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Antibacterial and Phytochemical Screening of Leaf and Seed Extract of *Ficus exasperata*

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Authors' contributions

This work was carried out in collaboration among all authors. Author OOJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OOJ, VOO and MFI managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Ficus exasperata belongs to the family Moraceae, and is commonly called forest sand paper tree/plant, widely spread in all eco-regions of Nigeria. This plant possesses antimicrobial agents and pharmacological compounds which aid in its efficacy for treatment of ailments. Hence, this study investigated the antibacterial activities and phytochemical screening of aqueous and ethanolic extract of leaves and seeds of Ficus exasperata. Leaves and seeds of the plant sample were processed to obtain fractions of crude extracts which were used against bacterial isolates such as, Staphylococcus aureus, Klebsiella aerogenes, Pseudomonas aeruginosa, Escherichia coli and Shigella spp. Phytochemical screening of the samples was also done to detect the presence of alkaloid, saponins, flavonoids, glycosides, tannin, terpenoids, sterol and phenols. Results obtained showed the susceptibility pattern against the bacterial isolates at concentrations ranging from 0.20 – 1.00 mg/mL. The ethanol extract of leaves of the plant sample showed high susceptibility pattern against *P. aeruginosa*, *S. aureus*, *E. coli* and *K. aerogenes*. The study shows that crude extracts of leaves and seeds of the plant sample were effective against the test organisms. The phytochemicals constituents were also present except sterol which is lacking in the

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seed sample due to the solvent used such as ethanol but may be present if other solvent is used. Antibacterial activity of crude extracts of *F. exasperata* leaves and seeds were as a result of presence of phytochemical constituents because they are fundamental biomedicals, which are considered biologically to be active compounds. This study provides an insight to the usefulness of extracts from *F. exasperata* leaves and seeds to be potential treatment against common clinical diseases.

Keywords: Antibacterial; crude extract; ficus exasperate; phytochemical; plant.

1. INTRODUCTION

Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern [1]. The clinical efficiency of many antibiotics in existence is being treated by the emergence of multi drugresistant pathogen [2]. Throughout the history of mankind, many infectious diseases have been known to be treated with herbal remedies .The natural herbal products either as pure compounds or as standardized plant extracts provided unlimited opportunities for new drug leads because of the un compared availability of diversities of chemical [3]. This results to a never ending and urgent need to discover new antimicrobial compounds with different chemical structure and new mechanisms of action for reemerging and new infectious diseases [4].

Ficus exasperata belongs to the family Moraceae, and is commonly called sand paper tree/plant, widely spread in all eco-regions of Nigeria. The plant has been ethnobotanically reported to have diverse medicinal uses [5]. The leaf extract is reported to have diverse medicinal uses such as treating hypertensive patients, haemostative, ophthalmia, coughs and haemorrhoids [6]. The root bark is reported to be

used in the treatment of high blood pressure [7]. The leaf is used to scratch skin parts affected by ringworm while the grounded leaves applied topically are used to treat boils [8]. Furthermore, the young leaves are prescribed as a common anti-ulcer remedy [9].

Various pharmacological actions such as antidiabetic, lipid lowering and anti-fungal activities have been reported for *Ficus exasperata* [10]. Other industrial uses of sand paper leaves are for polishing woods, stabilization of vegetable oils, suppression of foaming, supplement as food stock and antimicrobials [11]. The activities of the leaf extract of *Ficus exasperata* against some pathogenic organisms have been extensively investigated [6].

In African traditional medicine, different parts of plant such as; fruit, leaf, sap, bark, and root are considered medicinally important [12]. They are used as analgesic, anti-arthritic, diuretic, vermifuge, febrifuge, abortifacient, ecbolic, wound healing, ophthalmic and oral infections, nasopharyngeal afflictions, arthritis, rheumatism, gout, edema, kidney disorders, diarrhea, dysentery, hemorrhoids and venereal diseases [13].

