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Full Length Research Paper

Studies on the antibacterial activity of root extracts of *Carica papaya* L.

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The bioactive compounds of root extracts of *Carica papaya* L. were extracted, using water and organic solvents, and were investigated for antibacterial activity against some pathogenic bacteria using the cup plate agar diffusion method. The aqueous extracts did not show significant activity, but the organic extracts had significant activity with the methanol extracts demonstrating the highest activity against the test bacteria. The extracts demonstrated higher activities against all the gram-negative bacteria than the gram-positive bacteria tested, with the highest activity (14 mm zone of inhibition) demonstrated against *Salmonella typhi*. Increase in temperature enhanced the activity of the extracts, while alkaline pH decreased the activity. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts ranged between 50-200 mg/ml. Preliminary phytochemical analyses showed that the extracts contain alkaloids, tannins, saponins, glycosides and phenols. *Carica papaya* may be used for the treatment of gastroenteritis, uretritis, otitis media, typhoid fever and wound infections.

Key words: *Carica papaya* L., antibacterial activity, phytochemical analysis, antibiotics.

INTRODUCTION

The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs (Latha and Kannabiran, 2006). Infectious diseases are the world's major threat to human health and account for almost 50 000 deaths every day (Ahmad and Beg, 2001). The situation has further been complicated with the rapid development of multidrug resistance by the microorganisms 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 Asia, Latin America and Africa (Bibitha et al., 2002). *Carica papaya* L. (Family Caricaceae), commonly called pawpaw (English), Ibebe (Yoruba–Nigeria) or Okroegbe (Igbo–Nigeria), 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. The latex from the leaves has been used as antihelmints and for the treatment of infections of bacterial origin (Fajimi et al., 2001). This study was there-fore designed in order to investigate the antibacterial acti-vity of the root extracts of *C. papaya* against some infec-tious bacteria and to determine the chemical constituents that may be present in the extracts.

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MATERIALS AND METHODS

Processing of plant samples

Plant materials were collected from the wild in Yola North local government area of Adamawa state, Nigeria and were identified and authenticated at the Biological Sciences Department, Federal University of Technology Yola, Adamawa State, Nigeria. The fresh roots were harvested and properly washed in tap water (H_2O), and then rinsed in sterile distilled H_2O. The roots were divided into two equal parts; one part was dried in the hot air oven at 40°C for 3 days, while the second portion was blended fresh using an electric blender. The dried roots were pulverized, using sterile laboratory mortar and pestle, to obtain a powered form. These were stored in airtight glass containers protected from sunlight until required for analysis.

Table 1. Results of phytochemical analysis of root extracts of *Carica papaya.*

Phytoconstituent	Methanol extract	Hot water extract	Cold water extract
Saponins	++	++	-
Alkaloids	++	-	-
Tannins	++	-	-
Glycosides	-	++	++
Phenols	++	-	-

Key: ++ → present; - → absent

Bacterial isolates

Isolates of *Staphylococus aureus, Streptococcus pyogenase, Streptococcus pneumoniae, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi* and *Shigella flexneri,* were all clinical isolates obtained from the Specialist Hospital Yola, Adamawa State, Nigeria. Purity plates of each of the bacterial isolates were obtained by culturing on their respective selective media. Biochemical tests were performed to re-identify and confirm the identity of the isolates. Fresh plates of the test bacteria were made from the isolate cultures obtained on agar slants. Discrete colonies of fresh cultures of the different bacterial isolates were then picked and suspended in 5 ml Nutrient broth (NB, Oxoid), in well-labeled sterile Bijou bottles, and incubated for 24 h at 37ºC prior to antimicrobial susceptibility testing.

Extraction of plant material

Cold and hot extraction with H_2O, and soxhlet extractions with methanol (99%) and acetone, as described by Junaid et al. (2006), was carried out. Twenty (20) g of each sample was weighed into 100 ml of the solvent (water, methanol and acetone). For cold extraction the samples and solvent were stirred every 30 min for 3 h and allowed to stand for 24 h, while for hot extraction the samples and solvent were heated for 30 min and stirred every 30 min for 3 h and allowed to stand 24 h. After preparation of the crude extract as described, the organic extracts were diluted using 50% dimethylsulphoxide (DMSO), while the aqueous extracts were reconstituted using sterile distilled H_2O to obtain concentrations of 200, 150, 100 and 50 mg/ml.

Phytochemical screening

This was done on the different extracts to ascertain the presence of bioactive components present in *C. papaya* roots. The presence of alkaloids, resins, saponins, glycoside, tannins, flavonoids, cardiac glycoside, steroidal terpenes, anthraquinones and carbohydrates were determined, as described by Jigna et al. (2006).

