudomonas aeruginosa (ATC 27853)	0.781	0.781
Shigella dysenteriae	0.781	1.562
Bacillus cereus	0.391	0.391
Bacillus subtilis	0.391	1.562
Candida albicans	0.391	0.391
Aspergillus niger	0.391	0.391
Aspergillus flavus	0.391	1.563
Aspergillus fumigatus	0.391	1.563

Keys: FEA: Acetone leave extract of F. exasperata; FEM: Methanol leave extract of Ficus exasperata

4. DISCUSSION

Plants remain an inexhaustible source of novel antimicrobials. Africa with its tropical and subtropical climate is richly blessed with an array of plants that have naturally acquired secondary metabolites in order to survive the harsh environment [1,11]. Compounds with antimicrobial properties that also offer protection against drug resistant microorganisms have been isolated from medicinal plants [12,13]. The present study investigated the secondary metabolite profile and antimicrobial efficacy of leaves of *F. exasperata*.

The presence of the observed secondary metabolites in the leaf extracts validates the medicinal potentials of this plants as these compounds have been reported to play a protective role against pathogenic organisms [13]. The absence of saponin, steroids, phlobatannin and anthraquinone in the extracts might be attributed to solubility of the compounds in the extraction solvent used.

The antimicrobial activity of the extracts could be attributed to the observed phytochemicals in the extracts. In addition, the variation observed in the antimicrobial activity of the extracts might be linked to differences in the type and amount of phytochemicals present in the extracts. The structural differences in the cell wall of Gram positive and Gram negative bacteria may account for the higher susceptibility of Gram positive bacteria to the plant extracts. The complexity in the cell wall Gram negative bacteria gives them better buffering capacity thus making their cell wall less impermeable, whereas Gram positive bacteria have only an outer peptidoglycan cell wall which makes them more susceptible [14].

The higher antibacterial activity demonstrated by the extracts than antifungal activity is in consonance with findings of several authors [15,16] that have reported higher sensitivity of bacteria to antimicrobials. The chitinous cell wall of fungi promotes lesser susceptibility to antimicrobials than bacteria [17]. Antibiotics have been mostly reported to produce better performance against microorganisms than plants as a result their higher purity and smaller molecular sizes which aid their penetration into the cell wall of the organisms [18]. The better activity produced by the extract suggests that they can be explored for potent antimicrobial compounds.

5. CONCLUSION

The plant extracts produced an effective performance against the growth of the tested organisms especially *Staphylococcus aureus*. The plant extracts could therefore be exploited for the production of antimicrobial drugs especially for staphylococci infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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