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Phytochemical and Antibacterial Activity of Extracts of *Carica Papaya*

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Abstract

Ethanol and water extracts of powdered leaves and roots of *Carica papaya* were screened for basic phyto compounds and antibacterial activity using disc diffusion method against *Escherichia coli*, *Pseudomonas auruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. Preliminary phytochemical analysis showed positive test for saponins, tannins, flavonoids, cyanogenic glycosides in water extract and tannins, flavonoids and alkaloids showed positive in ethanol. Ethanol extracts of leaves inhibited the growth of *Salmonella typhi* at concentration of 18.80 and water extract 13.5 (mean zone of inhibition). The extracts demonstrated high activities against *Staphylococcus typhi*. The minimum inhibitory concentration (MIC) of the extracts ranged between 50 – 300 mg/ml, but MIC value ranging from 200 – 300 mg/ml showed more activity against the test organisms. The results of ethanol extracts from leaf showed that lead extracts are more effective against the test organisms than the root extracts.

Keywords: *Carica papaya*, Phytochemical and Antibacterial.

1.0 Introduction

The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academic, since many infectious agents are becoming resistant to synthetic drugs (Lathan and Kannabiran, 2006). Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths everyday (Ahmed and Beg, 2001). The situation has further been complicated by the rapid development of multidrug resistance by the micro-organisms to the antimicrobial agents available. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs (Pretorious and Watt, 2001). The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Africa (Bibitha *et al.*, 2002). *Carica papaya* (family, Caricaceae), commonly called paw paw (English), "Ibebe" (Yoruba), "Okroegbe" (Igbo), is a monosexual plant of Central American origin. Besides the fruits being edible, they have been reported along with the roots and leaves to be of medicinal value (Thomas, 1989). The latex from the leaves has been used as antihelminths and for the treatment of infections of bacterial origin (Fajimi *et al.*, 2001). Following the trend in research in medicinal plants, this study seeks to evaluate the antibacterial activities of leaf and root extracts of *Carica papaya* against some bacteria and to determine the chemical constituents that may be present in the extracts.

2.0 Material and Methods

2.1.1 Plant Material

The plant material was obtained from the Horticulture Unit of the National Root Crops Research Institute, Umudike, Abia State.

2.1.2 Test Organisms

The clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi* used in this study were obtained from the Federal Medical Centre, Owerri, Nigeria.

2.1.3 Preparation of Plant Extracts

The cold extraction method described by Harborne (1973) was adopted. 50 g of the powdered plant samples (leaves and roots) were soaked in 500 ml of ethanol. The mixture was agitated in a mechanical shaker over night. The extract was filtered and concentrated using a water bath and transferred to

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a soxhlet apparatus. The filtrate (crude extract) was then scooped into a small sample bottle for sensitivity test. The same procedure was followed for water using fresh samples.

2.1.2 Phytochemical Screening

i. Test for Saponins

a. *Frothing Test*: 5 g of the samples was diluted with 4 ml of distilled water and the mixture shaken vigorously and observed on standing for stable froth as evidence for the presence of saponins.

b. *Emulsion Test*: 2 drops of olive oil were added to the frothing solution and shaken vigorously to observe emulsion which indicates the presence of saponins.

ii. Test for Alkaloids

5 g each of the sample was boiled with 5 ml of 2 % hydrochloric acid on a steam bath. 1 ml portion of the filtrate reacted with 2 drops of the following reagents:

a. Wagner's reagent (iodine in potassium iodide solution) and observed for reddish brown precipitate. The presence of this indicates the presence of alkaloids.

b. Dragendoiff's reagent (Bismutt potassium iodide solution) and observed for orange colour precipitate, which indicates the presence of alkaloids.

iii. Tannins

Tannins was determined according to Trease and Evans (1996). 5 g of the sample was boiled in 20 ml of 45 % ethanol for 5 mins. The mixture was cooled and used for analysis.

Ferric Chloride Test: 1 ml of filtrate was distilled with 2.0 ml of distilled water and 2 drops of ferric chloride solution added and observed for transient greenish to black colour, which indicates presence of tannins.

iv. Cyanogenic Glycosides

The presence of cyanogenic glycosides was tested with alkaline and paper. 5 g of each sample was mixed with distilled water in a conical flask. A picrate paper (filter paper soaked in alkaline picrate solution) was filtered over the soaked sample in the flask and left to stand till the next day, and examined for colour change which was indicative of the presence of cyanogenic glycosides.

v Starch

The test for the presence of starch in the test sample was done using iodine test. 5 g of the sample was disposed in water and drops of iodine solution were added to each solution (extract). The presence of a blue black precipitate was recorded as indicative of the presence of starch.

vi Steroids

The test for steroids was done by the Liberman acid test. A portion of the organic extract (ethanol extract) was treated with drops of acetic anhydride. Concentrated H_2S_4 was then carefully added by the side of the test tube. The presence of a brown colour at the boundary of the mixture was taken as an indication of the presence of steroid (see Trease and Evans, 1996).

2.2 Determination of Antibacterial Activity

The test for sensitivity of each organism was done by the disc diffusion technique (Cheesebrough, 2000). A number of the sterile paper discs were mixed completely with a reconstituted extract in a sterile 10 ml glass beaker. The discs were allowed to remain in contact with the extract for at least an hour to enable them absorb the extract which became embedded on the discs. Each organisms was cultured by the spread plate technique (Pellezar and Chan, 1977). With a flamed wire loop, an inoculums was aseptically transferred from the pure slant culture of the organisms to a sterile Agar plate. The inoculum was spread evenly over the surface of the medium with the sterile glass hockey. Then using a flamed pair of forceps, the prepared sensitivity test discs were carefully picked and placed on top of the inoculated plate at some distance from one another. The plates were allowed to stand for about 5 minutes and incubated at 37^{o}C in an electronic incubator. They were observed daily for 24 to 48 hours for growth and possible clear zone around the disc as a mark of sensitivity to the test extract.