Determination of antimicrobial activity

Antibacterial activity of the aqueous and organic extracts of the plant sample was evaluated by the cup plate agar diffusion method (Aida et al., 2001). The bacterial cultures were adjusted to 0.5 McFarland turbidity standard and inoculated onto Mueller Hinton agar (MHA, Oxoid) plates (diameter: 15 cm). A sterile cork borer was used to make a well (6 mm in diameter) on the MHA plates. Aliquots of 100 µl of extract dilutions, reconstituted in 50% DMSO (for organic extracts) and distilled water (for aqueous extracts) at concentrations of 200, 150, 100 and 50 mg/ml, were applied in each of the wells in the culture plates previously seeded with the test organisms. The cultures were incubated at 37°C for 24 h. A well was made in each of the culture plates and filled with 20 µl of 10 mg/ml of ciprofloxacin and streptomycin as positive controls, and sterile filter paper soaked in sterile glycerol served as a negative control. Antimicrobial activity was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For each extract, three replicate trials were conducted against each organism.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate in test tubes. To 0.5 ml of varying concentrations of the extracts (5, 25, 50, 75, 100, 125, 150, 175 and 200 mg/ml) in test tubes, Nutrient broth (2 ml) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin and streptomycin). A tube containing Nutrient broth only was seeded with the test organisms, as described above, to serve as controls. The culture tubes were then incubated at 37°C for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile Nutrient agar by streaking. Nutrient agar plates only were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37°C for 24 h. After incubation the concentration at which no visible growth was seen was noted as the Minimum Bactericidal Concentration (MBC).

Effect of temperature and pH on antimicrobial activity of extracts

Five milliliters of 100 mg/ml of methanol extracts were constituted in test tubes and treated at 4°C in the refrigerator, and at 60 and 100°C in water bath for 30 min and then tested for antibacterial activity as described earlier.

To determine the effect of pH, methanol extracts in test tubes were treated at a pH of 2.5, 5 and 10, using 1 N HCl and 1 N NaOH, respectively, for 30 min. After 30 min of treatment, the extracts were neutralized (pH 7) using 1 N HCl and 1 N NaOH, respectively, and then tested for antimicrobial activity as described earlier.

RESULTS

Table 1 shows the results of the preliminary phytochemi-

Table 2. Antibacterial activity of root extracts of *C. papaya* on the test organisms.

Organism	Extract (mg/ml) / Zone of inhibition (mm)														
	50			100			150			200			250		
	WE	AE	ME	WE	AE	ME	WE	AE	ME	WE	AE	ME	WE	AE	ME
S. aureus	0	0	0	0	0	4	0	4	6	0	6	7	4	8	8
S. pyogenase	0	0	4	0	4	6	0	6	6	0	8	8	0	8	10
S. pneumoniae	0	0	4	0	4	6	0	6	8	0	8	10	2	8	10
B. cereus	0	0	2	0	2	4	0	2	4	0	4	5	0	6	8
E. coli	0	0	2	0	0	4	0	4	6	0	3	6	0	6	8
Ps. aeruginosa	0	0	6	0	4	8	0	6	10	0	6	12	4	8	14
Pr. mirabilis	0	0	6	0	4	10	0	6	10	0	8	10	4	10	12
S. typhi	0	0	6	0	0	8	0	4	10	0	8	12	4	4	14
Sh. flexneri	0	0	4	0	0	6	0	4	6	0	4	8	0	6	8

WE = water extract; **AE** = acetone extract; **ME** = methanol extract

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of extracts of *C. papaya.*

S/No	Organism	MIC (mg/ml)			MBC mg/ml)		
		WE	AE	ME	WE	AE	ME
1	*S. aureus*	+++	100	100	+++	200	200
2	*S. pyogenase*	+++	200	100	+++	+++	+++
3	*S. pneumoniae*	+++	150	100	+++	200	200
4	*B. cereus*	+++	150	200	+++	200	200
5	*E. coli*	+++	150	100	+++	150	150
6	*P. aeruginosa*	+++	150	100	+++	150	200
7	*P. mirabilis*	+++	150	100	+++	200	200
8	*S. typhi*	+++	50	50	+++	200	200
9	S. flexneri	+++	100	100	+++	200	200

+++ = profuse growth; **WE** = water extract; **AE** = acetone extract; **ME** = methanol extract

cal analyses of the different leave extracts of *C. papaya.* The result revealed methanol extracts possessed saponins, alkaloids, tannins and phenols, while the hot water extracts contained saponins and glycosides, and the cold water extracts contained only glycosides. Table 2 shows results of the antibacterial susceptibility test of the extracts against the test organisms. From the results, methanol extracts were the most effective and the highest activity was demonstrated against *S. typhi* (14 mm zone of inhibition), *P. aeruginosa* (14 mm zone of inhibition) and *P. mirabilis* (12 mm zone of inhibition), followed by the acetone extracts with the highest activity against *S. pyogenase* and *P. aeruginosa* (10 mm zone of inhibition each), and *S. aureus* and *S. pneumoniae* (8 mm zone of inhibition each) at 250 mg/ml. The hot water extracts demonstrated slight activity against *S. aureus, S. pyogenase, P. aeruginosa, P. mirabilis* and *S. typhi* (4 mm zone of inhibition in each case). The cold water extracts did not show any activity against the test organisms. Table 3 shows the results of MIC and MBC determination on the test organisms. The lowest MIC and MBC of 50 mg/ml was demonstrated against *S. typhi,* while the MIC and MBC values ranging between 100-200 mg/ml were demonstrated against the rest of the test bacteria. The effect of temperature on the antimicrobial activity of *C. papaya* root extracts (Table 4) showed that the activity of extracts increased with an increase in temperature. The activity of the extracts against *S. typhi* at 30ºC (untreated) (14 mm zone of inhibition) increased to 18 mm at 60 and 100ºC, respectively. At alkaline pH, however, the activity of the extracts decreased. The activity of the extracts against *S. typhi* (14 mm zone of inhibition each at an untreated pH of 4.3 and the pH treatment of 2.5) decreased at pH 10 (8 mm zone of inhibition) Table 5.

