Phytochemical analysis and comparison of in-vitro antimicrobial activities of the leaf, stem bark and root bark of *Ficus benghalensis*

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Abstract: In this study, the antimicrobial activities of aqueous extracts of leaf, bark and root bark of Ficus benghalensis plant were investigated against five bacteria (E.coli, S.aureus, B.subtilis, K.pneumoniae and P.aeruginosa and two fungi (T.rubrum and C.albicans). The results showed that the stem bark extract showed maximum antimicrobial activity on the five bacteria and two fungi used, K.pneumoniae and P.aeruginosa were resistant to the leaf and root extracts. C.albicans was also susceptible to all the extracts while T.rubrum was resistant to the leaf extract. Phytochemical screening showed the presence of Tannins, Flavonoids, Phenols, Saponins, Glycosides, Xanthoproteic, Carbohydrates and Alkaloids. There were various zones of inhibition in the antibiotics sensitivity of the bacteria used, while some were susceptible, others were not. The two fungi were also susceptible to Nystatin. The positive results for antimicrobial activities of the extract confirm the use of the plant in folk medicine in treatment of diverse ailments.

Keywords: Ficus benghalensis, phytochemical constituents, antimicrobial efficacy, dimethyl sulfoxide (DMSO), antibiotics

I. INTRODUCTION

Herbal medicine is the study and use of medicinal properties of plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions^[1] and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work^[2]. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.

In 2001, researchers identified 122 compounds used in modern medicine which were derived from ethno medical plant sources^[3], 80% of these compounds have had an ethno medical use identical to the current use of the active synthetic drugs^[4]. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, and quinine, opium. The use of herbs to treat disease is almost universal among non-industrialized societies^{[5], [6]} and is often more affordable than purchasing expensive modern pharmaceuticals. Researchers found that people in different parts of the world tended to use medicinal plants for medical purposes. In the early 19th century, when chemical analysis first became available ^{[7], [8]}, scientists began to extract and modify the active ingredients from plants. Later, chemists began making their own version of plant compounds and, over time, the use of herbal medicines declined in favour of drugs. Almost one fourth of pharmaceutical drugs are derived from botanicals.

The plant is a large evergreen tree distributed all over Southern and Eastern part of Nigeria but most common in deciduous forest South West. It is a grown in gardens and road sides for shades^[9]. It is a member of four sacred trees meant to be planted around the home and temples. It is found throughout the year, grows in evergreen except in dry localities where it is a leafless for a short time. It is hardy and drought-resistant; it withstands mild frost. It is epiphytic when young. It develops from seeds dropped by birds on old walls or on other trees and is therefore, considered destructive to forest trees, walls and buildings^[10]. The tree is commonly known as 'Opoto' in Yoruba, 'Banyan' tree in English, 'Bar' in Hindi and as 'Avaroha' in Sanskrit. The species of four *Ficus* yielding latex consist of Nyagrodha (*Ficus bengalensis*), Udumbara (*Ficus glomerata/Ficus racemosa*), Plaksha (*Ficus lacor/Ficus retusa*) and Ashvattha (*Ficus religiosa*)^[11] the bark and leaves of this group are used as astringent, haemostatic, anti-inflammatory, anti-septic; prescribed in diarrhoea, dysentery, and in the treatment of skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia, deficient lactation^[12]. A

very large tree up to 30m in height leaves spirally arranged on branchlets up to 10-30cm long and 7- 20cm wide, coriaceous, elliptic to ovate, apex obtuse, base rounded, with 5-7 basal nerves; petioles 2.5-5 cm long; stipules stout ^[13]. Fruits receptacles sessile, axillary in pairs 1.3-1.9 cm in diameter globose silky pubescent, scarlet or brick red when ripe; bracts 4-5, copular, 6mm, shortly connate, obtuse persistent and sepals 3-5, Male flowers dispersed with female, stamen 1, another oblong, parallel, unequal, and shortly mucronate. Ovary-obovoid globose, 1.5×1 mm, style erects or curved, tapering, gall flowers similar to female, pedicellate, achnes globose-ellipsoid, 2×1.5 mm dark brown flowers during the summer and fruits the rainy season. Smooth bark, light grey-white, 1.27cm thick wood moderately hard, grey or grayish-white ^[14], ^[15]. In the traditional system of medicine, the plant is used for various health problems and diseases.

