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In-vitro Studies of Antimicrobial Activity of Ethanolic Extract of *Ficus exasperata* on Selected Well Water Samples in Iworoko-Ekiti, Nigeria

F. O. Omoya[1] and A. G. Ogunleye[1*]

[1]Department of Microbiology, Federal University of Technology Akure, P.M.B. 704, Akure, Nigeria.

Authors' contributions

This work was carried out in collaboration among both authors. Authors FOO and AGO designed the study and wrote the protocol. Author AGO carried out the laboratory work, collected all data, performed the statistical analysis, did the literature search and wrote the first draft of the manuscript. Author FOO handled the critical revision of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: The incidence of waterborne diseases is still on the increase among rural dwellers in the developing countries. The States owned centralized water systems are usually limited mostly to some major parts of the urban areas and other larger communities.
Aim: The present study investigated the antibacterial property of the crude ethanolic extract of *Ficus exasperata* on bacterial isolates from well water samples in Iworoko-Ekiti, Nigeria.
Place of Study: Four selected wells used as drinking water sources in Iworoko-Ekiti, Ekiti State, Nigeria were used for the study which was conducted between June and September, 2015.
Methodology: Bacteriological analysis of the water well water samples was determined using plate count method. Ethanolic extraction (98% ethanol) of the plant bioactive components was carried out. The crude extract was tested against the bacterial pathogens isolated from the well water samples. Phytochemical analysis of the extracts was determined using standard methods.

**Corresponding author: E-mail: ogunleyeadefemi@gmail.com;*

Results: The *in vitro* antibacterial activity of the ethanolic extract of *Ficus exasperata* recorded highest zone of inhibition in *Pseudomonas aeruginosa* (18.68±0.58 mm) at 125 mg/ml concentration while lowest value was noted in *Klebsiella pneumoniae* and *Staphylococcus aureus* with inhibition of 1.00±0.00 mm each. Moreover, *Pseudomonas aeruginosa, Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae* required minimum concentration of 50 mg/ml to be inhibited whereas *Proteus mirabilis* was inhibited at 125 mg/ml. The phytochemical components of the extract include; alkaloid (49.9.00±0.10 mg/100), tannins (0.13±0.00 mg/100), glycosides (30.93±1.41 mg/100ml), flavonoids (1.36±0.00 mg/100 ml) and phenols (0.14±0.00 mg/100 ml).
Conclusion: The results obtained in this study revealed that ethanolic extract of *Ficus exasperata* could serve as potential source of antimicrobial agent and this justified its uses in the treatment of various infections in folk medicine.

Keywords: Ficus exasperata; well water; phytochemical; pathogens; antibacterial properties.

1. INTRODUCTION

The practice of using medicinal plants to treat diseases of various kinds, especially those of waterborne sources is more popular in the developing countries. African people have used different medicinal herbs to alleviate pains and for total cure of many diseases [1]. *Ficus exasperata* is one of such hopeful medicinal plants that have served humankind in the continent of Africa. The authors of this research paper have themselves benefitted immensely from this nature endowed blessing (*Ficus exasperata*). It is commonly called "sand paper leaf" as local name and **"***Ewe* Ee*pin*" by Yoruba people of West Africa. The plant is used medicinally for treating different human diseases. There are accounts that the plant is used for the treatment of the following diseases: sores and stomach pains [2], used to arrest bleeding [3], applied to leprous sores, chest complications and ejection of the after birth in cow and human according to Hallan and Adewole et al. [4] [5], also in haemostative ophthalmia, coughs and heamorrhoid treatments [6].

In Nigeria, there are reports that the plant is used for the treatment of diseases such as ulcer, diabetes mellitus, hypertension and certain cardiovascular dysfunctions [4,5]. *Ficus exasperata* was also documented to possess pharmacological actions such as antifungal [7] and anti-helminthic activities [2].

