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Research Article

Antibacterial Effect of *Carica papaya* Root Extract on Some Selected Pathogens from Clinical Isolates

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Abstract

Introduction: The search for alternate sources of antibiotics to combat the growing global challenge of antibiotic resistance is imperative thus this study was designed to determine the antibacterial activity of *Carica papaya* root extract on *Klebsiella* species, *Escherichia coli, Salmonella species, Pseudomonas species* and *Staphylococcus aureus.*

Materials and Method: Dried and pulverized papaya roots were mixed with extraction solvents (water and ethanol). Aseptically the extracts were filtered and inoculated into Nutrient agar to ascertain sterility of the solutions.

Results: Results showed high antibacterial activity for ethanol extract and the cold-water extract of Carica papaya with ethanol extract having more effect. It was observed that the extract was effective against the Gram- positive and Gram-negative test organisms investigated while the Antibiotic susceptibility test revealed that the microorganisms were resistant to one or two of the antibiotics used (Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Augmentin (50 µg), Nitrofurantoin (300 µg), Oflaxacin (5 µg), Ampicillin (10 µg) except for *Escherichia coli* which was resistant to all antibiotics used.

Conclusion: From the results obtained in this study, it can be inferred that Carica papaya root part is a potential source of natural therapeutic option that can be further exploited for the treatment of bacterial infection.

Keywords: Carica papaya; Antibacterial; Pathogenic Bacterial Isolates; Extracts

Introduction

The upsurge of resistant microbial pathogenic strains has become a global public health issue concerned about the potency of most commonly prescribed current antibiotics [1]. In Asia, Latin America, and Africa natural plants are commonly used locally as primary health remedies, due to their pharmacological properties [2]. Medicinal plants have been identified as producers of certain chemicals that are plainly toxic to bacteria which are inexpensive and inexhaustible sources of pharmacologically active substances [3].

Carica papaya is a member of the *Caricaceae* family and it is identified by different names such as the common name: pawpaw, it is also called papaya, pepol, kepaya, tinti, fan kua, etc. and various segments of the plant, such as the leaf, latex, fruits, seed, and root, have been known to possess certain compounds that have effect on living organism tissue or cell. The individual plant parts when grown on a commercial scale accounts for economic value [4]. The concentration of these constituent varies from structure to structure irrespective of the extraction of the active components from all plant part. *Carica papaya* plant parts documented to contain the highest concentration of the principles are often the choice for therapeutic uses and they can either be the leaves, the stem, the barks, roots, bulks, corms, rhizomes, woods, flowers, the fruits, or the seeds [5].

There has been a record of studies globally that have investigated the antimicrobial properties of plants and reported the efficacy of *C. papaya* as a therapeutic substitute [6]. Papaya has been used widely in folk medicine for many ailment for example the juice for warts, corns, cancers, tumors, and thickened skin; the root extracts for treating cancers of the uterus, syphilis, the tropical infection, hemorrhoids, e.tc; the unripe fruit is used as a mild laxative or diuretic, and employed in stimulating lactation, labor, or abortion, the ripe fruit is used for rheumatism and alkalinizing the urine, the seeds for intestinal worms or to stimulate menstruation or abortion; the leaves as a poultice for nervous pains and elephantoid growths, or smoked for asthma relief, and the latex for psoriasis, ringworm, indigestion, or applied externally as an antiseptic and its

root aqueous extract has been shown to have a purgative effect [7], in reproduction; various extracts of papaya have been reported to have antifertility activity in male [8] and female rats [9].

Since medicinal plants have been shown to contain positive attributes and not documented to cause damage or harm to the physiological state of people, then investigating them is imperative. Hence, the aim of this study is to investigate the antibacterial activity of the root part of *Carica papaya* in ethanol and cold-water extract, in comparison with commonly prescribed drugs.

Materials and Methods

Preparation of Plant extract

Apparently healthy, fresh root samples of *C. papaya* were harvested locally from Bells University campus, Ota, Ogun State. The root parts collected were cleaned with tap water and subsequently with distilled water. Plant extract were then dried in the oven at 50°C. The dried plant root was grounded using an electrical blender until a fine texture was achieved. Two grams (2g) were extracted by 20 ml solvent ratio 1:10. The containers were kept in the dark for five days. Whatman No 1 filter paper was used to filter the extract after which the filtrate was concentrated in a Vacuum (in vacuo). The amount of extract obtained was dissolved in Tween 20 making extracts of different concentrations as follows 4, 8, 16, 32, 64, 128, 256, 512 mg/ml.

Ethanol and cold-water extraction

Using the method described by Oyagade., *et al.* [10], ethanol and cold-water extract were performed by introducing 25 grams of the finely ground roots into 125 millimeter of cold water and 125 millimeter of ethanol.

