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A Preliminary Study on the Effect of Aqueous and Ethanolic Extracts of *Ficus benjamina 'variegata'* Fruits on Some Bacterial Isolates

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ABSTRACT: Aqueous and ethanolic extracts of *Ficus benjamina 'variegata'* fruits were investigated for antibacterial effects on *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klesiella pneumonia* and *Pseudomonas aeruginosa*. The minimum inhibitory concentrations (MIC) and minimum bacteriocidal concentrations (MBC) of the extracts were determined using test tube dilution susceptibility test and agar-well diffusion method respectively. The aqueous extract of *F. benjamina* fruits contained saponin and steroids while the ethanolic extract was made up of alkaloids, flavonoids, glycosides, phenolics, saponins and steroids. The aqueous extract of the fruit showed no activity on all the test organisms whereas the ethanolic extract inhibited all the test organisms. The MIC and MBC of the ethanolic extract on the test organisms ranged from 62.5 – 250 mg/ml and 125 – 500 mg/ml. The zone of growth of inhibition varied from 6 - 14.33mm. Overall, the study revealed that both the aqueous and ethanolic extracts of *Ficus benjamina 'variegata'* fruit did not possess antibacterial activities, notwithstanding the MBC and MIC displayed by the ethanolic extract which is not in the pharmacological range. Therefore, both the aqueous and ethanolic extracts of *F. benjamina* fruits should not be explored as antibacterial agent.

Keywords: *Ficus benjamina*, Antibacterial activity, Moracea, Minimum Inhibitory Concentration, Minimum Bacteriocidal Concentration

Introduction

Ficus benjamina (family-Moraceae), commonly known as weeping fig or Benjamina's fig (English), native to the tropics of South Africa, South East Africa, Australia, tropical Central and West Africa, Central and West Africa. It is an evergreen tree with dropping branches ranging from 6.10 m to 18.29 m high and a width of about 21.34 m (Frith *et al*., 1976). These trees are known for their tropical-looking foliage and pear-shaped edible fruits which are favourite of some birds, such as the superb fruit-dove, wompoo, orange-bellied fruit-dove (Frith *et al*., 1976).

There is folklore claims on the use of *Ficus benjamina 'variegata*' fruit in the killing of pathogens. This claim, however, has not been substantiated or refuted by scientific report. Therefore, this study was aimed at providing information on the effect of the solvent extracts (aqueous and ethanolic) of *Ficus benjamina 'variegata'* fruits on some bacterial strains.

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Materials and Methods

Test organisms

Pure cultures of *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Klebsiella pneumonea* and *Pseudomonas aeuruginosa* were obtained from the Department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria. The organisms were maintained on nutrient agar slants at 4^oC.

Plant material

Ficus benjamina 'variegata' fruits, collected from Government Reserved Area (GRA), Ilorin, Nigeria were identified at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Reagents and chemicals

The nutrient agar, peptone water and Muellar-Hinton agar were products of Biotec Laboratories Ltd., United Kingdom.

Preparation of aqueous extract

The fruits were washed in running tap water, oven dried at 40^oC for 72 h and pulverized in an electric blender. A portion (100 g) of the powder was extracted in 1000 ml of distilled water for 48hrs with constant shaking on an orbital shaker. This was then filtered through a Whatmann No. 1 filter paper to obtain the filtrate. The filtrate was concentrated on an electrothermal water bath to give a residue of 30.01 which corresponds to a yield of 3.00%. The residue was reconstituted in distilled water to give the required concentrations used in this study.

Preparation of ethanolic extract

A portion (100 g) of the powder was extracted in 1000 ml of 70% ethanol in a screw-cap bottle. This was stirred intermittently over a 72-hour period after which it was filtered through Whatmann No. 1 filter paper. The filtrate was concentrated using a rotary evaporator maintained at 40^oC to give 20.06 g of the residue which corresponds to a % yield of 2.01%. This was reconstituted to give the required concentrations used in this study.

Preparation of other chemicals

They were prepared according to the procedures outlined by the manufacturers.

Qualitative phytochemical screening

The aqueous and ethanolic extracts of *Ficus benjamina* fruits were screened for alkaloid (Harbone, 1973); steroids, anthraquinones, cardenolides and dienolides (Trease and Evans, 1989); saponins (Wall *et al*., 1954), phenolics and flavonoids (Awe and Sodipo, 2001); cardiac glycosides (Sofowora, 1993); tannins and triterpenes (Odebiyi and Sofowora, 1978).