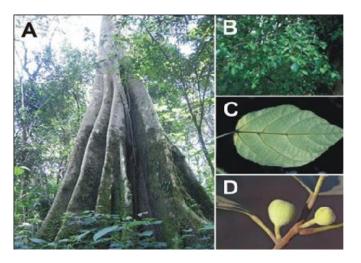


Fig. 1. (A) Ficus exasperata tree trunk, (B) Foliage, (C) Leaf, (D) Fruits

The leaves of F. exasperata are much valued in the treatment of a variety of diseases/disorders [5,14]. In French Guinea, a decoction of the leaves is used for stomach disorders. The leaves are used for treatment of hemostatic ophthalmia, coughs, hemorrhoids anxiety disorders, epilepsy, high blood pressure, rheumatism, arthritis, cancer, intestinal pains, colics, bleeding and wounds [11]. In Nigeria, Republic of Congo and Central African Republic the leaves are used as an antipyretic [15]. The leaves are macerated in water and the decoction is administered orally. The leaves are particularly valued in the treatment of malaria in Cameroonian folk medicine [16]. In some parts of Cameroon, leaves are used in the treatment of hemorrhoids and the water extract of the leaves is administered orally in diarrhea. One glass of extract made by macerating one handful of contused leaves in 1liter of water is given for 4 days in diarrhea [17].

In Nigeria, the young leaves are prescribed as a common anti-ulcer remedy. Few leaves that are chewed and swallowed three times for 4-8 weeks are believed to produce a complete cure of ulcer [18]. Dried leaves as such and the infusion are used to treat ulcers and stomachache [18]. A paste made of 50 leaves of *F. exasperata*, fifty (50) leaves of *E. coccinea* and ten (10) fruits of *Capsicum frutescens* is added to 1 liter of water, homogenized and filtered. 150 mL filtrate is given twice daily as a remedy for peptic ulcers [19].

The ethanol extract of F. exasperata leaves was reported to show moderate antibacterial activity against Escherichia coli and Staphylococcus albus with slight difference in minimum inhibitory concentrations (MIC) from S. albusis [4]. However, the crude plant extract in combination with the protein synthesis inhibitors exhibited significant antibacterial activity [18]. methanol leaf extract also inhibited the growth of P. aeruginosa, S. typhi, S. aureus, and E. coli [9]. The stem bark methanol extract inhibited the growth of P. aeruginosa and S. Typhi. Taiwo et al. [20] stated that, F. exasperata leaf, stem bark and root contain bioactive substances with the highest inhibitory activities against some human pathogenic organisms.

With prior knowledge on the recent microbial resistance to antibiotics and the opportunity to produce new potent antimicrobial drugs from plants, this study is conducted to evaluate the antimicrobial effects of the methanolic and ethyl acetate extract of the seed and leaves of Ficus exasperata on selected pathogenic

microorganism; and as well screen the plant extract for the presence of secondary metabolites. This study was also conducted in order to discover the level of potency of the plant extract in combating common clinical diseases.

2. MATERIALS AND METHODS

2.1 Collection of Sample

The leaf of *Ficus exasperata* (sandpaper) was spotted and collected at the Federal Polytechnic, Ado-Ekiti, Nigeria. The leaves were identified in the Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti. The fresh samples were washed under running tap water, air-dried at room temperature of 25°C for about 2 weeks and milled into fine powder using a Thomas Willey Milling machine and then stored in sterile container for further analysis.

2.2 Extraction of Bioactive Components from the Plant Material

Extraction method described by Fajilade and Oladunmoye [21] was employed. One hundred grams (100 g) of the powdered plant material (F. exasperata) was poured into different beakers and 500 ml of distilled water and ethanol. The content was stirred using a sterile glass rod and allowed to stand for 24 hours at room temperature (25°C \pm 1). This was then filtered using Whatman No.1 filter paper and the filtrate concentrated on steam bath at to give 120 g of the residue (green brown slurry) corresponding to a percentage yield of 40.

2.3 Source and Reactivation of Organisms

Staphylococcus aureus, Klebsiella aerogenes, Pseudomonas aeruginosa, Escherichia coli and Shigella spp. were obtained from the Microbiology Laboratory of Federal Polytechnic, Ado-Ekiti. The bacteria were re-suspended in test tubes containing Nutrient broth and the test tube was incubated at 37°C for 18–20 hours.

