

DETERMINATION OF ANTIBACTERIAL ACTIVITY OF *CARICA PAPAYA* (PAW-PAW) EXTRACTS

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ABSTRACT

Objective: The quest for suitable and affordable alternatives in the face of increasing antimicrobial drug resistance has led researchers into exploring the use of plant extracts in the treatment of infections.

Method: Antibacterial activity of extracts of carica papaya (paw-paw) fruit was investigated using isolates from wound culture. These include: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *proteus species*. These organisms were challenged with extracts from the seed, endocarp and epicarp.

Result: The extracts demonstrated antibacterial activity as shown by their zones of inhibition and this was more pronounced with alcohol extracts than that of water. *Staphylococcus aureus* was the most susceptible with a minimum inhibition concentration (MIC) of 1:64 while *Enterococcus faecalis* was the least sensitive. The extracts were also heat stable.

Conclusion: The demonstration of antibacterial activity against both gram positive and gram negative bacteria demonstrates that the paw-paw plant is a potential source for the production of antimicrobial drugs. Researchers should be encouraged towards utilizing this plant as well as others with such potential in order to curb the menace of antimicrobial drug resistance in the near future.

Key Words: Carica papaya extracts, antibacterial activity

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INTRODUCTION

Antimicrobial resistance to commonly available/affordable drugs due to inappropriate use of antimicrobials has led to the quest of affordable alternatives¹. This has brought about researches using extracts from roots, herbs and fruits of plants. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs². The local use of natural plants as primary health remedies, due to their pharmacological properties is common in Asia, Latin America and Africa³.

Carica papaya (paw-paw) is a monosexual plant of Central American origin and is also cultivated in the tropics. It is edible and as well has medicinal properties. Papain, the enzyme seen in papaya has been said to aid digestion, assist in treatment of cancer, reduce inflammation, prevent complications of diabetes mellitus, treatment of psoriasis, chronic skin ulcers, ringworms and in prevention of Human Papilloma Virus (HPV) and as well possess antibacterial activities^{2,4-11}.

The study was carried out to determine the antibacterial activity of extracts from the seed, epicarp and endocarp of both ripe and unripe

carica papaya fruits.

Materials and Methods

Bacterial isolates from clinical specimens from Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Medical Microbiology laboratory were used. The specimens were processed and identified using standard microbiological methods.

The organisms include:-

Staphylococcus aureus, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus species* and *Enterococcus faecalis*.

Control strains used were:-

Staphylococcus aureus ATCC 29213

Enterococcus faecalis ATCC 29212

Escherichia coli ATCC 35218

Pseudomonas aeruginosa ATCC 27853

Processing of raw extracts

Extraction of plant material.

Specimens of ripe and unripe carica papaya fruits were collected from the same plant, washed thoroughly with distilled water and cleaned with 95% ethanol to reduce the microbial load. The fruits (both ripe and unripe) were peeled and separated into epicarp, endocarp and seeds respectively (making 6 fractions). Each fraction was separated into three parts A, B, and C. The first part (A) was homogenized

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with water, second part (B) homogenized with 30% ethanol as solvents. The third part (C) was kept for detection of presence of alkaloids and glucosides. All these portions were handled separately. The homogenates were filtered with Whatman No 4 filter paper.

Phytochemical Screening

Screening was carried out in the different extracts to check for the presence of alkaloid and glycoside as indicated by formation of a precipitate¹².

Antibacterial Screening

Overnight cultures of the Test and Control organisms were emulsified in sterile peptone water and adjusted to 0.5 McFarlands standard. These were inoculated unto freshly prepared Mueller Hinton agar (MHA Oxoid) plates. Using a sterile cork borer, holes about 5mm diameter were made in the agar plates without puncturing the Petri dishes. Aliquots of both water and ethanol extracts of the specimens (i.e. ripe and unripe seeds, epicarp and endocarp extracts) were dropped into the wells.

Gentacin 10µg and Ciprofloxacin 5µg both Oxoid UK were used as positive controls.

On the other hand, the plant extracts were also inoculated on Mueller Hinton agar plates to check for sterility.

Effect of temperature on the antibacterial activity of the extracts.

Portions of the unripe epicarp were distributed into sterile tubes and each was heated to a varying temperature, between 30°C to 100°C for 30 minutes using a water bath. This was allowed to cool to room temperature. The antibacterial activity of the heated extracts was tested using *Staphylococcus aureus* and *E.coli*.

RESULTS

The antibacterial activity and phytochemical screening of *Carica papaya* extracts were investigated. It was noted that extracts from unripe fruit (seed, epicarp and endocarp) contained more alkaloid than those from ripe fruit, but there was little or no difference in their glucoside content as seen in Table 1.

