ANTIBACTERIAL SUBSTANCE FROM *CARICA PAPAYA* FRUIT EXTRACT

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ABSTRACT.—Ripe and unripe *Carica papaya* fruits (epicarp, endocarp, seeds and leaves) were extracted separately and purified. All the extracts except that of leaves produced very significant antibacterial activity on *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Shigella flexneri*. The MIC of the substance was small (0.2–0.3 mg/ml) for gram-positive bacteria and large (1.5–4 mg/ml) for gram-negative bacteria. The substance was bactericidal and showed properties of a protein. Other proteins previously found in *C. papaya* did not show antibacterial activity.

Although there was a number of reports on bacteria-inhibiting substances from plants (1,2,3), nothing is known about those of *Carica papaya* fruit origin. The microbial activity of vascular plants has been reviewed by Nickel (4), and similarly the antifungal activity of plants has been documented by Sehgal (5) and Stoessl (6). In his review, Nickel reported screening results which indicated antibacterial activity of pawpaw plant material against gram-positive bacteria and *Mycobacteria* (4). Nickel (4) also found most of the antimicrobial substances from higher plants either toxic to animal cells or not comparable therapeutically with the substances of microbial origin and, therefore, inadequate for human therapy. However, *Carica papaya* is an edible fruit for both human beings and mammals and does not produce toxic effects as food. It is very conceivable, therefore, that an antibacterial substance extracted from *C. papaya* would show no toxicity to normal animal cells if used for oral and external therapy.

This paper reports on the antibacterial activity of *Carica papaya* fruit extract against gram-positive and gram-negative bacteria, some of which are commonly found in wounds. The antibacterial activity of some enzymes previously found in *C. papaya* was also investigated.

MATERIALS AND METHODS

MICROORGANISMS.—*Staphylococcus aureus* (Oxford strains), *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Shigella flexneri, Salmonella typhimurium, Bacillus cereus* and *Streptococcus faecalis* used in this investigation were obtained from the Department of Microbiology, University of Nigeria culture collection Centre No. 384. Cultures were routinely maintained in nutrient agar (B.B.L.) slants at 4°.

EXTRACTION PROCEDURE.—Ripe and unripe *Carica papaya* fruits were separated into epicarp, endocarp, and seeds. After grinding and/or homogenizing (Waring Blendor model As-1) each part including the leaves, the homogenates were extracted with 30% ethanol at 4° for 24 h. Extracts were recovered by filtration under reduced pressure and centrifugation (5,000g for 30 min.). The crude extract of each part was subjected to salting out by ammonium sulfate (60% saturation) and gently adjusted to 30% acetone and placed at −10° for 30 min. followed by gel filtration with Sephadex G-100 (fig. 1). Fractions which showed significant bacteria-inhibiting capacity (fraction numbers between 40 and 50 containing 1 microgram ml^{-1} protein (7)) were pooled and concentrated by freeze-drying in an Edwards Freeze dryer (model EF03, Minor Royal Crawley, Sussex, England) at −80° until a 0.1 vacum pressure was achieved. At this pressure the primary drying was regarded as complete. The dry material was stored at 4° in a dessicator.

SENSITIVITY OF BACTERIAL SPECIES TO *Carica papaya* EXTRACTS.—Filter paper discs (Whatman, 6 mm diam.) were soaked with 4 mg/ml phosphate buffered saline (W/V) solutions of each purified extract (epicarp endocarp, seeds and leaves) and dried at 60°. The discs were then placed on soft nutrient agar (0.7%) petri plates which were previously seeded with 10^6 cells/ml suspension of each of eight bacterial species. After equilibration at 4° for 1 h (8) and incubation at 37° for 24 h, zones of inhibition were measured. Varying amounts up to 4 mg of papain, chymopapain and lysozyme were also tested for anti-bacterial activity. Antibacterial activity was expressed in terms of the diameter of the zone of inhibition calculated as the difference in diameter of the observed zones and the paper discs. Zones of inhibition 3 mm in diameter or larger were considered significant.

