

Antimicrobial Activity of *Carica Papaya* (Pawpaw Leaf) on Some Pathogenic Organisms of Clinical Origin from South-Western Nigeria

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Abstract

The bioactive compound of leaf and root extracts of *Carica papaya* was extracted, using water and organic solvents, and were investigated for antibacterial activity against some human pathogenic bacteria using the agar diffusion method. The aqueous extracts of the root 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 root extracts demonstrated higher activities against all the gram-positive bacteria than the gram-negative bacteria tested, with the highest activity (14 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa* while the aqueous leaf extract showed pronounced inhibition demonstrating higher activities against the test bacteria than the organic solvents. The extracts demonstrated higher activities against all the gram-positive bacteria than the gram-negative bacteria tested, with the highest activity (4.2 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa*. 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 root 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, urethritis, otitis media and wound infections.

Key words: Antimicrobial activity, *Carica papaya*. pathogenic organisms. South-Western Nigeria

Introduction

In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded across the countries. Therefore, the use and history of herbs dates back to the time of early man, who had the crudest tools as his implements and use stones to start his fire. They used herbs in their raw and cooked forms to keep fit. Since that time, the use of herbs has been known and accepted by all nations and has been known also as the first art of treatment available to man (Kafaru, 1994).

The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Ahmad, 2001).

There is no plant that does not have medicinal value. The active components are normally extracted from all plant structures, but the concentrations of these components vary from structure to structure. However, parts known to contain the highest concentration of the principles are preferred to therapeutic purposes and it can either be the leaves, stems, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits or the seeds (Kafaru, 1994).

Some of the active principles singly or in combination inhibit greatly the life processes

of microbes, especially the disease causing ones. They do this by binding their protein molecules, acting as chelating agents (selective binding polyvalent metal ions so that the latter loses its biological activities), altering their biochemical systems, preventing utilization of available interests to the microorganisms, other causes inflammation analysis of microbial cells (Garrod *et al.*, 1995). The bitter taste, pungent and repulsive smell in some plants; have been found to have repressive ability over the metabolic activities of a wide range of microorganisms (Mitscher *et al.*, 1992). Sofowora, (1982) and Baladrin *et al.*, (1985) defined medicinal plants as a plant in which one or more of the organs contains substances that can be used for therapeutic purposes or which its precursors for the manufacturing of drugs are useful for disease therapy. The use of medicinal plants predates the introduction of antibiotics and other modern drugs into the African continent. Since medicinal plants do not merely save people from feeling pain but also permit them to emerge unscathed, then they deserve investigation. The active components in these medicinal attribute are expected to be inimical to the growth of at least some microorganisms especially the disease causing ones e.g. *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* etc. therefore, many studies and researches had been done on the antimicrobial properties of many plants but for this study, the leaf of *Carica papaya* will be discussed.

Carica papaya belongs to the family Caricaceae. It has the following common names; pawpaw tree, papaya, papayer, tinti, pepol, chich put, fan kua, wan shou kuo, kavunagaci, kepaya etc. The parts that are usually used include the leaves, fruit, seed, latex, and root. The plant is described as a fast growing, erect, usually unbranched tree or shrub, 7-8m tall with copious latex, trunk of about 20cm in diameter. The plant is also described in a documented property forms and it act as analgesic, amebicide, antibacterial, cardi tonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge.

It is distributed throughout Asia, Nigeria etc (Afolayan, 2003). *Carica papaya* contains many biochemically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion. Papain is used in the treatment of arthritis. The leaves of *Carica papaya* is used as soap substitute which are supposed to remove stains. The papain, the proteolytic enzyme has a wealth of industrial uses. It has milk-clotting (rennet) and protein digesting properties. Active over a wide pH range, papain is used in medicine, combating dyspepsia and other digestive disorders. In liquid preparations, it has been used for reducing enlarged tonsils. Nearly 80% of American beer is treated with papain, which digests the precipitable protein fragmented and then the beer remains clear on cooling. Papain is also used for degumming natural silk. But most of the papain imported in the U.S is used for meat-tenderizers and chewing gums. Also used to extract the oil from tuna liver cosmetically, it is used in some dentifrices, shampoos and face-lifting preparations. Use to clean silks and wools before dyeing and to remove hair from hides during tanning (James, 1983). It is also used in the manufacture of rubber from latex (Morton, 1977). Recently, FDA has cleared chymopapain for intradiscal injection in patients with documented herniated lumbar intervertebral discs whose signs and symptoms have not responded to conservative therapy over an adequate period of time.