The incidence of waterborne diseases is still on high in Nigeria due to insufficient supply of potable water to the fast growing population. About 1.25 billion people in the world especially the tropics suffer from major water related diseases such as diarrhoea, cholera, typhoid, salmonelosis, dysentery, hepatitis, etc [8]. The people in the rural areas which make up the bulk population of the people in Nigeria do not have access to public centralized water system and therefore depend on other water sources such as well, springs and rivers. In Iworoko-Ekiti, pipe-borne water is not available due to the damage done to the laid tap water pipes as a result of the on-going expansion and construction of dual carriage way that link the Ekiti State University Campus Community and neighbouring villages with Ado-Ekiti Metropolis, the State capital. Majority of people living in this suburb area are living on untreated well water which is a potential source of pathogenic microorganisms. It is therefore imperative to search for cheap and alternative drug source rather than depending on expensive and sometimes toxic commercial antibiotics which are fast losing their effectiveness due to the emergence of resistant bacterial strains. This study investigated the effects of *Ficus exasperata* extract on bacteria isolated from well water sources in Iworoko-Ekiti, Nigeria as alternative antibacterial therapy.

2. METHODOLOGY

2.1 Study Area and Sampling

This study was carried out in Iworoko-Ekiti, one of the communities in the suburb of Ado-Ekiti. It lies on latitude $7^{o}43'53$ N and longitude $5^{o}15'48$ E and it is about one kilometre away from Ekiti State University Ado-Ekiti. Due to its proximity to the University Campus, different privately owned hostels were erected in the town. Well water forms the major source of drinking water in these areas and most of the wells under study are privately owned but are usually open to general public.

2.2 Sampling Design

The sampling was restricted to the wells in privately owned student's hostels around the

University. The sampling units were divided into four zones; the northern zone which covered Prince and Princess Villa area (PP), the southern zone covered God's Grace Villa area (GG), the eastern zone also covered Egunlusi Villa (EG) while the western zones covered Ocean Way Villa and its environment (OW).

One well water sample was collected from a randomly selected well in each zone which contained about two wells that are opened to the public in each of the zone. The water samples were collected using the same method as inhabitants normally used. They were aseptically bottled and labeled to indicate the sources from which they were collected. All the studied wells were covered however they were all close to one source of contaminant or the other such as surface water; refuse dumpsite or septic tank (suck away). The four litre containers used as fetcher to draw water from these wells are usually left on the well. All the wells are not less than 10 years old after sinking.

2.3 Collection of Water Samples

Well water samples were collected using the same fetcher used by the inhabitants. Contamination of the water samples was avoided before and after collection. The samples were collected in clean, sterile 100 ml screw-cap bottles and were labeled to indicate the sources from which they were collected. They were transported to Microbiology Postgraduate Laboratory Obanla Campus, Federal University of Technology Akure in an ice-pack container. The bacterial examination of the well water samples and identification of the different species of the bacteria in the analyzed water samples were carried out as promptly as possible.

2.4 Collection of Plants Materials and Preparation of Extract

Leaf of *Ficus exasperata* was collected from the Teaching and Research Farm, Federal University of Technology Akure, Ondo State, Nigeria. The identification and authentication of the plant was carried out in the Herbarium of the Department of Plant Science, Ekiti State University Ado- Ekiti, Ekiti State. The leaf was collected and dried in a container in the laboratory for a period of five weeks. The dried leaf material was pounded with a wooden mortar and pestle until they became powdery form. The extract of the leaf was prepared with ethanol (98 %). A mass 200 g of dried powdered leaf was weighed into different conical flasks and was labeled. The 750 ml of ethanol was added into conical flask. The extraction was allowed for a period of 3 days after which the mixture was filtered with muslin cloth. Filtrate obtained was concentrated using a rotary evaporator (model RE-52A Union Laboratories, England) under reduced pressure. The concentrate was then stored in the refrigerator until used.