Taxonomic classification of Ficus benghalensis

Kingdom: *Plantae* Sub Kingdom: *Tracheobiota* Super division: *Spermatophyta* Division: *Magnoliophyta* Class: *Magnoliopsida* Subclass: *Hamamelidae* Order: *Urticales* Family: *Moraceae* Genus: *Ficus* Species: *Ficus benghalensis*

II. MATERIALS AND METHOD

Collection of the plant materials

The plant materials were collected in November 2012, at Okada in Edo State, Nigeria. The leaves, barks and roots were identified and authenticated by Mr. B.U Ikhile, Department of Phamacognosy, Igbinedion University Okada.

Preparation of the materials: - The materials were initially separated from the main plants body, rinsed with distilled water and air dried at room temperature in laboratory and then homogenized into fine particles and stored in air tight bottles and was used for all the extraction process.

Extract Preparation

Each of the dried pulverized powder (leaf, stem-bark and root-bark) was weighed (600g) into a large beaker and filled up with 2000ml of cold water and covered, it was left un-interrupted for 24hrs, it was sieved using muslin cloth followed by sterile filter paper into a sterile conical flask, the solution was subjected to hot air water bath evaporation at 35°C, the crude extract was obtained and stored at 4°C for further use ^[16].

The percentage yield of the crude aqueous extracts is calculated as follows % yield = $\frac{\text{weight of extract}}{\text{weight of sample}} \times 100$

Leaf = $\frac{12.47g}{600g} \times 100$, Bark = $\frac{16.90g}{600g} \times 100$, Root = $\frac{14.32g}{600g} \times 100$

A. PHYTOCHEMICAL SCREENING OF PLANTS

Phytochemical analysis of plants was carried out for all the extracts as per the standard methods.

1. Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered ^[17]. Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The yellow coloured precipitate was indicated that presence of alkaloids.

2. Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered it. The filtrates sample was used to test for the presence of carbohydrates. Filtrates samples were hydrolyzed with the dilute HCl, and neutralized with alkali then heated with Fehling's A and B solutions^[18]. The red coloured precipitate indicated that presence of reducing sugars.

3. Detection of glycosides:

Sample extracts were hydrolyzed with the dilute HCl, and then subjected to test for glycosides.

4. Detection of steroids and terpenoids

In 1 ml of methanol plant extract 1ml of chloroform was added and 2-3 ml of acetic anhydride was mixed then 1-2 drops of concentrated H_2SO_4 was added. Then dark green colouration of the solution indicated that the presence of steroids and pink or red colouration of the solution indicated that presence of terpenoid.

5. Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes only. Then formation of 1 cm layer of foam indicated that presence of saponins. Foam Test: 0.5g of plant extracts was shaken with 2 ml of water. The foam was produced persists for 10 minutes it was indicated that the presence of saponins.

6. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. The bluish black colour was indicated that presence of phenols.

7. Detection of proteins and amino acids

Xanthoproteic Test: The extracts were treated with few drops of concentrated nitric acid. Then the formation of yellow colour indicated that presence of proteins.

8. Detection of flavonoids

In Methanol extract 10% NaOH was added and dilute HCl was added to that solution. The change of colour from yellow to colourless provides the positive result.

B. ANTI-MICROBIAL TEST

The microbial strains are standard which were obtained from University College Hospital, Ibadan. The bacterial strains studied were *Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*. The fungi used were *Candida albicans* and *Trichophyton rubrum*

Bacterial cultures

The overnight bacterial culture used was prepared by sub-culturing, a loopful was streaked with a sterile wire loop and inoculated into 5ml nutrient broth present in the test tubes and were incubated at 37° C for 24 hrs.

Fungal cultures

Fungal culture was prepared by streaking from fungal slopes with the aid of a sterile loop and inoculated into 5ml Tryptone soy broth present in the test tubes and incubated for 48 hrs.