Preparation of Inocula and Growth Media

The method described by Barry and Thornsberry (1991) was used. Briefly, pure cultures of isolates from overnight nutrient agar culture plate were transferred into each of the test tubes containing sterile 1 ml peptone water. The organisms were standardized by visual comparison of its turbidity with that of McFarland 0.5 standard of 1.5×10^8 bacterial suspension per ml, prepared by adding 0.5 ml of 1.175% (w/v) barium chloride to 99.5 ml of 1% sulphuric acid.

Antimicrobial susceptibility test

(A) Determination of Minimum Inhibitory Concentration

This was carried out on both the aqueous and ethanolic crude extracts. In the determination of Minimum Inhibitory Concentration (MIC), the peptone water test tube dilution susceptibility test was performed using the method described by Barry and Thornsberry (1991). Briefly, about 15 g of both extracts were diluted to give a 'neat' (100%) concentration. 1 ml of 'neat' concentration of both extracts was introduced into 1 ml of standardized inoculated peptone water to give 500 mg/ml concentration. This was serially diluted to give the desired concentrations of 15.62mg/ml, 31.25mg/ml, 62.50mg/ml, 125mg/ml and 250 mg/ml. The positive control tube

contained 1 ml each of standardized inoculated peptone water and diluent (distilled water or 70% ethanol) depending on the extract used while the negative control contained only 1 ml of peptone water which was inoculated. This was carried out in triplicates on all the five isolates. All the tubes containing the isolates were then incubated for 24 h at 37^{o}C after which they were examined for growth. The least concentration of the extract that showed no growth (evidenced by absence of turbidity) was recorded as the MIC.

(B) Determination of the Minimum Bacteriocidal Concentration and the Zones of Inhibition

This was done by adopting the ditch plate method described by Barry and Thonsberry (1991). All the test tubes that showed no growth were then sub-cultured in Petri dishes on Muellar-Hinton agar medium to determine the Minimum Bacteriocidal Concentration (MBC). The control test tubes were also sub-cultured. Sterile molten Muellar-Hinton agar was dispensed into Petri dishes in the inoculating room and allowed to solidify. The test organisms were then streaked on the surface of each solid agar plate in a form that will produce confluent growth. After inoculation, three wells of 6 mm diameters were punched on each Petri dish with sterile cork borer. A known volume (0.2 ml) of the extract of known concentrations was dispensed into each well; another well containing 0.2 ml of the positive control while the third well was designated as the negative control. The experiment was carried out in triplicates. The plates were again incubated for 24 h at 37^{0}C after which the zones of inhibition were measured. The presence of zones of inhibition was taken as an indication of antimicrobial action while the absence of any measurable zone of inhibition was interpreted as no case of antimicrobial action. Antimicrobial activity was expressed in terms of average diameter of zones of inhibitions computed using the expression:

Zone of inhibition (mm) due to antimicrobial action alone = (x - 6)

where: x (mm) = observed zone of inhibition
6 (mm) = diameter of the cork borer

The least concentration for each test organism that showed zones of inhibition was regarded as the MBC.

Results

Saponins and steroids were detected in the aqueous extract of *F. benjamina 'variegata'* fruit whereas alkaloids, saponins, cardenolides and dienolides, phenolics, cardiac glycosides, flavonoids and steroids were present in the ethanolic extract.(Table 1).

The aqueous extract of *F. benjamina 'variegata'* fruits at the concentrations investigated in present study did not inhibit the growth of any of the test organisms as turbidity was observed in all the set-up. In contrast however, there was absence of turbidity at some concentrations of the ethanolic extract. The zone of inhibition produced by the ethanolic extract of *F. benjamina 'variegata'* fruits varied for *S. aureus* (6-10.67 mm), *S. typhi* (6-14.33 mm), *E. coli* (6-13.33 mm), *K. pneumonia* (6-8.33 mm) and *P. aeruginosa* (6-7.67 mm) (Table 2).

The MIC for the test organisms produced by the ethanolic extract ranged from 62.5-250 mg/ml whereas the MBC varied from 125-500 mg/ml (Table 3). Again, *S. typhi* was the most sensitive among the test organisms to the ethanolic extract whereas *P. aeruginosa* was the least sensitive. The ethanolic extract was also bacteriostatic at concentrations lower than that at which they are bactericidal.