2.4 Determination of Degree of Antibacterial Potency

Agar well diffusion method described by Balouiri et al. [22] was adopted to evaluate the antimicrobial activity of the plant extract. The agar plate surface was inoculated by spreading 0.5 mL of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6

mm was punched aseptically with a sterile cork borer, and a volume (0.3–1.20 mg/mL) of the extract solution at desired concentration is introduced into the well. Then, agar plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters (mm) with a meter rule.

2.5 Antibiotic Susceptibility Test

The antibiotics susceptibility of the bacteria isolates were determined by disc diffusion method according to CLSI [23]. The antibiotic discs were aseptically, carefully and firmly placed on the inoculated plates using sterile forceps. The plates were then inverted and incubated for 24 hours at temperature of 37°C. After incubation, the plates were examined for growth and the diameters of zone of inhibition were measured and the results were interpreted with reference to CLSI [23]. The bacterial isolates were screened for resistance to ten (10) gramantibiotic discs which positive comprise Chloramphenicol (CH 30µg), Ciprofloxacin (CPX 10μg), Amoxil (AM 20μg), Gentamycin (CN 10μg), Streptomycin (S 30μg), Rifampicin (RD 20 μg), Erythromycin (Ε 30μg), Levofloxacin (LEV 20µg), Norfloxacin (NB 10µg) and Ampiclox (APX 20µg). Gram-negative discs contains additional constituent such as Ofloxaxin (OFL 10μg), Pefloxacin (PEF 10μg), Ciprofloxacin (CPX 10µg), Augmentin (AU 30µg), Gentamycin Streptomycin (S 30µg), (CN 10µg), Cephalosporin (CEP 10µg), Nalidixic acid (NA 30µg), Septrin (SXT 30µg) and Ampicillin (PN 30µq).

2.6 Phytochemical Screening of Plant Extract

2.6.1 Test for alkaloids

0.4 g of the plant extract was diluted with 8 mL of 1% HCl and the mixture was then boiled and filtered. 2 mL of filtrate was treated separately with (a) few drops of potassium mercuric iodide (Mayer's reagent) and (b) potassium bismuth (Dragendroff's reagent). Turbidity or precipitation with either of these reagents was taken as evidence for existence of alkaloids.

2.6.2 Test for saponins

The ability of saponins to produce emulsion with oil was used for the screening test. 20 mg of plant extract was boiled in 20 mL of distilled water in a water bath for 5 min and then filtered.

10 mL of the filtrate was mixed with 5 mL of distilled water and was shaken vigorously for froth formation. 3 drops of olive oil was mixed with froth, shaken vigorously and was observed for emulsion development.

2.6.3 Test for terpenoids

Presence of terpenoids in the plant extract was carried out by taking 5 mL (1 mg/mL) of the extract and mixed with 2 mL of chloroform, and then followed by 3 mL of concentrated H_2SO_4 . A reddish brown coloration of the interface confirmed the presence of terpenoids.

2.6.4 Cardiac glycosides determination

Five (5) mL (10 mg/mL in methanol) of the plant extract was mixed with 2 mL of glacial acetic acid having one drop of FeCl $_3$ solution. To the mixture obtained 1 mL of concentrated H_2SO_4 was then added to form a layer. The presence of brown ring of the interface indicated deoxyl sugar characteristic of cardiac glycosides.

2.6.5 Test for flavonoids

Fifty (50) mg of the plant extract was suspended in 100 mL of distilled water to get the filtrate. 5 mL of dilute ammonia solution was added to 10 mL of filtrate followed by few drops of concentrated $\rm H_2SO_4$. Presence of flavonoids was confirmed by yellow coloration.

2.6.6 Test for tannins

50 mg of the plant extract was boiled in 20 mL of distilled water and filter. A few drops of 0.1% FeCl₃ was added in the filtrate and observe for colour change; brownish green or a blue-black coloration was taken as evidence for the presence of tannins.

2.6.7 Test for PhenoIs

A portion of the extract was treated with aqueous 5% ferric and observed for formation of deep blue or black colour.

