

Phytochemical and Anti-bacterial Studies of the fruit extract of *Ficus benjamina* (L.)

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Abstract— This work aimed at identifying the phytochemical composition of *Ficus benjamina* and to test the various extract fractions against some selected gram positive and negative bacteria. Fresh fruits of *Ficus benjamina* (L) were collected from Labo Tarkar's mosque reserve area of Katsina local government area, Katsina State, Nigeria. 1.4 Kg of the air dried and powdered fruit was percolated with ethanol (5.0dm³) for two weeks. It was filtered and concentrated on rotary evaporator at 40°C to yield crude extract (23.79g). This extract was fractionated by maceration using chloroform, ethyl acetate and acetone yielding their soluble fractions with weights of 13.32g, 2.42g and 3.26g respectively. The fractions were subjected to various phytochemical tests. Ethanol fraction contains saponins, flavonoids etc; Chloroform fraction contains alkaloids, flavonoids etc; Ethyl acetate fraction contains alkaloids, tannins etc; Acetone fraction contains alkaloids, saponins etc; aqueous fraction contains flavonoid, tannins etc. The fractions were further subjected to the antimicrobial screening. Ethanol fraction possessed highest zone of inhibition of 23mm against *Streptococcus pyogenes* at 250mg/ml concentration, followed by ethyl acetate fraction with 20mm against *Streptococcus pyogenes* at 250mg/ml concentration, followed by acetone fraction with 19mm against *Streptococcus pyogenes* at 250mg/ml concentration and the least zone of inhibition was on ethanol fraction with 6mm against *Pseudomonas auriginosa* at 250mg/ml concentration. It was observed that different classes of secondary metabolites were detected from different fractions of *Ficus Benjamina*. Also a remarkable inhibition was recorded against *Streptococcus pyogenes* from the ethanol fraction which further justify the ethanol medical claim of the plant.

Index Terms— Anti-microbial assay, *F. benjamina*, Maceration, Phytochemicals, percolation

1. INTRODUCTION

According to online medical dictionary, natural products are naturally occurring compounds that are end products of secondary metabolism; often, they are unique compounds for particular organisms or classes of organisms.

The productive source of new compounds and drugs of natural origin are the medicinal plants, most of the natural products isolated from medicinal plants are secondary metabolites, which include alkaloids, tannins, flavonoids, steroids, terpenoids, phenylpropanoids[1] (Harvey, 2008) and anthraquinones[2] (Ayo, 2010). Some of the products have nutritive value, antifungal and antibacterial activities [3](Prasad and Bisht, 2011). It is clear that plants play a vital role in our lives, with the primary use of serving as food and source of medicine.

Infectious diseases of microbial origin caused by *Staphylococcus aureus*, *Bacillus cereus*, *Shigella spp*, *Salmonella typhi*, *Klebsiella pneumonia*, *Escherichia coli* constitute the major causes of morbidity and mortality in several countries [4](Kloos and Keim, 1993). Such microbial infections could lead to diarrhea, pneumonia, typhoid fever, among others [5](Oladiji, *et al.*, 2012).

Medicinal higher plants have been used extensively as a source for numerous active constituents for treating human diseases and they as well, have high content of therapeutic value [6] (Nostro, *et al.*, 2000). The *in vitro* anti-bacterial or antifungal assay is the first aim to evaluate the importance of these plants since the antibiotic resistance has become a global concern [7] (Westh, *et al.*, 2004).

2.0 Experimental details

2.1 Extraction

The fruits were air dried and ground using mortar and pestle. 1.4 kg of the powdered fruits was percolated with 96% ethanol (4.9 dm³) for two weeks [8] (Fatope, *et al.*, 1993). It was decanted and evaporated to dryness using a rotary evaporator (R110) at 40°C. The crude residue was weighed (23.79 g), it was labeled as F₁ and kept in a freezer.