2.5 Bacteriological Examination of the Water Samples

The total bacterial, coliform, *Escherichia coli* and *Salmonella-shigella* counts were assessed. The bacteriological enumeration was done after serial dilution of the well water samples. One milliliter of the well water sample was dispensed into a test tube containing 9.0 ml of sterilized distilled water. A volume of 1 ml of the diluent was thereafter transferred into the next test tube containing 9.0 ml of sterilized water sequentially until a dilution 10^5 was obtained. Bacterial plate count was carried out using Oxoid nutrient agar (NA) manufactured by Thermo Scientific Incorporation, United States. MacConkey agar was used for the enumeration of the enteric coliforms and Eosin Methylene Blue agar (of Biomark Laboratory, Pune 411 041, India) was used to determine the *Escherichia coli* counts as described by Barrow and Feltham [10]. All the media were prepared according to the manufacturer's instruction. All plates were incubated at 37^oC for 24 h and plates were counted by colony counter to obtain the colonies counts of the viable bacteria.

2.6 Characterization and Identification of Isolates

Pure cultures of the bacterial isolates were observed for morphological characteristics, gram-stained and were subjected to various biochemical tests [9]. Pure culture was then characterized and subsequently identified using Cowan and Steel's Manual for the identification of Medical Bacteria [10].

2.7 Antibacterial Assay of the *Ficus exasperata* Leaf Extract

The antibacterial activity of extract was determined using agar well diffusion method as described by Esimore et al. [11]. Different concentrations of the plant extract were prepared using 30% Dimethyl sulphoxide (DMSO) as the

reconstituting solvent. It was thereafter filtered using 0.4 µm sterilized membrane pore filter paper. The control was prepared by using 0.1 ml of the reconstituting solvent and distilled water at the ratio (3:7). The plates were incubated in 37°C for 24 h.

2.8 Determination of Minimum Inhibitory Concentration (MIC)

Agar well diffusion was used to monitor the antimicrobial effect of the different concentrations of the extract [12]. Concentrations of extract used ranged from 6.25 mg/ml to 125 mg/ml while the control contained mixture of distilled water and DMSO in ratio 7 to 3 respectively. The minimum inhibitory concentration was obtained by taking the lowest concentration that did not permit any visible growth of the tested organisms.

2.9 Phytochemical Analysis of the Plant

Phytochemical analysis for qualitative detection of alkaloids, tannin, saponin, flavonoids, glycosides and phenol was performed on the extract using standard methods [13] [14] [15].

2.10 Test for Saponins

The ability of saponins to produce frothing in aqueous solution was used as screening test for saponins. About 0.5 g of each plant extract was shaken with distilled water in a test tube, frothing which persisted on warming was taken as evidence for the presence of saponins [14].

2.11 Test for Tannins

A mass of 5 g of the plant extract was stirred with 100 ml of distilled water, filtered and ferric chloride reagent was added to the filtrate. A blue-green precipitate indicated the presence of tannins [13].

2.12 Test for Alkaloids

A mass of 0.5 g of the extract was diluted with 10 ml of acid alcohol, boiled and filtered. Two milliliter of diluted ammonia was added to 5 ml of the filtrate. Five milliliter of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Meryer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Meryer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was taken as positive for the presence of alkaloid [13].

2.13 Test for Flavonoids

A mass of 2 g of powdered sample was detanned with acetone. The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the detanned sample. The mixture was filtered while hot. The filtrate was cooled and 5 ml of 20 % sodium hydroxide was added to equal volume of the filtrate. A yellow solution indicates the presence of flavonoids [15].

2.14 Test for Phlobatannins

Deposition of red precipitate when aqueous extract of plant was boiled with aqueous HCl acid was taken on evidence of phlobatannins [16].

2.15 Test for Steroids

A volume of 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of the sample with 2ml tetraoxosulphate (VI) acid. The change from violet to blue or green indicated the presence of steroid.