2.7 Statistical Analysis

Analysis of variance was computed using Statistical Package for the Social Sciences (SPSS) 15 software for each attribute and the Duncan multiple range test was used to separate the means where significant difference existed. Statistical significance was considered at p<0.05.

3. RESULTS AND DISCUSSION

3.1 Results

Tables 1 to 4 present the calculated mean zones of inhibition (mm) of aqueous and ethanolic extracts of *Ficus exasperata* leaf and seed on the gram positive and negative bacterial used for this study respectively. The mean zones of inhibition of growth of the isolates are a function of relative antibacterial activities of the extracts. The zone of inhibition is simply the area on the agar plate that remains free from microbial growth. The size of the zone of inhibition is usually related to the level of antimicrobial activity present in the sample or product - a larger zone of inhibition usually means that the antimicrobial is more potent.

The extracts showed selective levels of activities against the isolates. On Table 1, it was observed that the potency of the seed extract of *F. exasperata* increases with increase in the concentration. The highest rates of inhibition were observed across all the microorganisms tested at 0.2, 0.4, 0.6, 0.8 and 1.0 g/mL, while concentrations 0.2 g/mL and 0.4 g/mL recorded no antimicrobial potency for *Klebsiella aerogenes*, while the highest inhibition against bacterial growth was recorded for *Pseudomonas aeruginosa* (26.30 mm).

The ethanolic seed extracts of *F. exasperate* it was observed that the potency of the ethanolic seed extract in Table 2 showed that different concentration of the extract was active against the test organism except *Shigella* spp. at concentration (0.2 and 0.4 g/mL) meanwhile the highest zone of inhibition was observed in *Staphylococcus aureus* with (38.20 mm) at 1.0 g/mL concentration while the lowest inhibitory concentration was observed in *Shigella* spp. with (10.80 mm) at 0.6 g/mL concentration. Generally, it appears that the growth of bacteria from strains is inhibited on ethanolic seed extract more than the aqueous extract under the treatment.

The aqueous leaf extract of *F. exasperata* inhibit high antibacterial activity against selected pathogens as shown in Table 3. The leaf extract was highly effective, maximum range (7.80 mm to 24.50 mm). *Pseudomonas aeruginosa* and *Shigella* spp. had the highest inhibitory concentration (24.50 mm) at 1.0 g/mL concentration. It was observed that at 0.2 g/mL concentration *Klebsiella aerogene*, *Escherichia coli* and *Pseudomonas aeruginosa* were not active while the lowest inhibitory concentration was observed in *Klebsiella aerogenes*.

The results on Table 4, however, vary considerably from that of Table 3.

Table 1. Antibacterial activity of aqueous extracts of *F. exasperata* seeds against bacterial pathogens

Test organisms	Dia	ameter of zon	es of inhibitio	n (mm)	
	0.20	0.40	0.60	0.80	1.00
Pseudomonas aeruginosa	17.30±0.01	20.10±0.03	21.40±0.02	22.20±0.01	26.30±0.03
Staphylococcus aureus	20.10±0.03	22.40±0.01	23.50±0.01	24.00±0.01	25.10±0.01
Shigella spp.	14.50±0.01	15.20±0.01	20.30±0.03	21.10±0.03	22.40±0.02
Escherichia coli	12.60±0.02	16.30±0.01	16.50±0.02	17.20±0.04	20.50±0.03
Klebsiella aerogenes	0.00 ± 0.00	0.00±0.00	14.50±0.02	15.50±0.01	19.70±0.01

Values are mean of three determinations

Table 2. Antibacterial activity of ethanolic extracts of *F. exasperata* seeds against bacterial pathogens

Test organisms	Diameter of zones of inhibition (mm)							
-	Concentrations (mg/mL)							
	0.20 0.40 0.60 0.80 1.00							
Pseudomonas aeruginosa	17.60±0.02	21.50±0.01	23.40±0.04	28.50±0.03	19.80±0.02			
Staphylococcus aureus	21.20±0.02	23.20±0.01	30.00±0.00	35.10±0.02	38.20±0.02			
Shigella spp.	0.00±0.00	0.00 ± 0.00	10.80±0.02	13.70±0.03	17.10±0.04			
Escherichia coli	18.60±0.01	22.30±0.04	28.20±0.01	30.10±0.02	32.50±0.01			
Klebsiella aerogenes	20.30±0.02	21.10±0.02	26.10±0.04	29.00±0.00	31.50±0.02			

