

Phytochemical screening and antibacterial activities of *Dillenia indica* and *Ficus exasperata*

ABSTRACT

It has been proven that antimicrobials of plant origin work more efficiently with fewer side effects. This study aimed to identify the phytochemicals in extracts of *Dillenia indica* and *Ficus exasperata* and also to examine their antibacterial effect on clinical bacterial isolates (*Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes*). Dry leaves of *D. indica* and *F. exasperata* were extracted in ethanol, methanol, distilled water and hot water. The 100% extract solution was further diluted to different concentrations (75%, 50%, and 25%). The extracts were concentrated using a rotary evaporator. Using the agar well diffusion method, the isolates were subjected to the antibacterial action of various concentrations of these extracts. The diameter of the zones of inhibition were measured in millimeters. The data obtained were analyzed by Analysis of Variance (ANOVA) to determine significant ($P < 0.05$) effects. Significant differences between means were determined using Duncan's Multiple Range Test (DMRT). The phytochemical screening indicated the presence of alkaloids, phenols, glycosides, flavonoids, saponins, quinones, and anthraquinones. Terpenoids were absent. The extracts of the plants inhibited the growth of the bacteria tested with varied effectiveness. The diameter of the zones of inhibition were concentration dependent in all extracts. The maximum antibacterial activities were observed in the organic solvent extracts. When the activity of the plant extract was compared with that of the standard antibiotics used in this work, it was observed that the plant extract compared favourably with those of these standard antibiotics. In conclusion, the results showed that leaf extract of *D. indica* and *F. exasperata* have antibacterial activity. Based on the antibacterial activity of *D. indica* and *F. exasperata* extracts as revealed by this research, the extracts of these plants can be used in the development of new pharmaceuticals which can be useful as strong therapeutic agent against bacterial pathogenic infections.

Key words: *Dillenia indica*, *Ficus exasperata*, phytochemicals, zone of inhibition, leaf extracts

1.INTRODUCTION

“Natural medicines have been used to boost health since time immemorial, and the success of modern medical science largely depends on drugs originally obtained from natural sources” [1]. “In the past, a large number of antimicrobial compounds were discovered from synthetic and natural products for the treatment and control of infectious agents” [2]. In traditional disease management, a variety of therapeutic plants and components have been used [3]. They are valuable economic resources as well as crucial in health care [4]. According to [5], nearly 80% of the world's population lives in low-development, low-income areas where traditional medicine is used to treat a variety of health concerns. Antibiotic resistance and related toxicity issues have recently emerged, limiting the use of antimicrobial drugs [6]. “As a result, crude plant extracts are used as herbal medicine to treat human infectious diseases” [7]. “Chemical synthesis and the search for natural products from living organisms such as higher plants have been identified as primary sources for finding a new bioactive compound to treat human diseases caused by pathogenic microbes, as more than 80% of the world's population relies on traditional medicine for their primary healthcare needs” [8].

“Bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* cause several human infections” [9]. “*E. coli* is a Gram-negative,

rod-shaped non-spore-forming bacteria. It's commonly linked to urinary tract infections (UTIs) [10] and post-operative wound infection. *S. aureus* is a Gram positive bacteria that causes a variety of infections, including skin infections, bacteremia, endocarditis, pneumonia, and food poisoning. *S. pyogenes* is regarded as one of the most successful human pathogens globally” [11]. “*S. pyogenes* is a Gram-positive coccus that almost exclusively affects humans and can induce non-invasive and invasive illness, as well as non-suppurative sequelae. This includes pharyngitis, scarlet fever, streptococcal toxic shock syndrome, acute rheumatic fever, post-streptococcal glomerulonephritis [12], superficial skin infections (impetigo), and deep skin infections, such as erysipelas and cellulitis” [13].

Dillenia indica, commonly known as Elephant Apple [14], is a tropical tree that grows in tropical regions [15]. It is a member of the Dilleniaceae family. It's a huge evergreen shrub with spreading branches and thick bark that reaches 6-15 meters tall [16]. Various portions of *D. indica* have traditionally been used to treat indigestion, asthma, influenza, diarrhea, jaundice, weakness, and rheumatic pain. In the indigenous system of medicine, the plant's leaf, bark, and fruit are used. It has a variety of therapeutic benefits, including pain relief and body temperature regulation [17].