2.16 Test for Terpenoids

A volume of 5 ml of extract was mixed with 2 ml of $CHCl_3$ in a test tube. Thereafter, 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed for the presence of terpenoids [13].

2.17 Statistical Analysis

Data obtained were presented as mean (of 3 replicates) ± SE (standard error of mean). Significance difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using SPSS window 7 version 16 software. For all the tests, the significance was determined at the level of $P<0.05$.

3. RESULTS AND DISCUSSION

The bacteriological analyses results in Table 1 below revealed the values of the total plate, coliform and *Escherichia coli* counts which range

from 1.83 × 10^4 cfu/ml to 4.65 × 10^4 cfu/ml, 1.00×10^3 to 3.20×10^3cfu/ml and 8.30 × 10^2 cfu/ml to 16.4 × 10^2 cfu/ml respectively. These values are remarkably high far above the standard limit set by WHO for drinking water and this poses risk to the health of the people depending on the wells as sources of water for drinking [17]. High bacterial loads recorded in this study could be linked to the proximity of these wells to the point source of contamination such as septic tank, shallowness of the wells, dumpsites, polluted surface water as well as various human and animal activities such as the use of multiple and unkempt fetchers, washing of clothes around the well and animal droppings falling on the wells' casings. All these were observed during samples collection.

Various species of bacteria were identified from the pure cultures obtained on Nutrient agar, Eosine methylene blue agar, Macconkey agar and *Salmonella-shigella* agar. These include *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Bacillus substilis, Proteus mirabilis* and *Staphylococcus aureus* and their distribution are shown in Table 2. However, *Escherichia coli* and *Klebsiella pneumoniae* were the most frequently encountered pathogens in all the water samples (Table 2). This could be attributed to the proximity of the wells to toilet facilities or animals droppings since these microorganisms are normal microflora of human (and animal) gut. They could be accidentally introduced into well due to unhygienic practices and seepage from nearby septic tanks. Therefore, the presence of enteric coliform especially *E. coli* makes the water unsuitable for human consumption going by the guidelines set by World Health Organization for safe potable water (0 coliform/100 ml) [17].

Accordingly, the total coliform counts for all the samples were exceedingly higher than the standard set by Environmental Protection Agency for coliform bacteria in drinking water (zero total coliform per 100 ml of water) [17]. World Health Organization has documented that since these pathogens are mainly of feacal origin, any water that contains them should not be used for drinking or cleaning purpose [17].

Table 3 shows the antibacterial activity of *F. exasperata* extract on the bacterial isolates. The ethanolic extract of *Ficus exasperata* showed appreciable antibacterial activity against the bacteria isolates: *E. coli*, *K. pneumoniae, P. mirabilis*, *P. aeruginosa, B. substilis, S. typhi* and *S. aureus* at different concentrations. At lower concentrations (6.25 mg/ml, 12.5 mg/ml and 25 mg/ml) no inhibition was observed in all the test isolates. However, *P. aeruginosa, E. coli* and *S. typhi* were found to be sensitive to concentrations of the crude extract at 50 mg/ml and upwards. *P. aeruginosa* showed highest zone of inhibition (18.50±0.5 mm) at 125 mg/ml concentration. *K. pneumoniae* and *S. aureus* still demonstrated mild sensitivity at 75 mg/ml concentration giving rise to zones of inhibitions of 1.0±0.0 mm inhibition each, while at this same concentration 3.00±0.00 mm, 5.00±0.00 mm and 6.53±0.50 mm zones of inhibition were recorded against *E. coli, S. typhi* and *P. aeruginosa* respectively. However, better inhibition was observed with the crude extract at higher concentrations; 8.00±0.00 mm, 8.50±0.50 mm and 18.50±0.50 mm were marked against *E. coli, S. typhi* and *P. aeruginosa* respectively. *P. mirabilis* was sensitive to the extract only at 100 mg/ml with zone of inhibition of 2.00±0.00 mm, whereas none of the bacterial isolates was sensitive to the control.