Values are mean of three determinations

Table 3. Antibacterial activity of aqueous extracts of *F. exasperata* leaves against bacterial pathogens

Test organisms		Diameter of z	zones of inhib	ition (mm)				
	Concentrations (mg/mL)							
	0.20	0.40	0.60	0.80	1.00			
Pseudomonas aeruginosa	0.00±0.00	7.80±0.02	15.50±0.01	17.40±0.02	24.50±0.03			
Staphylococcus aureus	12.50±0.02	16.40±0.03	18.20±0.02	19.70±0.01	23.70±0.02			
Shigella spp.	9.80±0.02	14.60±0.02	17.30±0.01	19.3±0.001	24.30±0.02			
Escherichia coli	0.00±0.00	11.50±0.03	15.40±0.01	17.10±0.02	20.60±0.03			
Klebsiella aerogenes	0.00±0.00	8.50±0.03	9.50±0.04	14.50±0.01	19.40±0.01			

Values are mean of three determinations

Table 4. Antibacterial activity of ethanolic extracts of *F. exasperata* leaves against bacterial pathogens

Test organisms	Diameter of zones of inhibition (mm)								
-	Concentrations (mg/mL)								
	0.20	0.20 0.40 0.60 0.80 1.00							
Pseudomonas aeruginosa	20.40±0.01	23.50±0.02	28.10±0.02	31.10±0.02	36.00±0.01				
Staphylococcus aureus	38.30±0.03	43.10±0.02	47.50±0.01	50.00±0.01	52.00±0.01				
Shigella spp.	0.00±0.00	0.00±0.01	35.60±0.03	38.20±0.01	45.20±0.03				
Escherichia coli	22.50±0.01	26.20±0.03	30.50±0.01	32.50±0.03	35.10±0.01				
Klebsiella aerogenes	18.60±0.02	23.30±0.03	26.40±0.02	27.40±0.01	30.20±0.02				

Values are mean of three determinations

Table 5. Antibiotic sensitivity test for gram negative bacteria

Organism	S	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT
Shigella spp.	-	-	-	21.50	-	19.60	15.50	15.50	21.10	-
Klebsiella spp.	-	-	-	22.20	-	21.50	19.40	-	-	22.50
E. coli	15.40	-	-	18.00	-	15.40	19.60	18.50	21.50	-
Pseudomonas spp.	34.50	-	-	34.10	-	35.30	39.10	-	19.40	-

S – Streptomycin; PN – Amplicin; CEP – Ceporex; OFX – Tarivid; NA – Nalidixic; PEF – Reflacine; CN – Gentamycin; AU – Augmentin; CPX – Ciproflox; SXT – Septrin

Table 6. Antibiotic sensitivity test for gram positive bacterium

Organism	RD	AML	S	NB	СН	CPX	Е	LEV	CN	APX
Staph. aureus	-	17.50	20.50	24.70	-	21.30	18.50	19.30	18.70	15.90

RD – Rifampicin; AML– Amoxil; S – Streptomycin; NB – Norfloxacin; CH – Chloramphenicol; CPX – Ciproflox; E – Erythromycin; LEV – Levofloxacin; CN – Gentamycin; APX – Ampliclox

Table 7. Phytochemical screening of leaf and seed extracts using ethanol and aqueous

Parameter	Seed H ₂ O	Seed ethanol	Leaf H₂O	Leaf ethanol
Alkaloid	+	+	+	+
Saponin	+	_	+	_
Flavonoid	+	+	+	_
Sterols	_	_	_	_
Tannin	_	_	_	_
Glycoside	+	+	_	_
Phenol	_	_	+	_
Terpenoids	_	_	_	_

Positive = +

Negative = -

The ethanolic extract of the leaf of *F. exasperata* indicated the highest inhibition growth zone in *Staphylococcus aureus* (52.00 mm), followed by *Shigella* spp. (45.20 mm) at 1.0 g/mL extract concentration. The extract was potent against only four bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella aerogenes*) at concentration 0.2 – 1.0 g/mL, while at 0.2 and 0.4 g/mL for *Shigella* spp. no potency or inhibition was recorded. Table 5 and 6 show the antibiotics sensitivity of bacteria isolates; and the phytochemical screening analysis of plants extracts using ethanol and aqueous was showed in Table 7.