2.3 Determination of Minimum Inhibitory Concentration (MIC) Extract

The minimum inhibition concentration was deter-

mined as the least concentration of the extract which inhibited each test organism. Each extract from water and ethanol was separately reconstituted in sterile distilled water and diluted to concentrations of choice (50, 100, 200, 300 mg/ ml). The regenerative concentrations were used in the preparation of test discs (for sensitivity test). After incubation, the plates were observed for inhibition zones. The least concentration which caused inbibition was the minimum inhibition concentration.

3.0 Results

The average percentage yield of water and ethanol extracts of the leaf of root samples of *Carica papaya* was 2.276 and 2.764d respectively for both water and ethanol. The % yield for root was 2.302 in distilled water and 3.016 in ethanol (see Table 1). The roots therefore yielded slightly more than the leaves in both solvents, while in ethanol, the yield was more in root and leaf samples. Leave extracts in water gave positive results for tannins, saponins, flavonoids, cyanogenic glycosides (see Table 2), alkaloids and steroids were however absent from the extract of the plant sample. The ethanol extract also gave positive result for tannin, flavonoid, alkaloid while saponin and cyanogenic glycoside were absent in the sample. However, starch was present in both water and ethanol extracts of roots. Ethanol leaf extracts were most effective and showed the highest activity against *Salmonella typhi* (18.80 $\pm$ 1.13) mean zone of inhibition), followed by (13.50 $\pm$ 0.00 mean zone of inhibition) by *Pseudomonas aeruginosa*. Leaf water extract showed activity against *Salmonella typhi* (13.5 $\pm$ 0.70) more than the other test organisms. From the results of the antibacterial screening of ethanol and water extracts (Table 4). The root extracts on water showed activity against *Salmonella typhi* (10.50 $\pm$ 0.12 mean zone of inhibition) more than other test organisms while *Salmonella typhi* (15.00 $\pm$ 0.24 mm mean zone of inhibition) was more sensitive to ethanol extract of root sample. The minimum inhibitory concentration of the active principles against test organisms ranged between 200 – 300 mg/ml for leaf extract (water) and 50 – 300 mg/ml for ethanol extract against *Pseudomonas aeruginosa* and *Salmonella typhi*, while the other test organisms were sensitive to MIC value ranging from 200 – 300 mg/ml. similarly, root extracts in water and ethanol showed MIC value of 50 – 300 mg/ml against *Salmonella typhi* only, while the other test organisms showed activity from MIC value, ranging from 100 – 300 mg/ml.

Table: Percentage Yield of Extracts

Samples	w1	w2	w2-w1	% Yield
Leaf (distilled water)	49.672	50.810	1.138	2.276
Leaf (ethanol)	49/677	51.059	1.138	2.764
Root (distilled water)	49.674	50.825	1.151	2.302
Root (ethanol)	48.568	50.076	1.508	3.016

4.0 Discussion

The importance of phytochemical constituents in plants has been a subject of discussion amongst the intellectuals and traditional medicine practitioners. The pharmaceutical quality and the vast spectrum of some plant active principles have been reported by Umar *et al.*, (2000). Doughari *et al.*, (2007) in his studies reported on the medicinal values of these plants which are corroborated by the results of this study. The higher susceptibility of the best organisms to ethanol extracts is not surprising as previous

Table 2: Phytochemical Screening of *Carica papaya* (Root and Leaves)

Samples	Tannin	Saponin	Flavonoid	Alkaloid	Cyangenic Glycoside	Steroids	Starch
Leaves (water)	+ve	+ve	+ve	+ve	+ve	-ve	-ve
Ethanol	+ve	+ve	+ve	+ve	-ve	+ve	-ve
Roots	+ve	+ve	+ve	-ve	+ve	-ve	+ve
Ethanol (water)	+ve	+ve	+ve	+ve	-ve	-ve	+ve

Key: ***+ve*** **-** ***Present*** ***-ve*** **-** ***Negative***

studies have reported ethanol to be a better solvent than water (Obi and Onuoha, 2000). The activity and spectrum of extracts as a result of the nature of solvents lends more weight to the findings of Obi and Onuoha (2000), who reported high recovery of alkaloids and essential oils with ethanol than with water. Thus, it could be that some of the active principles responsible for some of its medicinal properties may not be extractible using water as solvent and also it may be due to better solubility of the active components in organic solvents eg ethanol (de Boer, *et al.*, 2005). The test organisms were susceptible to the extracts but the gram-negative organisms (*Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli*) were more susceptible to the extracts than the gram-positive (*Staphylococcus aureus*). This result is in agreement with earlier report by Doughari *et al.*, (2007). The activity of the extracts was comparable to those of antibiotics. The fact that the extracts were active against both gram positive and gram-negative bacteria tested may indicate a broad spectrum activity. The low MIC value observed for *Salmonella typhi* is a good indication of the efficacy against this bacterium. This outcome is remarkable considering the fact that typhoid fever, caused by *Salmonella typhi* is on the rise and also becoming resistant to other antibiotics for its treatment in Nigeria. The antibacterial mechanism of the test plant extracts is not fully understood, but like other antibacterial or antimicrobial agents, their effectiveness may be associated with the damage of cell wall or membrane, inhibition of protein synthesis, inhibition of nucleic acids, and replication of the pathogens.

5.0 Conclusion

This study supports the traditional application of the plant and suggests that the plant extract possess compounds with properties that can be used as antibacterial agents for the treatment of gastro enteritis, typhoid fever and wound infections. Further work should be embarked upon to determine its toxicological effects and its antimicrobial mechanism.

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