This result agrees with the work carried out by some authors who evaluated the antimicrobial activities of *Ficus exasperata* plant observed that *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* were all susceptible to its extract [18]. Antibacterial property of hexane and ethanolic extract of *F. exasperata* leaf against clinical bacterial isolates have been determined

Table 1. Total Bacteria, coliform and *Escherichia coli* counts of the well water samples from Iworoko-Ekiti

Well water Sample	Total plate count (cfu/ml) × 10^4	Coliform count (cfu/ml) × 10^3	*Escherichia coli* count (cfu/ml) × 10^2	*Salmonella-shigella* (cfu/ml) × 10^2
PP	4.65^d±0.15	3.20^d±0.04	16.4±0.04	9.80±0.02
GG	3.67^c±.045	1.37^c±0.05	6.65±0.03	4.88±0.03
OW	2.38^b±.045	1.13^b±0.04	8.30±0.10	0
EG	1.83^a±0.00	1.00^a±0.03	0	0

Values with the same alphabet along the column are not significantly different P<0.05

Legend: ***PP-*** *Prince and Princess Villa;* ***OW -*** *Ocean way Villa;* ***GG-*** *God's grace Villa;* ***EG-*** *Egunlusi Villa*

Table 2. Occurrence and distribution of the bacterial isolates

	Isolate	PP	OW	GG	EG
1	*Klebsiella pneumoniae*	+	+	+	+
2	*Escherichia coli*	+	+	+	+
3	*Pseudomonas aeruginosa*	+	+	-	-
4	*Salmonella typhi*	+	-	+	-
5	*Bacillus substilis*	-	-	+	-
6	*Proteus mirabilis*	-	-	+	+
7	*Staphylococcus aureus*	+	-	-	+

Legend: PP*- Prince and Princess Villa;* ***OW*** *- Ocean way Villa;* ***GG****- God's grace Villa;* ***EG****- Egunlusi Villa;* ***+****: Present; - : Absent*

Table 3. Antibacterial activity of *Ficus exasparata*

Concentration (mg/ml)	Zones of Inhibition (mm)						
	Pseud. aeruginosa	***E. coli***	***K. pneumoniae***	***S. typhi***	***B. substilis***	***P. mirabilis***	***Staph. aureus***
6.25	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
12.5	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
25.0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
50.0	1.83±0.02^{b}	2.00±0.00^{b}	0.00±0.00^{a}	2.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
75.0	6.50±0.50^{c}	3.00±0.00^{c}	1.00±0.00^{b}	5.00±0.00^{c}	0.00±0.00^{a}	0.00±0.00^{a}	1.00±0.00^{b}
100.0	12.00±0.00^{d}	6.50±0.50^{d}	2.50±0.50^{c}	6.00±0.00^{d}	1.90±0.10^{b}	2.00±0.00^{b}	3.00±0.00^{c}
125.0	18.50±0.50^{e}	8.00±0.00^{e}	4.00±0.00^{d}	8.50±0.50^{e}	4.00±0.00^{c}	4.00±0.00^{c}	4.50±0.50^{d}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Values with the same alphabet along the column are not significantly different P<0.05

and were found to be potent [19]. In this work the inhibition caused by the extract was observed to be concentration-dependent like the previous work published by other authors; hence the extract with highest concentration showed the greatest inhibition.

Table 4 shows the minimum inhibitory concentration of the crude extract *F. exasperata* on the bacterial isolates. *P. aeruginosa, E. coli*, *K. pneumoniae* and *S. typhi* required a minimum concentration of 50 mg/ml to be inhibited while *S. aureus* was inhibited at 75 mg/ml concentration. *P. mirabilis* was inhibited at minimum concentration of 125 mg/ml of the crude extract. This also corroborates with the observation of Engwa et al. in whose work this *Ficus* species achieved the minimum inhibition of the test bacteria from 50 mg/ml concentrations upwards [19].