Preparation of Bacterial seeded plate

Dilution of 0.2ml of 10^{-6} each of these cultures was introduced into 20ml molten Mueller Hilton agar in the bottles, it was mixed well and poured into plates and allowed to set. Four equidistant ditches were made on each plate of bacteria with the aid of cork borer {6mm}, three wells were used for methanol extract (leave, stem-bark and root-bark) and the fourth well serves as control. This procedure was duplicated for the aqueous extract

Preparation of Fungal seeded plate

Dilution of 0.2ml of 10^{-6} each of the two cultures was introduced into 20ml molten potato dextrose agar in the bottles, it was mixed well and poured into plates and allowed to set. Four equidistant ditches were made on each plate of fungi with the aid of cork borer {6mm}, three wells were used for methanol extracts (leave, stem-bark and root-bark) and the fourth well serves as control. This procedure was done in pairs for the aqueous extract

Antimicrobial screenings

The crude extract was reconstituted with 30mg/ml, each extract was screened for antimicrobial activity using the seeded bacterial and fungal plates. The method employed was agar diffusion ^[19] technique in which the anti-microbial activities of different extracts from different partitions were placed in plates containing a single

microorganism. 40μ l of the extract was placed in the well with the use of pasture pipette. The plates were left on the bench for an hour at room temperature to allow diffusion of the extract into the agar. The plates were incubated at 37°C for 24hrs for bacteria and 30°C for 48hrs for fungi. The negative control used was DMSO^[20] for both fungi and bacteria and 50µg/ml for Nystatin. After specified time of incubation, the plates were brought out of incubator and the diameter of zone of inhibition was measured with ruler calibrated in millimeters.

Antibiotic sensitivity test

To determine the antibacterial activity of standard antibiotics, the disc diffusion method was applied. Paper disc impregnated with antibiotics of known concentrations was placed on the surface of agar plate inoculated with the test organism with the aid of sterile forceps. The plates were incubated at 37°C for 24hrs, the zone of inhibition was later observed around the disc.

RESULTS AND DISCUSSION

ANTIMICROBIAL SENSITIVITY TEST

Table III shows the inhibitory activity of the leaf, bark and root bark of *Ficus benghalensis* on the bacteria at 30mg/ml. The bark of the plant had more potency on bacteria than the other parts (i.e. leaf and root). *S. aureus* had the highest zone of inhibition in the bark and leaf. For the DMSO, there was no zone of inhibition on any of the bacteria used. *Klebsiella pneumoniae* had the lowest zone of inhibition in leaves and root bark extract.

ANTIFUNGAL SENSITIVITY TEST

Table IV shows the inhibitory activity of the leaf, bark and root bark extracts of *Ficus benghalensis* on the fungi used at 30mg/ml. The extracts also showed better potency on the fungi. No zone of inhibition in leaf aqueous extract of Trichophyton rubrum

ANTIBIOTIC SENSITIVITY TEST

The inhibitory effect of standard antibiotic disc of various concentrations tested on the bacteria was shown in Table V

PHYTOCHEMICAL SCREENING OF EXTRACT

Table II below indicates the phytochemical groups present in each sample

Table I PERCENTAGE EXTRACT YIELD

The percentage yield of the extracts

EXTRACT	LEAF (%)	BARK (%)	ROOT (%)
AQUEOUS	2.078	2.817	2.387

TABLE: II PHYTOCHEMICAL ANALYSIS OF THE EXTRACTS

Group	Leaf	Bark	Root
Alkaloids	+	-	+
Carbohydrates	+	+	-
Glycosides	+	+	+
Terpenoids	+	+	+
Saponins	+	+	+
Phenols	+	+	+
Xanthoproteic	+	+	-
Flavonoids	+	+	+
Tannins	+	+	+

KEYS

+: Present -: Absent

Table III ANTIBACTERIAL SENSITIVITY TEST OF THE EXTRACTS

AQUEOUS INHIBITION ZONE (MM)							
EXTRACT	LEAF	BARK	ROOT				
E. coli	10	19	15				
S. aureus	12	24	8				
P. aeruginosa	R	10	R				
B. subtilis	12	17	12				
K. pneumoniae	R	14	R				