The medicinal folk uses the leaves poultice onto nervous pains and elephantoid growths. The leaf smoked for asthma relief in various remote areas. Javanese believe that eating papaya prevent rheumatism. Dietary papaya does reduce urine acidity in humans while the flowers have been used for jaundice. The young leaves and to lesser degree other parts contain carpain an active bitter alkaloid which has a depressing action on heart. The plant is strong amoebicide (Reed, 1976).

The efficacy of treatment with *C. papaya* is dependent on the quantity of the different compounds in the preparations. In Indonesia, papaya leaves are used as feed for animals after

parturition-2 leaves boiled in water fed every 2 days for 1 week. It also has been reported that papaya leaf extracts is used as a profilaxis against malaria, though no studies on this use could be found in the literature (Satrija *et al.*, 1994).

In Nigeria, it is used for smooth upper respiratory tract ailment and tumour (uterus). In Ivory Coast, it is used for treating madness. In Trinidad, it is used for treating scorpion bites and hypertension. In cote d'Ivoire and Sama, it is used for toothache and tuberculosis in Mexico. In Honduras and Turkey, it is used for liver ailments, constipation and laxative. In Philippines, India, Malagasy and Malaya, it is used for treating arthritis and rheumatism. In Java, Panama, Sri Lanka and Turkey, it is used for treating abortifacient. In Honduras, Japan, Panama and West Africa, it is used for the treatment of diarrhea and dysentery.

Materials and Methods

Sterilization of Materials

All glasswares were washed with detergent and rinsed with distilled water properly. These were then air dried before wrapping with aluminium foil and sterilized in hot air oven at 170°C for 2 hours. Prepared media such as nutrient agar and nutrient broth were sterilized in an autoclave at 121°C for 15 minutes. Cork borer, glass rods and forceps were sterilized by dipping in 70% ethanol which was then flamed in Bunsen flame. The inoculating loop was also sterilized by heating in Bunsen flame. The inoculating loop was also sterilized by heating to redness using naked flame before and after each use.

Collection and Maintenance of Test Organisms

The test organisms that were used were all human pathogenic organisms from clinical origin. These isolates include *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Proteus mirabilis*. They were obtained from the

Department of Microbiology and Parasitology laboratory in University of Ilorin Teaching Hospital (U.I.T.H). The organisms were collected on sterile agar slants and incubated at 37°C for 48 hours. They were then kept as stock cultures in the refrigerator set at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmatory purposes.

Collection of Plant Materials

The plant *Carica papaya* was used for this project work and it was identified as pawpaw leaf. These were then collected in a sterile polythene bag, rinsed, sundried and made into a powdery form before use.

Preparation of Plant Material

The pawpaw leaf *Carica papaya* was separately extracted with cold ethanol, cold methanol and hot water. These were prepared using the method as described by Oyagade *et al.*, 1999. These were carried out by suspending 25 grams of the finely ground leaves in 125 milliliters of distilled water or 95% ethanol or methanol. The hot water extraction was done at 80°C in a water bath for 1/2 hours. The ethanolic and methanolic extraction was done at 28±1°C for 120 hours. The extracts were then decanted and filtered through a Whatman filter paper. The filtered extract was then sterilized using a membrane filter and evaporated to dryness at 45°C. The residues obtained were reconstituted in 95% ethanol at stock concentration of 0.2 g/ml. The extract solution was then stored in the refrigerator at 4±2°C until used (Omojosola and Awe, 2004).

Standardization of Test Organisms

All inoculums were standardized using the McFarland nephelometer method (Albert *et al.*, 1991). To prepare this, 11 (eleven) large test tubes, 1% each of barium chloride and sulphuric acid were also used. The protocol for preparing this solution is as stated in the

Table 1.

Table 1 shows the various concentrations of 1% each of barium chloride and sulphuric acid that would be added in the various tubes. The reaction gives rise to turbid solutions but the degree of turbidity differs in each test tubes. These are then kept on the work bench for use. After then, liquid broth of the test organisms were made in other test tubes and their turbidity were used to match the standard solutions turbidity such that any one that has turbidity similar to the standard solution is considered as having the corresponding number of bacterial suspension per ml. However for this project, the standard that was used is the one that corresponds to 15×10^8 /bacteria suspension per millilitre. To prepare this 0.5ml of already prepared nutrient broth was pipette into a sterile test tube aseptically and the pure culture of the particular test organism was dissolved into it until the bacterial suspension was corresponding to the standard.