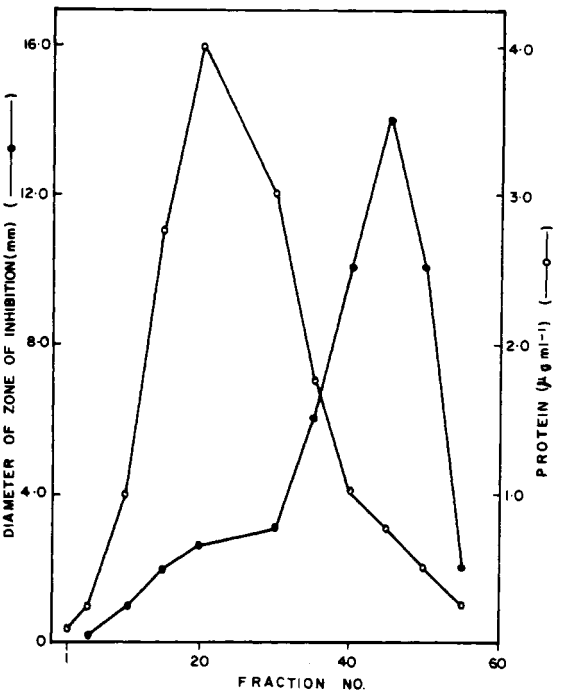

FIG. 1: Antibacterial profile of Sephadex G-100 gel filtration of an ammonium sulfate precipitate of *C. papaya* fruit extract. The extract was adjusted to pH 7.0 with 0.01 phosphate buffered saline (PBS) and salted out with ammonium sulfate (60% saturation). The precipitate collected by centrifugation (5,000g, 30 min) was dissolved in PBS and dialyzed against PBS for 24th at 4°. The non-dialytic fluid was adjusted to contain 1 microgram ml^{-1} protein with PBS and gently mixed with acetone to 30% concentration and placed at −10° for 30 min. Following centrifugation (5,000g, 30 min), the precipitate collected was dissolved in PBS and dialyzed against PBS at 4° for 24th. After the fluid was adjusted to 1.0 microgram ml^{-1} protein with PBS, the solution (5ml) was chromatographed on a Sephadex G-100 column (26 x 700 mm, Pharmacia, previously equilibrated with PBS), and eluted with PBS. Fractions (5ml each) were collected, pooled and lypholized.

DETERMINATION OF MINIMAL INHIBITION CONCENTRATION (MIC) OF EXTRACT.—The MIC of the extract was estimated for the five sensitive bacterial species. For comparison, MICs of penicillin and tetracycline (POLFO Tarchomin, Poland) were also determined for five bacterial species. Eighteen-hour culture of the test bacteria was inoculated into nutrient broth (initial cell density of 10^6 cells/ml), and varying amounts of the extract or penicillin G or tetracycline were added. These were incubated at 37° for 24 h and examined for growth or absence of growth. Smears were also prepared from each sample of the 24 h broth cultures for morphology and gram-reaction of the cells.

EFFECT OF TEMPERATURE AND PH ON THE ANTI-BACTERIAL ACTIVITY OF EXTRACTS.—Suspensions of purified extracts were heated at 30°, 60° and 80° for 30 min and others were adjusted to pH 2.0, 4.5 and 8.0. The antibacterial activity of each suspension was determined by use of the disc method already described.

OTHER TESTS.—The extract was separately treated with RNase, lipase, pronase and trypsin before the antibacterial activity was determined.

RESULTS

ANTIBACTERIAL ACTIVITY OF EXTRACTS OF *Carica papaya* FRUIT.—The extracts from the epicarp, endocarp and seeds of both ripe and unripe fruits produced very significant degrees of antibacterial activity on *S. aureus*, *B. cereus*, *E. coli*,

Ps. aeruginosa and *S. flexneri* (table 1). The extracts gave variable sizes of zones of inhibition against the bacterial species. The leaf extract failed to inhibit growth of any of the bacterial species.

Similarly, amounts up to 4 mg of papain, chymopapain, and lysozyme did not produce significant zones of growth inhibition on *S. aureus*, *B. ceraus* and *E. coli* strains.

MIC OF EXTRACT COMPARED WITH PENICILLIN G AND TETRACYCLINE.—The MICs of the extract, penicillin G and tetracycline on the five bacterial species are shown on table 2. The MIC was smaller for the gram-positive bacteria (*S. aureus* and *B. cereus*) than for the gram-negative bacteria. The MIC of the extract is several orders of magnitude larger than that of penicillin or tetracycline.

SOME CHARACTERISTICS OF THE ANTIBACTERIAL SUBSTANCE.—The antibacterial substance in the extracts resisted heating at 80° for 30 min, acid pH (pH 2 to 6.0), and treatment with RNase or lipase. The activity was lost or significantly reduced in pH 8.0 (table 3) or by treatment with proteolytic enzymes such as pronase or trypsin.

BACTERICIDAL EFFECT OF PAPAYA EXTRACT.—After 24 h growth of *S. aureus*, *B. cereus*, and *E. coli* in a medium containing the extract (2mg/ml), subsequent subcultures of these bacteria showed no growth (table 4). The cell morphology appeared distorted and the cells were gram-variable.