Ficus exasperata, popularly known as sand paper tree, is a member of the Moraceae family [18]. “*Ficus exasperata* is found in all types of vegetation in West Africa. It grows up to 20-30 meters tall as a deciduous shrub or small medium-sized tree. Alkaloids, flavonoids, tannins, saponins, and cyanogenic glycosides have been discovered in the leaves and stem bark of *F. exasperate*” [19]. Coughs, digestive problems, colics, bleeding, ulcers, wounds, bacterial, fungal infections, and other diseases have all been reported to be treated using *Ficus exasperata* leaf extract [20]. *Ficus exasperata* has been shown to have anti-hypertensive, antioxidant, anti-inflammatory, anti-

ulcer, anti-lipidic, anti-bacterial, and anti-fungal properties. The objective of the study was to identify the phytochemicals in the extracts of *Dillenia indica* and *Ficus exasperata* and to examine their antibacterial effect on clinical bacterial isolates *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes*

2. MATERIALS AND METHODS

Plants were collected from the Botanical Garden of University of Ibadan, Ibadan Nigeria. The test organisms used for this study were bacteria strains. Clinical isolates of two (2) Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and one (1) Gram negative bacteria (*Escherichia coli*) were collected from Nigerian Institute of Medical Research (NIMR), Lagos, and incubated in the incubator at 37.0°C.

2.1. Preparation of Extracts

Preparation of the extracts were done according to [21]. Fresh leaves of *Dillenia indica* and *Ficus exasperata* were harvested and dried in the shade for two weeks. The dry leaves of the plants were finely grounded with blender so as to release the phytochemicals in the leaves and of the plant. The finely grounded powder of the plant were weighed and thereafter poured into beakers. The mouths of the beakers were wrapped with aluminum foil in order to prevent external contamination. The beakers were labeled appropriately using a masking tape. 50 g of the grounded plant material was soaked in 400 ml of distilled water for 72 hrs. The solution was sieved using Cheese Cloth to remove debris, after which the solution was centrifuged using the Centrifuge, and thereafter filtered using Whatman No 1 Filter Paper. The plant extract solution (100%) was diluted appropriately with water to give 75%, 50%, and 25% concentrations of the aqueous extracts while distilled water served as control. Hot water extract was prepared by boiling distilled water to obtain hot water extract of the different concentrations. The resulting

extracts were stored in the refrigerator to prevent fungal attack. The procedure described above for the preparation of aqueous extract was carried out for methanolic and ethanolic extracts using the organic. The plant extract solution (100%) was diluted appropriately with water to give 75%, 50%, and 25% concentrations of the methanolic extracts while distilled water served as control. The ethanolic and methanolic extracts were concentrated at 40 °C using a rotary evaporator.

2.2. Phytochemical screening of *Dillenia indica* and *Ficus exasperata* plant extracts

“Phytochemical screening for alkaloids, phenols, glycosides, flavonoids, saponins, terpenoids, quinones and anthraquinones were carried out according to the methods of”[22, 23].

2.3. Antibacterial activity of the plant extracts

“The antibacterial activity of the plant extracts was carried out according to the agar well diffusion assay by [24] against two Gram positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and one Gram negative (*Escherichia coli*) clinical isolates bacteria. Agar-diffusion tests are often used as qualitative methods to determine whether a bacterium is resistant or susceptible”.

The agar diffusion seeded plate method was employed to assess the antimicrobial activity of *Dillenia indica* and *Ficus exasperata* extracts. Peptone Broth was prepared according to the manufacturer’s instruction, and it was used to grow the organism. Bijou bottles containing freshly prepared Peptone Broth were cooled to 45 °C and inoculated aseptically with 0.1 ml of the test organisms and thereafter incubated at 37 °C for 24 hrs. Sterile Petri dishes were prepared and labelled for each extract and organism. Mueller Hinton Agar was prepared according to the manufacturer’s instruction. The agar was poured into the petri dishes aseptically and was allowed to solidify. A standardized concentration of inoculum with fixed volume was spread evenly on the surface of the gelled agar plate. A sterile cork borer was used to bore 6mm-8mm diameter

holes (wells) on the solidified seeded agar plates. A micropipette was used to introduce 0.1 ml of different concentration of extracts into the bored wells. The plates were left for about 30 minutes to allow for diffusion of the extract into the agar and incubated at 37 °C for 24 hrs. The diameters of zone of inhibition produced by the extracts were measured and their mean values were reported.