The phytochemical analysis of the *Ficus* plants showed that the leaf contained alkaloid, saponin, flavonoid, steroid and cardiac glycoside (Tables 5). Hill had attributed the medicinal values in the medicinal plants to these chemical substances present in them [20]. The quantitative phytochemicals analysis results revealed that alkaloids content was 49.9.0±0.10 mg/100 g in *F. exasperata* (Table 6). The high alkaloid content could have contributed to the antibacterial property of the extract. The concentration of 0.13±0.00 mg/100g of tannin was recorded while saponin content was 18.45±0.0 mg/100g. Edible plant materials containing tannins have been used to cure diarrhoea and dysentery [21]. There are also records that medicinal plants rich in tannin had been used for the treatment of urinary tract infections [22]. The presence of tannins in *F. exasperata* justifies their use in traditional medicine for the healing of many different diseases. Moreover, apart from antibacterial activity potential of saponin, it also reduces the uptake of glucose and cholesterol in the gut through intra-lumenal physicochemical interaction which could confer protection against heart diseases [23]. Phenols and flavonoids proportion were 0.14±0.0 mg/100g and 1.36±0.00 mg respectively. There had been reports on the antimicrobial property of phenolic substances [24] and they are believed to be responsible for the plant defenses against the attack from fungi and other microorganisms in the soil. Apart from antimicrobial potential of flavonoids, it presence in diet also reduces the risk of various degenerative diseases in human such as coronary heart diseases [25], cancers as well as prevention of menopausal symptoms [26] [27]. Many plants containing alkaloids and flavonoids have been documented to possess diuretic, antispasmodic, anti-inflammatory and

analgesic effects [22]. The presence of all these bioactive compounds would have contributed to the antibacterial activity demonstrated by *Ficus exasperata*.

Table 4. Minimum inhibitory concentration of the crude plant extract

Bacterial isolate	*Ficus exasperata* (mg/ml)
Pseudomonas aeruginosa	50
Escherischia coli	50
Klebsiella pneumoniae	50
Salmonella typhi	50
Bacillus substilis	100
Proteus mirabilis	125
Staphylococcus aureus	75

Table 5. Qualitative analysis of the phytochemicals in *Ficus exasperata*

Phtochemical	*Ficus exasperata*
Alkaloid	+
Saponin	+
Phlobatanin	-
Anthraquinones	-
Flavonoids	+
Steroids	+
Terpenoids	-
Cardiac glycosides	+

Legend: *+ = Present - = Absent*

Table 6. Quantitative analysis of the phytochemicals in *Ficus exasperata* extract

Component	*Ficus exasperata* extract (mg/100ml)
Alkaloids	$49.9.00\pm0.10^{e}$
Terpernoids	0.00 ± 0.00^{a}
Flavonoids	1.36 ± 0.00^{b}
Steroids	0.00 ± 0.00^{a}
Glycosisides	30.93 ± 1.41^{d}
Tannins	0.13 ± 0.00^{a}
Phenols	0.14 ± 0.00^{a}
Saponin	18.45 ± 0.00^{c}

Values with the same alphabet along the column are not significantly different P<0.05

4. CONCLUSION

The quality evaluation of water sources in the privately owned student hostels around Ekiti State University Ado-Ekiti, Nigeria revealed that both the total bacterial counts and the total coliform counts were found to exceed the limits recommended by World Health Organization and Nigeria Standard for Drinking Water Quality [28] [17]. Consequently, this rendered the water sources unfit for drinking purpose as they pose a great health risk to the life of the consumers. Moreover, the *in-vitro* antibacterial activity of *F. exasperata* extract as it was observed in this study justified it use in ethno-medicine. The plant can find application as alternative source of treatment of waterborne bacterial infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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