Table 1: Phytochemical constituents of aqueous and ethanolic extracts of *Ficus benjamina 'variegata'* fruits

	Phytochemicals	***Ethanolic Extract***	**Aqueous Extract**
1	Alkaloids	+	-
2	Tannins	-	-
3	Anthraquinones	-	-
4	Saponins	+	+
5	Cardenolides & Dienolides	+	-
6	Phenolics	+	-
7	Cardiac Glycosides	+	-
8	Flavonoids	+	-
9	Triterpenes	-	-
10	Steroids	+	+

\+ Present
\- Not detected

Table 2: Zones of inhibition on the microorganisms by the ethanolic extract of *Ficus benjamina 'variegata'* fruits
Zones of inhibition (mm)

Groups	***Staphylococcus aureus***	***Salmonella typhi***	***Escherichia coli***	***Klebsiella pneumonea***	***Pseudomonas aeuruginosa***
Positive Control	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00
Negative Control	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00
62.50 mg/ml	-	6.00± 0.00	6.00± 0.00	-	-
125 mg/ml	6.00± 0.00	9.60± 0.33	9.33± 0.33	-	-
250 mg/ml	9.33± 0.33	12.67± 0.33	11.67± 0.33	6.00± 0.00	6.00± 0.00
500 mg/ml	10.67± 0.33	14.33± 0.33	13.33± 0.33	8.33± 0.33	7.67± 0.33

Each value is a mean ± SEM of three determinations

Table 3: Minimum Inhibitory Concentration and Minimum Bacteriocidal Concentration of the ethanolic extract of *Ficus benjamina 'variegata'* fruits on the test organisms

Parameters	***Staphylococcus aureus***	***Salmonella typhi***	***Escherichia coli***	***Klebsiella pneumonea***	***Pseudomonas aeuruginosa***
Minimum Inhibitory Concentration (mg/ml)	125.00	62.50	62.50	250.00	250.00
Minimum Bacteriocidal Concentration (mg/ml)	250.00	125.00	125.00	500.00	500.00

Discussion

Plant-based *in vivo* research has made significant rewarding progress in many important areas such as development of cancer (Harvey, 1999), male sexual dysfunction (Yakubu *et al*., 2005), antibiotics (Adebayo *et al*., 1989), and is still contributing to the search for more botanicals with antibacterial activity. For example, several studies had implicated plants with antibacterial or antimicrobial substances/activities, although, these activity varies with the extracts (Zaria *et al*., 1995, Ibekwe *et al*., 2001, Akujobi *et al*., 2004). This study revealed that ethanol extracted more of the phytoconstituents of *F. benjamina* than water and thus showed more effect on the isolate than the water extract. The differences in the effect of the solvent extracts appear to be related to qualitative and quantitative diversity of compounds that might have been extracted (Geyid *et al*., 2005). The stronger extraction capacity of organic solvents had earlier been documented, which could have produced a greater number of phytoconstituents responsible for the effect in the present study (Parekh and Chanda, 2006). This is also an indication that the antibacterial bioactive agents are soluble only in non-polar organic solvent as against the polar solvent (Glasby, 1991). The findings in this study is in accordance with those of Majekodunmi *et al* (1996) and Martinez *et al* (1996) that a high activity of extractable natural products was obtained in ethanol compared with aqueous extracts. In addition, the report of Brain and Turner (1975) indicated that the efficacy of an extraction procedure depends on the accessibility of the constituents to the solvent. According to many workers, water extract usually show little or no activity against bacteria (Aliero and Afolayan, 2006; Koduru *et al*., 2006). This study therefore has also complemented such findings.

Infectious diseases of microbial origin caused by *Staphylococcus aureus, Bacillus cereus, Shigellia spp, Salmonella typhi, Klebsiella pneumonia, Escherichia coli* constitute the major causes of morbidity and mortality in several countries (Kloos and Kein, 1993). Such microbial infection and pathophysiology of water and electrolyte transport could lead to diarrhea, pneumonia and typhoid. Although the extracts exhibited some effects on the different bacterial strains in the present study as evidenced by the MIC and MBC, the concentrations at which the extract was able to induce its activity are not in the pharmacological range. Yakubu *et al* (2012) had reported that a good antimicrobial agent should demonstrate activity at concentration range of 0.1-10 mg/ml. Although, the extract most especially the ethanolic showed some antibacterial activity, the use of concentrations outside the pharmacological range may not warrant equivocal pronouncement as an antibacterial agent. The antibacterial activity of the extract was concentration dependent with bacteriostatic effect occurring at concentrations lower than bacteriocidal.

Overall, this study has substantiated the folkloric antibacterial use of *Ficus benjamina* fruits only at concentrations higher than the pharmacological range. It has also revealed that the antibacterial activity resides in the ethanolic extract and not in the aqueous extract. Work is still in progress on evaluating the activity within the pharmacological range.

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