3.2 Discussion

There is need to develop new antimicrobial agents and antibiotics from plant materials, owing to the fact that microorganisms are developing resistance to many drugs and the death rate from infectious diseases have increased tremendously. Plant materials have long been used for the treatment of infectious diseases such as asthma, sexually transmitted infections, skin infections and many others. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body [24].

This study has revealed that the extracts from Ficus exasperata seed and leaf have antimicrobial effects on the clinical strains of the five microorganisms used for this study. The antimicrobial effect of the seed is noted, especially against Staphylococcus aureus. In addition, antibacterial properties of the leaf against Staphylococcus aureus is also worth mentioning. This could be interpreted to mean that the seed and leaf of F. exasperata may be relevant in the treatment of the diseases caused by Staphylococcus aureus.

Studies by Adebayo et al. [9] indicated that the ethanolic extract of Ficus exasperata root recorded a high rate of inhibition of microbial growth of Escherichia coli, Salmonella typhi and Klebsiella aerogene, which are responsible for causing stomach illnesses. They further reported the relatively large zone of inhibition by the extract of the leaf ethanolic against Pseudomonas aeruginosa, which corresponds to the findings of this work. This suggests that the plant can be used in the treatment of the diseases caused by this microorganism. A downward trend in the potency of the extracts on inhibition of the microbial growth

corresponding to the decreasing extract concentration was also observed in the work done by Adebayo et al. [9].

The phytochemical screening analysis of plants extracts using ethanol and water was showed in Table 1, from the phytochemical analysis alkaloid were found in sandpaper leaf and seed in both ethanol and aqueous solvents. The ethanol extract of sand paper seed and leaf shows the absence of saponin while saponin is found to be present in the aqueous extracts of the seed and leaf.

In all plant extracts found flavonoids except in ethanol extract of the leaf. Glycoside and reducing sugar were observed to be present in both aqueous and ethanol extracts of the seed and absent in both extracts of the leaf. The aqueous and ethanol extracts of the sand paper leaf and seed showed the absences of sterols, tannin and terpenoids. Saponin though positive in aqueous extracts of the leaf and seed, persistent frosting was intense in the aqueous seed extract then the leaf [24]. This compound has been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties [25].

The presence of alkaloids has been implicated in its detoxifying and antihypertensive properties as a result of its stimulatory effects [25]. Tannins suggests the ability of a plant to play a role as anti-diarrhea and anti-haemorrhagic agents, therefore, the absence of tannin in the extracts shows that it will lack the ability to perform this functions.

The presence of phenol in the aqueous extract of the leaf indicates that the plant might play an important role as dietary antioxidants. Phenolic compounds prevent oxidative damage in living systems. Glycoside, were present in aqueous and ethanolic extracts of the seeds only. This may be due to the mode of extraction. Glycosides have been used for centuries as stimulants in cases of cardiac failure [26]. Conclusively, this study shows that Ficus exasperata was effective against the test pathogens and it justifies the ethno pharmacological uses of both plants in the treatment of microbial infections.

4. CONCLUSION

The phytochemical analysis of the seed and leaf extracts revealed the presence of some

secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides, saponins, and steroids in both aqueous and ethanolic extracts. The diameter of zone of inhibition of extracts against test microorganisms (in mm) was highest for the ethanolic extract, followed by aqueous extract. The zones of inhibition for the extracts are in the range of 7-52 mm. On the whole, it can be confidently stipulated that the study has established some scientific bases for the folkloric use of the seed and leaf of Ficus exasperata in managing acute inflammation, infections and many other tropical diseases. This study provides an insight to the usefulness of extracts from F. exasperata leaves and seeds to be potential treatment against common clinical diseases.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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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