2.4. Antibiotic sensitivity test

The antimicrobial discs used for this research were the products of Celtech Diagnostic, Belgium Inc. The modern antibiotics discs employed were Amoxicillin Clavulanate (30µg), Gentamycin (10µg), Cefotaxime (25µg), Cefuroxime (30µg), Ceftriaxone Sulbactam (45µg), Cefexime (5µg), Ofloxacin (5µg), Ampiclox (10µg), Imipenem (10µg), Nitrofurantoin (300µg), Nalidixic Acid (30µg), Levofloxacin (5µg), Ciprofloxacin (5µg), Azithromycin (15µg), and Erythromycin (10µg) for the bacteria.

“The antibiotic susceptibility testing procedure employed was according to” [25]. The test organism was emulsified in peptone water until the turbidity was comparable with 0.5% McFarland’s standard. A loopful of the suspension was transferred onto a Mueller Hinton agar plate, and then a sterile cotton swab was used to streak the entire surface of the plate. Sterile forceps were used to apply the antibiotic discs to the surface of the agar plate and incubated at 37 °C for 18-24 hrs. Zone diameters around the antibiotic discs were measured. Isolates that were resistant to 3 or more antibiotics were labeled as multidrug resistant (MDR).

2.5. Statistical analysis

The data obtained were analyzed Analysis of Variance (ANOVA) to determine significant ($P < 0.05$) effects. The significant differences between means were determined using Duncan’s

Multiple Range Test DMRT. The result of the study was presented as Mean \pm Standard Error of the Trials.

3. RESULTS

In the present study, the phytochemicals occurring in the various solvent extracts of *Dillenia indica* and *Ficus exasperata* (aqueous, methanolic and ethanolic extracts) were analyzed qualitatively by phytochemical screening. The phytochemical screening indicated the presence of alkaloids, flavonoids, glycosides, saponins and quinones in methanolic and ethanolic extracts. Phenols and glycosides were present in the aqueous, methanolic and ethanolic extracts whereas, terpenoids were absent in all the extracts. (Tables 1 and 2)

Tables 3 and 4 show the antibacterial activity of different concentrations of *Dillenia indica* and *Ficus exasperata* extracts respectively. The highest zone of inhibition for *Escherichia coli* was recorded in ethanolic extract with a zone diameter of 17.66 mm at 100%, while the lowest zone of inhibition was recorded in distilled water extract (Table 3). The highest zone of inhibition for *Staphylococcus aureus* was seen in ethanolic extract with a zone diameter of 17.66 mm, followed by methanolic extract with zone of inhibition of 17.33 mm, while the least zone of inhibition was recorded in hot water extract. The highest zone of inhibition for *Streptococcus pyogenes* was recorded in methanolic extract with zone diameter of 29.00 mm, followed by ethanolic extract with zone of inhibition of 17.66 mm. Methanolic extract of *Ficus exasperata* had the highest zone of inhibition with diameter of 16.66 mm on *E. coli*, while the highest zone of inhibition for *S. aureus* was recorded for ethanolic extract with zone diameter of 18.33 mm, followed by methanolic extract with a diameter of 12.33 mm. For *S. pyogenes*, the highest zone of inhibition was recorded in methanolic extract with zone diameter of 16.00 mm, followed by ethanolic extract with diameter of 12.66 mm (table 4)

When the activity of the plant extract was compared with that of the standard antibiotics used in this work, it was observed that the **plant extracts** compared favorably with those of these standard antibiotics as seen in Table 5.