TABLE 1. Inhibition of bacterial growth by *Carica papaya* fruit extracts

Bacterial species	Diameter of zone of inhibition (mm)[a]			
	Epicarp	Endocarp	Seed	Leaves
S. aureus	8.0	12.0	15.0	0
B. cereus	5.5	9.5	15.0	0
E. coli	5.0	7.0	9.0	0
Ps. aeruginosa	5.0	6.0	8.0	0
S. flexneri	5.0	5.0	5.0	0
S. typhimurium	1.5	1.5	1.5	0
P. vulgaris	1.5	1.5	1.5	0
S. faecalis	0	0	0	0

[a]The numbers represent an average of five determinations using paper discs containing 4mg of each extract per ml PBS (W/V).

A zone of inhibition with a diameter in excess of 3 mm is regarded as significant. 0, represents absence of zone of growth inhibition.

TABLE 2. Minimum inhibition concentration of *Carica papaya* extract compared with penicillin G and tetracycline

Bacterial species	Minimal inhibition concentration		
	Penicillin mg. $\times 10^{-5}$/ml	Tetracycline mg. $\times 10^{-2}$/ml	Papaya extract mg/ml
S. aureus	0.12–0.24	0.2	0.2
B. cereus	0.12–0.24	0.2	0.3
E. coli	0.12–0.24	0.2	1.5
Ps. aeruginose	0[a]	0	2.5
S. flaxneri	0	0	4.0

[a]0, No zone of growth inhibition observed.

TABLE 3. The effect of temperature and pH on the inhibition of bacterial growth by *Papaya* extract

Bacterial species	Temperature			Diameter of zone of inhibition (mm) pH		
	30°	60°	80°	2.0	4.5	8.0
S. aureus	15.0	12.0	10.0	15.0	14.0	0
B. cereus	13.0	10.0	8.0	13.0	12.0	0
E. coli	9.0	7.0	6.0	9.0	8.0	0

TABLE 4. Effect of *Papaya* extract on growth and cell-morphology.

Bacterial species	Growth (after treatment)	Morphology of cells (after treatment)	Gram reaction (before treatment)	Gram reaction (after treatment)
S. aureus	No growth	Swollen	+ve	variable
B. cereus	No growth	Short and Swollen	+ve	variable
E. coli	No growth	Short and Swollen	−ve	variable

DISCUSSION

Our results indicated that *Carica papaya* fruit contains an antibacterial substance which is bactericidal on several species of gram-positive and negative bacteria. However, the bacteria varied widely in the degree of their susceptibility. Although the MIC of the fruit extract was many fold greater than that of penicillin and tetracycline, suggesting lower activity, the results showed that small amounts [0.2–0.3 mg/ml (W/V)] of the extract inhibited growth of gram-positive bacteria such as *S. aureus* and *B. cereus*. On the other hand, a wider range and higher concentrations [1.5–4 mg/ml (W/V)] were required for the inhibition of the gram-negative bacteria tested (table 2).

The antibacterial substance in the extracts resisted heating at 80° for 30 min, acid pH and treatment with RNase or lipase. The activity was lost or significantly reduced in pH 8.0 (table 3) or by treatment with proteolytic enzymes such as pronase or trypsin. The results suggested that the active principle or the antibacterial substance in papaya fruit is a protein. The isolation of a protein-like substance from *C. papaya* fruit extract is in accord with reports of other workers who have also isolated from papaya fruits and seeds, proteolytic and hydrolytic enzymes such as papain (9, 10) chymopapain (11), proteinase (12) and thioglucosidase (13). However, although further chemical and biological characterization and comparative studies of the antibacterial substance should be carried out prior to its classification, the extracted antibacterial substance does not appear to be identical with these previously isolated proteins. For example, the activity of the protein-like substance was lost at pH 8.0, whereas the activity of papain is stable (14). The substance was stable at acid pH, as is chymopapain (11). However, neither papain nor chymopapain showed antibacterial activity.

After 24 h of growth in a medium containing the extract, the cell morphology appeared distorted, and the cells were gram-variable suggesting that the site of action of the antibacterial substance was at the cell wall. The bactericidal substance appears to exert antibacterial activity by inhibiting the growth of and by killing the sensitive bacteria.

The findings showed significant antibacterial activity of a protein-like substance extracted from *C. papaya* fruit against both gram-positive and gram-negative bacteria, of which some are found in wounds (*S. aureus* and *E. coli*).

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