DISCUSSION

The presence of bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explains the demonstration of antibacterial activity by the plant extracts used in this study (Srinivasan et al., 2001). The results of this study

Table 4. Effect of temperature (ºC) on the antibacterial activity of root extracts of *C. papaya* on the test organisms.

Test organism	Temperature (ºC) / Zone of inhibition (mm)											
	NT (30ºC)			4ºC			60ºC			100ºC		
	WE	AE	ME	WE	AE	ME	WE	AE	ME	WE	AE	ME
S. aureus	0	8	8	0	8	8	0	10	12	0	10	12
S. pyogenase	0	10	10	0	10	10	0	12	14	0	12	14
S. pneumoniae	0	8	10	0	10	10	0	10	14	0	10	14
B. cereus	0	6	8	0	6	8	0	8	10	0	8	12
E. coli	0	6	8	0	6	8	0	8	10	0	10	12
P. aeruginosa	0	8	14	0	10	14	0	10	16	0	10	16
P. mirabilis	0	10	12	0	10	12	0	12	14	0	12	14
S. typhi	0	4	14	0	4	14	0	6	18	0	6	18
S. flexneri	0	6	8	0	6	8	0	8	10	0	8	10

Table 5. Effect of pH on the antibacterial activity of root extracts of *C. papaya* (250 mg/ml) on the test organisms

Test organism	pH / Zone of inhibition (mm)											
	NT(4.3)			2.5			5			10		
	WE	AE	ME	WE	AE	ME	WE	AE	ME	WE	AE	ME
S. aureus	0	8	8	0	10	10	0	10	14	0	4	6
S. pyogenase	0	10	10	0	10	12	0	12	14	0	4	8
S. pneumoniae	0	8	10	0	10	12	0	10	14	0	4	6
B. cereus	0	6	8	0	6	8	0	8	12	0	4	6
E. coli	0	6	8	0	6	10	0	8	10	0	4	6
Ps. aeruginosa	0	8	14	0	10	14	0	10	16	0	4	8
Pr. mirabilis	0	10	12	0	10	12	0	12	14	0	4	8
S. typhi	0	4	14	0	6	14	0	6	14	0	4	8
Sh. flexneri	0	6	8	0	8	10	0	8	10	0	4	6

NT = non-treated extract**; WE** = water extract; **AE** = acetone extract; **ME** = methanol extract.

showed that the organic extracts were more effective than aqueous extracts and the methanol extracts demonstrated the highest activity. This may be due to the better solubility of the active components in organic solvents (de Boer et al., 2005). Among the gram-positive and gram-negative bacteria tested, the gram-negative bacteria were more susceptible to the extracts. This result, however, is at disparity with an earlier report indicating that plant extracts are more active against gram-positive bacteria than gram-negative bacteria (Jigna and Sumitra, 2006). There may be several factors that will predispose bacteria to antibacterial agents such as previous encounters with the agents or the nature of medium used, which may affect the diffusability of the agent. The activity of the extracts was comparable to those of antibiotics. The demonstration of activity against the test bacteria provides scientific bases for the local usage of these plants in the treatment of various ailments. The fact that the extracts were active against both gram-negative and gram-positive bacteria tested may indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms. The low MIC value observed for *S. typhi* is a good indication of high efficacy against this bacterium. This outcome is remarkable considering that typhoid fever (caused by *S. typhi*) is on the rise and also becoming recalcitrant to first-line antibiotics for its treatment in developing countries, including Nigeria. High MIC may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds. Temperature stability of plant extracts has been reported earlier (Doughari, 2006). This may be an indication that the bioactive compounds are heat stable and explains the ethno-botanical application process of the plants where boiling at very high temperatures for extended time periods are often practiced without the concoctions loosing their efficacy. Acid stability is an indication that the extracts when refined can be favorable for oral administration, since the stomach contains acidic secretions.

CONCLUSION

The demonstration of antimicrobial activity against both

gram-negative and gram-positive bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. The results of the study also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of gastroenteritis, uretritis, otitis media, typhoid fever and wound infections. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are the future challenges.

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