2.2 Fractionation of Ethanol Fraction (F₁):

The dried ethanol fraction (F₁), was macerated with Chloroform in parts using 20 cm³ each, four times. The chloroform soluble fraction was filtered and evaporated on rotary evaporator (R110) at 40°C to afford Chloroform soluble fraction, F₂. The chloroform insoluble fraction was then macerated with ethylacetate in parts using 20 cm³ each, four times to obtain the ethyl acetate soluble fraction F₃. It was filtered and evaporated to dryness on rotary evaporator (R110) at 40°C. The ethyl acetate insoluble fraction was macerated four times in parts using 20 cm³ of acetone each. The acetone soluble fraction F₄ was also evaporated to dryness on rotary evaporator (R110) at 40°C. The weight, texture and color of fractions are shown in Table 1.

2.3 Phytochemical Analysis

The fractions were subjected to phytochemical screening, to determine the classes of secondary metabolites present in the plant materials. These include phenol glycosides, anthraquinone glycosides, flavonoids, saponins, tannins, and alkaloids (Table 2).

2.3.1 Test for Phenolic Glycosides

3 drops of 500mg/ml each fraction was added to 3 drops of 1M sulfuric acid in a boiling tube, a red colour was observed which disappeared upon addition of 2 cm³ distilled water. This confirms the presence of phenolic glycoside.

2.3.2 Test for Anthraquinone Glycosides

5 drops of 500 mg/ml of each fraction was boiled with 1ml of 0.1M sulphuric acid for 5 minutes and then filtered while hot. The filtrate was cooled and shaken with equal volume of dichloromethane. The dichloromethane layer was then separated and shaken with about 3 cm³ of 1M ammonia. A reddish-pink colour was observed; this confirmed the presence of anthraquinones.

2.3.3 Test for Flavonoids

Each fraction (2.0 g) was dissolved in 50% methanol (2 cm³) by heating. Magnesium metal (0.01 g) and concentrated hydrochloric acid 50% (5 drops) were added. Appearance of a red colour indicates the presence of flavonoids.

2.3.4 Test for Saponins

Each fraction (0.5g) was vigorously shaken with 5 cm³ distilled water and allowed to stand for a while. A persistent frothing indicates the presence of saponins.

2.3.5 Test for Tannins

Each fraction (0.5g) was treated with 5% FeCl₃ (3 drops). A dark black colored precipitates in a very dark solution, which turns green-black to blue-black coloration on dilution indicates the presence of tannins.

2.3.6 Test for Alkaloids

Each fraction (0.5 g) was stirred with 1% HCl (5 cm³) on steam bath. The solution was cooled and filtered. The filtrate 1 cm³ was treated with 2-3 drops of Dragendoff's reagent. Formation of an orange red precipitate/turbidity with Dragendoff's reagent indicates the presence of alkaloids.

2.4 Antimicrobial Screening

2.4.1 Preparation of Test Solution and Disc Concentration

The stock solutions were prepared by dissolving 0.5 g of each extract in 2 cm³ of the DMSO. For each fraction, concentrations of 250 mg/disc, 125 mg/disc, and 62.5 mg/disc were prepared separately in sterilized Bijour bottles containing 100 sterile improvised whatman No. 1 filter paper discs that have absorbance potency of 0.01 cm³ in each case. The filter papers were impregnated with plant extract of desired concentration. The serial dilution was as follows:

2.4.2 Concentration of 250 mg/disc:-

From the stock solution, 1 cm³ was taken into a bijour bottle containing the discs and labeled 250 mg/disc.

2.4.3 Concentration of 125 mg/disc:-

1 cm³ of DMSO was added into the remaining stock solution making 2 cm³; 1 cm³ was taken into another bijour bottle containing the discs and was labeled 125 mg/disc.

2.4.4 Concentration of 62.5 mg/disc:-

1 cm³ of DMSO was further added to the remaining stock solution, making the volume 2 cm³ again, from which 1 cm³ was taken and transferred into another bijour bottle containing the discs and was labeled 62.5 mg/disc.