Antimicrobial Assay of Extracts

The agar well method of the agar diffusion technique was used to determine the antibacterial activity of the plant extracts. One millilitre of the different standardized organisms were introduced separately and thoroughly mixed with 30milliliters of molten nutrient agar each in a sterile Petri dish and allowed to set then labelled. A sterile 8mm cork borer was then used to punch holes (i.e. 5wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labelled accordingly; 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml while the 5th well contained the extractant i.e. the solvent used for the extraction to serve as control. These were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37°C for 48hours. After incubation, the diameter of the zones of inhibition around each well

were measured to the nearest millimetres along two axis i.e. 90° to each other and the mean of the two readings were then calculated.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts

The MIC of the extracts was determined by using the broth dilution technique (Adebayo *et al.*, 1989). The MIC helps to measure more exactly the concentration of an antibiotic necessary to inhibit growth of a standardized inoculums under defined conditions (Geo *et al.*, 2001). Serial dilutions of the extract in liquid medium were prepared. These were then challenged with small inoculums of an overnight broth culture of the test organisms. The culture was then challenged with small inoculums of an overnight broth culture of the test organisms. The culture was then incubated at 37°C for 48 hours. The smallest concentration that inhibits the growth was taken as the MIC. The determination of the value of MBC follows the determination of MIC by the broth dilution technique. The MBC is the lowest concentration of the antibacterial agent that kills at least 99.9% of the test organism (Geo *et al.*, 2001). To determine this value, about 0.5 ml of the sample was removed from the test tubes used in the determination of MIC in which there was no desirable growth was spread over the surface of the oven dried nutrient agar plates. The lowest concentration of the agent that prevented the growth of less than 0.1% of the test organism on the recovery plate was taken to be the MBC value for the extract.

Results and Discussion

Nature of Extracts

The colours of the methanolic and ethanolic extracts were both green while that of the hot water was brown (Table 2). 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 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 against the root extract of *C. papaya*, the Gram-negative bacteria were more susceptible especially *P. aeruginosa* 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 while that of the leaf extract of *C. papaya* was next to the most sensitivity with the Gram-negative bacteria especially *Proteus mirabilis* (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. aureus* is a good indication of high efficacy against this bacterium. This outcome is remarkable considering that boil, breast abscess and surgical wound infection etc (caused by *S. aureus*) 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 losing their efficacy.

The impotency of the order extracts on the test organisms especially those of the methanolic and ethanolic leaf extract of all the concentrations used and the water extract for the root of *C. papaya* at concentrations 50, 100, 150 and 200mg/ml could be as a result of the following as stated by Bernice (1997);

1. Location of harvest should never be areas treated with insecticides.
2. Time of collection should be when the leaf or plant sprouts most and collection should also be during the day.
3. Drying method. Sometimes, active agents of leaves are destroyed by direct exposure to sunlight or by drying in the hot air oven especially if the active agent is volatile.
4. Once dried, herbs should be dated, labelled and stored in area not exposed to light, moisture or heat.
5. It could also be due to the extractant used and the method used to obtain the active component (Unaeza and Abrikwa, 1989).

Therefore, since this work had revealed the ineffectiveness of methanolic and ethanolic leaf extracts and aqueous and ethanolic (50mg/ml) root extracts, further research should be made using the leaves dried at room temperature and not sun drying nor oven drying to see if this had contributed to the impotency of the extracts.

It could be however concluded that 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, urethritis, otitis media, 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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Table 1. Protocol for test tubes for Mcfarland nephelometer in 10ml.

test tube number	1%barium chloride (ml)	1% sulphuric acid (ml)	Corresponding bacter suspension ($\times 10^8/\text{ml}$)
0.5	0.05	9.95	1.5
1	0.1	9.9	3
2	0.2	9.8	6
3	0.3	9.7	9
4	0.4	9.6	12
5	0.5	9.5	15
6	0.6	9.4	18
7	0.7	9.3	21
8	0.8	9.2	24

9	0.9	9.1	27
10	1.0	9.0	30

Source: Albert *et al.*, (1991).

Table 2. Nature of *Carica papaya* extracts.

Extracts	Colour
Methanolic	Green
Ethanolic	Green
Hot water	Brown

Table 3. Minimum Inhibitory Concentration Of *Carica papaya*.

Test Organisms	Minimum Inhibitory Concentration (mg/ml)		
	Hot water	Ethanol	Methanol
<i>Pseudomonas aeruginosa</i>	0.7	-	-
<i>Klebsiella pneumonia</i>	0.4	0.4	-
<i>Escherichia coli</i>	0.8	-	-
<i>Streptococcus mutans</i>	-	-	-

<i>Staphylococcus aureus</i>	1.2	0.8	-
<i>Proteus mirabilis</i>	0.8	-	0.8