Table 2 shows the results of the preliminary screening of antibacterial activity of the extracts (from ethanol and distilled water) using disc diffusion method. Antibacterial activity was detected in the endocarp, epicarp and seeds. The test organisms except *Enterococcus faecalis* showed some degree of inhibition. *E. coli* however, showed resistance to the extracts of ripe epicarp and seed.

The effect of temperature on the activity of the extracts was determined using ethanol extracts of unripe epicarp. The extracts were relatively stable at

temperature between 30°C -50°C while their activity was markedly reduced from temperatures of 60°C and above Table 3.

Effect of extract on bacterial cell morphology using Gram reaction is shown in Table 4. There was no reaction noted in *Staphylococcus aureus* and slight changes in cell morphology were seen in *E.coli* and *Enterococcus faecalis*.

Table 1: Chemical Components of the Extracts.

Source of Extract	Test for Alkaloid	Test for Glucoside
Seed of unripe fruit	+++	+
Epicarp of unripe fruit	++	+(slow)
Endocarp of unripe fruit	+	++
Seed of ripe fruit	++	+
Epicarp of ripe fruit	+	+
Endocarp of ripe fruit	+	+

Table 2: Primary Screening for Antimicrobial Activity Using the Disc Diffusion Method.

Micro-organism	Used Solvent	Seed		Endocarp		Epicarp		Mean
		UR	R	UR	R	UR	R	
<i>Staph. aureus</i>	DW	8	7	8	6	7	5	6.8
	EE	12	10	20	114	28	10	15.7
<i>E. coli</i>	DW	6	-	10	9	14	-	6.5
	EE	10	-	17	12	22	-	10.2
<i>P. aeruginosa</i>	DW	8	6	8	5	7	6	6.7
	EE	11	8	10	8	12	9	9.7
<i>Proteus specie</i>	DW	7	6	9	7	8	6	7.2
	EE	8	-	6	6	10	7	6.2
<i>E. faecalis</i>	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-

Table 3: Effect of Temperature on the Activity of the Extract.

Microorganism- *Staphylococcus aureus*

Temperature of the abstract (°)	30	40	50	60	70	80	100
Zone of inhibition (mm)	26	24	24	20	19	19	17

Microorganism- *Escherichia coli*

Temperature of the abstract (°)	30	40	50	60	70	80	100
Zone of inhibition (mm)	20	20	18	16	16	14	12

Table 4: Effect of Extract on Bacterial Cell Morphology.

Micro-organism	Growth	Cell Morphology	Gram Reaction	
<i>Staph. aureus</i>	No growth	-	Before treatment	After treatment
<i>E. coli</i>	A little growth	Circular patches with irregular edges	Gram +ve cocci	Gram variable rods
			Short Gram -ve	
<i>Enterococcus faecalis</i>	Growth	Cocci	Gram +ve cocci	Gram variable

Key: -ve = Negative
+ve = Positive

DISCUSSION

The results from the study show that paw-paw extracts have antibacterial activity. This is probably the reason why some people use paw-paw for the treatment of wounds in the villages.

The extract is bacteriostatic on *Escherichia coli* and bactericidal on *Staphylococcus aureus* but has no effect on *E. faecalis*. This somewhat contradicts the findings of Viera¹³, where it was shown that the extract was bactericidal both to *Staphylococcus aureus* and *Escherichia coli*. The variation could have resulted to the concentration of ethanol used. In this study, 30% ethanol was used for extraction while 50% ethanol was used by Viera. The higher concentration of ethanol may have produced a more potent extract that was bactericidal to both gram positive and gram negative organisms.

It is worthy of note that Ethanol extract was more potent than distilled water extract as seen in Table 2. This may be due to the fact that ethanol is a better solvent for extraction than water.

The extract from seed, epicarp and endocarp of both ripe and unripe fruits had antibacterial activity but that from endocarp of unripe fruit had the most activity. This could be due to its high content of papain latex. It was also observed that the antibacterial activity of the extracts were relatively unaffected by temperatures between 30°C - 50°C and this is commendable as it falls within the body temperature.

It is believed that with rising degree of antimicrobial resistance against the commonly available and affordable antimicrobials, research should shift to the affordable alternatives which include the use of roots, herbs, fruits, etc for the treatment of common ailments.

It is recommended that Government, Non governmental organizations (NGOs) and philanthropists should encourage researchers in this field so that the spread of antimicrobial resistant pathogens can be curbed.

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