2.4.5 Preparation of 30µ/ml Chloramphenicol (control)

0.03 mg was weighed and dissolved in 1 ml injection water to afford 30µ/ml

2.4.6 Organisms Used

Two gram positive organisms, *Streptococcus pyogenes*, *Staphylococcus aureus*, and two gram negative organisms, *Escherichia coli*, and *Salmonella typhi*, were used. The test microorganisms were clinical isolates obtained from Microbiology Department of Aminu Kano Teaching Hospital, Kano, which were further identified and confirmed in the Microbiology department, Bayero University, Kano.

2.4.7 Inoculums Standardization

As described by standard sensitivity test [9] (National Committee for Clinical Laboratory Standards, NCCLS, 2000), loops of the confirmed isolates were introduced in Peptone water in separate sterilized bottles and kept overnight in an incubator (37 °C). Few colonies of the overnight growth of the isolates to be tested were dispersed in sterile normal saline to form a turbid culture suspension that match 0.5 McFarland turbidity.

2.4.8 Preparation of Media

Nutrient Agar (12 g) was dissolved in 400 cm³ of distilled water, according to manufacturer's instructions. The conical flask containing the nutrient agar solution was autoclaved for 15 minutes at 121°C. About 20 cm³ of the sterilized nutrient agar was transferred into petri-dishes under septic condition. The petri-dishes were allowed to cool and solidify. Control antibiotic (chloramphenicol) was obtained from a pharmaceutical store to serve as control.

2.4.9 Antimicrobial Susceptibility Test (AST)

Antibacterial activities of the fractions were determined using disc diffusion method of antimicrobial susceptibility test as described by [9] (National Committee for Clinical Laboratory Standards, NCCLS, 2000). Standardized inocula of the isolates were swabbed onto the surface of the solidified and oven-dried nutrient agar in separate petri-dishes under sterilized environment. The prepared discs of the different concentrations of the extracts were then placed onto the surface of the inoculated media at intervals. The positive control discs (chloramphenicol) were placed at the center of the bacteria inoculated media. The plates were incubated for 24 hours at 37 °C for bacteria cultures, after which the antimicrobial activity was observed by measuring the diameter of the clear inhibition zone around the discs and the values obtained recorded in mm.

3. Results and Discussion

The physical characteristics of the fruit extracts of *Ficus benjamina* was determined. Table 1 shows the characteristics.

Table 1
Weight, texture and appearance of *Ficus benjamina* fruit extracts

Fractions	Weight (g)	Texture	Appearance
F ₁	23.79	Gummy	Dark brown
F ₂	13.32	Gummy	Light brown
F ₃	2.42	Gummy	Dark brown
F ₄	3.26	Gummy	Dark brown

- F₁ – Ethanol extract
- F₂ – Chloroform fraction
- F₃ – Ethyl acetate fraction
- F₄ – Acetone fraction

3.1 Results OF Phytochemical Analysis

The phytochemical screening of the various fractions of the fruit extracts of *F. benjamina*, revealed the presence of alkaloids, tannins, saponin, flavonoids, anthraquinone glycoside and phenol glycosides (Table 2). Ethanol fraction contains alkaloids, tannins, saponins, flavonoids, anthraquinone glycosides and phenolic glycoside; Chloroform fraction contains alkaloids, flavonoids, anthraquinone glycosides and phenolic glycosides; Ethyl acetate fraction contains alkaloids, tannins, flavonoids, anthraquinone glycosides and phenolic glycosides; Acetone fraction contains alkaloids, saponins, flavonoids, anthraquinone glycosides and phenolic glycosides; aqueous fraction contains alkaloids, tannins, flavonoids, anthraquinone glycosides and phenolic glycosides.