Ethanol extraction was performed at 28 ± 1°C for 120 hours. The cold water and ethanol extracts were then decanted and filtered through a Whatman filter paper. Afterwards the filtrate was sterilized using a membrane filter and evaporated to dryness at 45°C. Reconstitution of the residues was in 95% ethanol at stock concentration of 0.2 g/ml. The extract solutions were stored in the refrigerator at 4 ± 2°C for further test [11].

Test Organisms

The microorganisms employed in this study were all human pathogenic organisms of clinical origin. These isolates include *Escherichia coli, Pseudomonas species, Klebseilla species, Staphylococcus aureus,* and *Salmonella species*. They were obtained from the Department of Microbiology and Parasitology Laboratory in State (General) Hospital, Ota, Ogun State. These organisms were collected and inoculated aseptically onto sterile agar slants and incubated at a temperature of 37°c for 48 hours. They were then preserved as stock cultures in the refrigerator (at 4°c). Biochemical analysis was performed for each of the test organisms to confirm the organisms.

Standardization of Test organisms

Using the McFarland nephelometer method as described by Akah., *et al.* [7], all inoculums were standardized. Bacterial suspension was prepared according to 0.5 McFarland standards by measuring the turbidity. Using a sterile wire loop, isolated colonies of the organisms on Mueller Hinton Agar plates were aseptically picked and emulsified in 9 ml of sterile distilled water in McCartney bottles. The mixture was then homogenized and thus referred as the stock solution.

Antimicrobial Assay of Extracts [12]

About 0.1 ml of the overnight broth culture was transferred into 9.9 ml of sterile distilled water to produce 1 in 100 (10^{-2}) dilution. 0.2 ml of this dilution was transferred aseptically into 20 ml sterile Mueller-Hinton agar and swirled then poured into sterile Petri dishes. With the aid of a sterile cork borer of 8 mm diameter, equidistant wells were made in the seeded agar and the wells labeled accordingly. Using a micro pipette approximately 100 µL of the reconstituted extracts at the various dilutions (4 to 512 mg/ml) were placed into each well to fill up.

The plates were allowed to stand for an hour to allow for proper diffusion of extracts into the medium after which they were incubated in an upright position for 24 hours at 37°C. Zones of inhibition were measured to nearest millimeter (mm). These experiments were performed in triplicate.

Results

According to the results derived from this study, it was observed that the ethanol extracts had higher antibacterial activity against the clinical isolates than the cold-water extract and this is represented in tables 1 and 2 respectively. The results showed zone of inhibition within the intermediate range of 15 - 19 mm through the susceptible (≥ 20 mm) range. Varying degrees of susceptibility was observed in the isolates to the various extracts. While some isolates were resistant, some others showed intermediate degree and some were susceptible to the extracts. Antibiotic susceptibility test revealed that the microorganisms were resistant to one or two of the antibiotics used (Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Augmentin (50 µg), Nitrofurantoin (300 µg), Oflaxacin (5 µg), Ampicillin (10 µg) except for Escherichia coli which was resistant to all antibiotics used.

Clinical Isolate	Diameter of zone of inhibition							
	4mg/ml	8mg/ml	16mg/ml	32mg/ml	126mg/ml	256mg/ml	512 mg/ml	T.20
S. aureus	-	14 ± 0.15	11 ± 0.14	12 ± 0.47	11 ± 0.37	18 ± 0.37	20 ± 0.97	-
Klebsiella sp.	-	-	14 ± 0.27	10 ± 1.37	11 ± 1.09	16 ± 1.12	19 ± 0.27	-
Escherichia coli	-	10 ± 0.47	15 ± 1.12	15 ± 1.27	17 ± 0.57	16 ± 1.18	15 ± 1.78	-
Pseudomonas sp	-	13 ± 0.79	13 ± 0.07	10 ± 0.11	13 ± 0.97	18 ± 0.87	19 ± 1.67	-
Salmonella sp.	-	11 ± 0.12	12 ± 1.17	13 ± 0.27	16 ± 1.21	14 ± 0.67	21 ± 0.45	-

Table 1: Antibacterial activity of cold water extract of Carica papaya root against clinical isolates.

NOTE: all readings are average of triplicate experiments and are represented in millimeter (mm)

T.20 - Tween 20 (control).