Table 1: Phytochemical **screening** of *Dillenia indica* **plant extract**

Phytochemicals	Hot Water Extract	Distilled Water Extract	Methanolic Extract	Ethanollic Extract
Alkaloids	–	–	+	+
Phenols	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	–	–	+	+
Terpenoids	–	–	–	–
Saponins	–	–	–	+
Quinones	–	–	+	+
Anthraquinones	–	+	–	–

KEY: + indicates the presence of the phytochemical compound

– indicates the absence of the phytochemical compound

Table 2: Phytochemical screening of *Ficus exasperata* plant extract

Phytochemicals	Hot Water Extract	Distilled Water Extract	Methanolic Extract	Ethanollic Extract
Alkaloids	–	+	+	+
Phenols	–	+	+	+
Glycosides	+	+	+	+
Flavonoids	–	–	+	+
Terpenoids	–	–	–	–
Saponins	+	+	–	+
Quinones	–	–	+	–
Anthraquinones	–	–	+	–

+ indicates the presence of the phytochemical compound

– indicates the absence of the phytochemical compound

Table 3: Antibacterial activity of *Dillenia indica*

<i>Dillenia indica</i>		<i>E. coli</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
Extracts	Extract Concentration	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)
Hot Water Extract	100%	11.66 ± 0.88	-	-
	75%	9.33 ± 1.76	-	-
	50%	8.66 ± 1.33	-	-
	25%	5.00 ± 2.64	-	-
Water Extract	100%	-	3.00 ± 3.00	-
	75%	-	2.66 ± 2.66	-
	50%	-	-	-
	25%	-	-	-
Methanolic Extract	100%	13.00 ± 0.57	17.33 ± 0.33	29.00 ± 0.577
	75%	11.33 ± 1.20	14.00 ± 1.00	17.00 ± 1.52
	50%	10.00 ± 1.15	11.33 ± 0.66	12.00 ± 2.00
	25%	8.33 ± 0.66	8.00 ± 1.15	7.33 ± 0.88
Ethanollic Extract	100%	17.66 ± 3.71	17.66 ± 0.33	17.66 ± 3.17
	75%	13.33 ± 2.84	9.00 ± 4.58	6.33 ± 3.17
	50%	5.66 ± 2.96	3.66 ± 3.66	3.00 ± 3.00

	25%	-	-	2.66 ± 2.66
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Table 4: Antibacterial activity of *Ficus exasperata*

<i>Ficus exasperata</i>		<i>E. coli</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
Extracts	Extract Concentration	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)
Hot Water Extract	100%	9.00 ± 5.77	1.66 ± 1.66	8.66 ± 1.33
	75%	4.66 ± 2.60	-	5.66 ± 2.84
	50%	2.66 ± 2.66	-	2.66 ± 2.66
	25%	-	-	-
Water Extract	100%	-	-	8.66 ± 4.66
	75%	-	-	5.00 ± 5.00
	50%	-	-	3.66 ± 3.66
	25%	-	-	-
Methanolic Extract	100%	16.66 ± 3.28	12.33 ± 1.45	16.00 ± 3.05
	75%	3.00 ± 3.00	-	-
	50%	-	-	-
	25%	-	-	-
Ethanollic Extract	100%	4.00 ± 4.00	18.33 ± 6.00	12.66 ± 6.74
	75%	-	8.00 ± 4.16	3.33 ± 3.33

	50%	-	3.66 ± 3.66	-
	25%	-	3.33 ± 3.33	-

Table 5: Antibiotic susceptibility pattern of Gram-Negative and Gram-Positive clinical isolates

Bacterial Isolates	Zone of Inhibitions by Antibiotics (mm)											
	LBC	ZEM	ACX	CRO	NA	CXM	OFX	IMP	CTX	AUG	GN	NF
Gram Negative												
<i>E. coli</i>	25.00 (0.00)	20.00 (1.00)	9.33 (4.04)	10.33 (0.59)	9.33 (5.13)	1.00 (1.73)	(15.66) (4.04)	(1.00) (1.73)	R	5.66 (5.13)	7.66 (0.57)	3.66 (1.15)
Gram Positive												
<i>S. aureus</i>	17.00 (2.08)	5.33 (1.50)	10.67 (2.96)	3.00 (0.00)	6.00 (1.00)	R	R	13.67 (0.66)	18.33 (0.88)	3.00 (1.73)	14.66 (1.76)	R
<i>S. pyogenes</i>	14.33 (1.15)	1.00 (1.73)	5.00 (0.00)	1.00 (1.73)	R	R	R	10.66 (0.57)	9.00 (3.60)	7.66 (6.42)	19.33 (1.15)	10.33 (8.96)