Note: Sensitivity at 30mg/ml

ANTIFUNGAL SENSITIVITY TEST OF THE EXTRACTS Table IV

	INHI			
EXTRACT	LEAF	BARK	NYSTATIN	
C. albicans	8	12	10	16
T.rubrum	R	10	8	14

KEY

Nystatin=30µg

R = **R**esistant

TABLE V ANTIBIOTICS SENSITIVITY TEST ON BACTERIAL ISOLATES

a) For Gram positive bacteria

Antibiotics										
Bacteria	Bacteria PEF CN APX Z AM R CPX S SXT E							Е		
S.aureus	R	R	R	R	R	R	7	R	R	R
B.subtilis	6	R	R	R	R	5.5	5.5	5	R	R

b) For Gram negative bacteria

Antibiotics										
Bacteria	PEF	CN	SP	AU	AM	СН	CPX	S	SXT	OFX
E.coli	6.5	4	8	R	5	R	7	5.5	R	7.5
P.aeruginosa	8.5	5.5	6.5	R	4.5	R	9.8	R	6.4	5
K.pneumoniae	5.4	6.8	7.8	R	5	R	8.4	5	R	6.9

KEY

R= **Resistance**

Full names of the antibiotics with their concentrations

	ANTIBIOTICS	Conc. (Gram +ve)	Conc. (Gram -ve)
PEF	Pefloxacin	10µg	30µg
CN	Gentamycin	10µg	10µg
APX	Ampiclox	30µg	
SP	Sparfloxacin		10µg
Ζ	Zinacef	20µg	
AU	Augmentin		30µg
AM	Amoxacillin	30µg	30µg
R	Roceptin	25µg	
СН	Chloramphenicol		30µg
CPX	Ciprofloxacin	10µg	10µg
S	Streptomycin	30µg	30µg
SXT	Septrin	30µg	30µg
Е	Erythromycin	10µg	
OFX	Tarivid		10µg

Phytochemical screening the extracts of leaf bark and root bark of *F.bengalensis* has been summarized in **Table II**. Tannins, Flavonoids, Phenols, Saponins and Glycosides were present in all the extracts. Xanthoproteic and Carbohydrates were present leaf and bark. The Alkaloids were present in leaf and root. This conforms to research demonstrated by Babu *et al.* (2010), in preliminary phytochemical screening of *F*.

bengalensis^[21]. They showed the presence Tannins, Saponins, Flavonoids, Terpenoids in all parts of *F.bengalensis* Alkaloids and

The leaf and root extracts had no antibacterial activity against *P.aeruginosa and K. pneumonia*, from the five bacterial strains investigated; the most susceptible bacterial strains were *S.aureus* and *B.subtilis. E.coli* was less susceptible. Gram-negative bacterial strains were more resistant than Gram-positive bacterial strains. Similarly, both the methanol and aqueous extracts had inhibitory activity against two fungi (*C.albicans* and *T.rubrum*) except for the aqueous leaf extract that was resistant to *T. rubrum*.

Most of the antibiotics were inhibitory on the test organisms except Septrin, Chloramphenicol and Augmentin on Gram negative. However, on Gram positive, *S. aureus* showed resistance to all antibiotics except Ciprofloxacin. On the other hand, *B. subtilis* was susceptible to Pefloxacin, Roceptin, Ciprofloxacin and Septrin. [22]

CONCLUSION

Ficus benghalensis is an important medicinal plant that has a number of bioactive compounds. The aqueous extracts of the plant have revealed some potential phytochemicals like alkaloids, glycosides, Terpenoids, flavonoids, and tannins. The extracts had antimicrobial activity against selected microorganisms with the bark having the highest potency, this is very similar to commercial antibiotics indicating that bioactive compound from the plant can be used for the production of new antibiotics. However, it is imperative that more clinical and pharmacological studies should be conducted to investigate the unexploited potential of this plant.

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