Table 2
Result of Phytochemical screening of the various fractions of *Ficus benjamina*

Fractions	Alkaloids	Tannins	Saponin	Flavonoids	Anthraquinone Glycoside	Phenol glycosides
F ₁	+	+	+	+	+	+
F ₂	+	-	-	+	+	+
F ₃	+	+	-	+	+	+
F ₄	+	-	+	+	+	+
F ₅	+	+	-	+	+	+

- F₁ – Ethanol extract
- F₂ – Chloroform fraction
- F₃ – Ethyl acetate fraction
- F₄ – Acetone fraction
- F₅ – Aqueous fraction

Antimicrobial screening was carried out on all the four fractions against some selected gram positive bacteria (*Streptococcus pyogenes*, and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*.) The ethanol extract (F₁), chloroform (F₂), ethyl acetate (F₃) and acetone (F₄) of the fruits have showed remarkable zones of inhibition. Ethanol fraction has 6 mm, 10 mm and 12 mm zones of inhibition against *Escherichia coli* at (62.5, 125 and 250) mg/ml concentrations respectively; 15mm and 23 mm against *Streptococcus pyogenes* at (125 and 250) mg/ml concentrations respectively; 6 mm and 9 mm against *Staphylococcus aureus* at (125 and 250) mg/ml concentrations respectively; and 6 mm against *Pseudomonas aeruginosa* at 250 mg/ml. Chloroform fraction has zero (0 mm) zone of inhibition against *Escherichia coli* at all the three concentrations; 14 mm and 14 mm against *Streptococcus pyogenes* at (62.5 and 125) mg/ml concentrations respectively; 10 mm and 12 mm against *Staphylococcus aureus* at (62.5 and 125) mg/ml concentrations respectively; and zero (0 mm) zone of inhibition against *Pseudomonas aeruginosa* at all the three concentrations. Ethyl acetate has 10 mm and 11 mm zones of inhibition against *Escherichia coli* at (125 and 250) mg/ml concentrations respectively;

Table 3
Results of antimicrobial test

Isolates	Zone of inhibition against fractions (mm)														
	Ethanol (mg/ml)			Chloroform (mg/ml)			Ethyl acetate (mg/ml)			Acetone (mg/ml)			Chloramphenicol (mg/ml)		
	62.5	125	250	62.5	125	250	62.5	125	250	62.5	125	250	62.5	125	250
	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)
E. coli	6	10	12	-	-	-	-	10	11	-	10	14	-	-	25
Strep.	-	15	23	14	14	-	-	16	20	13	13	19	-	-	27
Staph.	-	6	9	10	12	-	9	10	10	-	10	12	-	-	24
Pseudo.	-	-	6	-	-	-	-	-	-	-	-	10	10	-	19

- E. coli = *Escherichia coli*
- Strep.= *Streptococcus pyogenes*,
- Staph.= *Staphylococcus aureus*,
- Pseudo = *Pseudomonas aeruginosa*,
- Chloramphenicol (control)

16 mm and 20 mm against *Streptococcus pyogenes* at (125 and 250) mg/ml concentrations respectively; 9 mm, 10 mm and 10 mm zones of inhibition against *Staphylococcus aureus* at (62.5, 125 and 250) mg/ml concentrations respectively; and zero (0 mm) zone of inhibition against *Pseudomonas auriginosa* at all the three concentrations. Acetone has 10 mm and 14 mm zones of inhibition against *Escherichia coli* at (62.5 and 250) mg/ml concentrations respectively; 13 mm, 13 mm and 19 mm zones of inhibition against *Streptococcus pyogenes* at (62.5, 125 and 250) mg/ml concentrations respectively; 10 mm and 12 mm zones of inhibition against *Staphylococcus aureus* at (125 and 250) mg/ml concentrations respectively; 10 mm and 10 mm zones of inhibition against *Pseudomonas auriginosa* at (125 and 250) mg/ml concentrations respectively. The potency was comparable to the control antibiotic, chloramphenicol possessing inhibitions against *Escherichia coli* of 25 mm, *Streptococcus pyogenes* of 27 mm, *Staphylococcus aureus* of 24 mm and *Pseudomonas auriginosa* of 19 mm respectively at 30 mg/ml.

4.0 Conclusion

The results of phytochemistry showed that the plant is bioactive. The results of anti-microbial assay also showed that the microorganisms were inhibited at higher concentrations.

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