Amoxicillin Clavulanate (AUG), Gentamycin(GN), Cefotaxime(CTX), Cefuroxime (CXM), Ceftriaxone Sulbactam (CRO), Cefexime (ZEM), Ofloxacin (OFX), Ampiclox (ACX), Imipenem/Cilastatin (IMP), Nitrofurantoin (NF), Nalidixic Acid (NA), Levofloxacin (LBC), Ciprofloxacin (CIP), Azithromycin (AZN), and Erythromycin (ERY)

4. DISCUSSION

The major phytochemicals found in the extracts were phenols, saponins, flavonoids, glycosides, quinones, anthraquinones and alkaloids. “Quinones are important naturally occurring pigments which are widely distributed and known to exhibit a variety of physiological activities as antimicrobial and anticancer compounds. Several benzoquinones, naphthoquinones, and anthraquinones have shown antiviral and antibacterial activities” [26]. “Several studies have reported antibacterial activity of flavanonoids. For example, the result of in vitro investigation of flavanonoids from *Paulownia tomentosa* fruits showed strong antibacterial activity against Gram positive bacteria” [27]. “It has also been demonstrated that flavonoid from the stem bark of medicinal plant inhibit activity of *E. coli* [28]. [29] reported excellent activity of flavonoids isolated from *Combretum apiculatum* toward *S. aureus*”. “In another study, this compound from the leaves of *Cryptocarya chinensis* was potent against *Mycobacterium tuberculosis* [30] demonstrated that flavonoids have promising antibacterial activities when used alone or in combination with conventional antibiotics. Flavanoids from the *Mundulea sericea*, have been reported to have significant antibacterial activity against *S. aureus*” [31]. [32,33] “showed antibacterial activity of flavonoids against *E. coli* and *S. aureus*. [34] reported that *Dorstenia* species deactivated *S. aureus* via depolarization of membrane and inhibition of DNA, RNA, and protein synthesis”. According to these authors, this compound rapidly reduced the bacterial cell density and caused lysis of *S. aureus*.

“The result showed that phytochemicals in the different extracts varied with extraction solvent. This was consistent with the observation of [35] who indicated that the recoveries of bioactive phytochemical compounds from plants are potentially affected by the conditions of extraction methods and different solvent formulations”. “Other investigators also asserted that the concentration of these compounds, which may or may not be detected in a phytochemical analysis, depends on the nature of the chemical used as solvent in the extraction process, as well the growth and storage conditions” [36]. Based on all these reasons which may affect the detection of these compounds in the plants, it is therefore not surprising that some of these compounds were not detected in some extracts. The inhibitory effects of these medicinal plants on the microorganisms may therefore, be due to the presence of the above phytochemical components.

“In this study, the presence of saponins, glycosides, alkaloids, phenols and flavonoids in the methanolic and ethanolic extract of *Ficus exasperata* leaves is in line with earlier reports by” [37 – 39].

“Extensive works have been carried out on the antibacterial activities of plant extracts [40]. The results of the antibacterial activity showed that the extracts had varying degrees of activity against the test isolates used in this study”. [41, 42] reported that the antibacterial activity of the plant extracts may be due to the presence of metabolic toxins and broad spectrum antimicrobial compounds that may act against bacteria. “The ethanolic extract of the stem bark of *Ficus exasperata* has been reported to have a broad spectrum of activity against Gram-positive and Gram-negative bacteria as well as the fungus *Candida albicans*” [43]. “The antibacterial activity of *S. aureus* in this study is in accordance with a previous study. For example, it has been reported that the ethanolic extract of *Berberis hispanica* roots was active against *S. aureus*” [44].

“The sensitivity of *E. coli* confirmed the activities obtained in previous screening against the *E. coli*” [45]. The extracts were observed to inhibit the growth of both Gram positive and Gram negative bacteria, and thus showed it possess a broad spectrum activity. When the activity of the plant extract was compared with that of the standard antibiotics used in this work, it was observed that the plant extract compared favourably with those of these standard antibiotics.

5. CONCLUSION

In this study, the extracts of the plants had antibacterial activities against the clinical isolates used.

The extract was observed to inhibit the growth of both Gram positive and Gram negative bacteria, supporting the significant use of plant extracts in treating infections related to these bacteria. The plant extracts should be studied further as future alternatives to control diseases associated with common pathogenic bacteria.

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