Clinical Isolate	Diameter of zone of inhibition							
	4mg/ml	8mg/ml	16mg/ml	32mg/ml	126mg/ml	256mg/ml	512mg/ml	T.20
S. aureus	0 ± 0.00	14 ± 0.21	16 ± 1.21	12 ± 0.23	11 ± 0.17	16 ± 1.50	20 ± 0.12	-
Klebsiella sp	0 ± 0.00	19 ± 0.43	14 ± 0.69	10 ± 0.72	11 ± 0.26	14 ± 1.25	19 ± 0.09	-
Escherichia coli	11 ± 0.17	16 ± 0.82	15 ± 0.38	15 ± 0.55	17 ± 1.58	13 ± 0.58	17 ± 0.55	-
Pseudomonas sp.	15 ± 0.21	13 ± 0.59	17 ± 0.91	10 ± 0.89	13 ± 1.12	15 ± 1.26	20 ± 0.45	-
Salmonella sp.	10 ± 0.19	18 ± 0.75	12 ± 0.33	13 ± 0.99	16 ± 0.76	16 ± 0.44	22 ± 1.06	-

Table 2: Antibacterial activity of ethanol extract of Carica papaya root against clinical isolates.

NOTE: all readings are average of triplicate experiments and are represented in millimeter (mm).

T.20 -Tween 20 (control).

Clinical Isolate	Antibiotics							
	CAZ (30µg)	CRX (30µg)	GEN (10µg)	CPR (5 µg)	OFL (5 µg)	AUG (30µg)	NIT (300µg)	AMP (10 µg)
S. aureus	-	-	21	28 ± 0.23	22 ± 0.18	-	24 ± 0.12	-
Klebsiella sp.	24 ± 0.21	-	22 ± 0.19	24 ± 0.21	22 ± 0.19	-	13 ± 0.14	-
Escherichia coli	-	-	-	-	-	-	-	-
Pseudomonas sp.	-	-	13 ± 0. 17	15 ± 0.18	-	-	-	-
Salmonella sp.	26 ± 0.38	30 ± 0.25	-	26 ± 0.19	24 ± 0.21	-	-	-

Table 3: Antibiotic Susceptibility test results of Clinical isolates.

NOTE: all readings are average of triplicate experiments and are represented in millimeter (mm).

Key: CAZ - Ceftazidime; CRX - Cefuroxime; GEN - Gentamicin; CPR - Ciprofloxacin; OFL - Ofloxacin; AUG - Augmentin; NIT - Nitrofurantoin; AMP- Ampicillin; - - Resistant.

Discussion

The results of this study demonstrated that the cold-water extracts were less effective (Table 1 and 2) and this is in agreement with the works of Doughari., *et al.* [13] and Kitonde., *et al* [14]. The antimicrobial effectiveness of plant extracts have been attributed to the presence of secondary metabolites in the investigated plant part which probably plays a vital role in its usefulness as a medicinal plant [15].

It was revealed that the plant extract was active against the selected test pathogens which comprised of both Gram-positive and Gram-negative bacteria indicative that the plant extract has a broad spectrum of activity since it inhibited both the growth of *Staphylococcus aureus*, a Gram- Positive Bacteria and *E. coli, Salmonella sp. Klebsiella* sp., and *Pseudomonas* sp. (Gram-negative bacteria. Zones of inhibition that are within the intermediate range (15 - 19 mm) to the susceptible range (≥ 20 mm) were observed thus

affirming the use of this plant as broad spectrum medicine and a strong basis for the development of novel drugs. This is based on the Clinical and Laboratory Standards Institute [16].

The results of the antibacterial screening also showed that at varying concentrations, the extracts had different activity on all the five organisms tested with the highest activity at a concentration of 512 mg/ml and the lowest activity at 4 mg/ml. The resistance pattern of antibiotic susceptibility test performed indicates that the isolates are multidrug resistant organisms [17]. Where it was observed that the Organisms were resistant to one or more antibiotics tested especially observed in E. coli which demonstrated resistance to all antibiotics employed as shown in Table 3.

There is a dire need to search for alternate sources of antibiotics as the upsurge in Bacteria resistance cannot be over emphasized and Bacteria resistance implies that they have antibiotic resistance genes which could either be chromosomal or plasmid borne. It has been well reported that plasmids are major vectors for the spread of both antibiotic resistance and virulence determinants among bacterial populace [18]. Genetic materials can be interchanged between bacteria population through transformation, conjugation or transduction processes or by mobile genes (transposons) and this has been highlighted as a major factor in the rapid evolution of microorganisms such as bacteria to gain resistance to antibiotics [19].

Conclusion

According to this study *Carica papaya* demonstrated broad spectrum antibacterial activity against all tested isolates - *Escherichia coli, Pseudomonas* species*, Klebseilla* species*, Staphylococcus aureus*, and *Salmonella* species. which is based on the Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility test interpretive category thus *Carica papaya* root part is a potential source of natural therapeutic drug that may be further exploited for the treatment of bacterial infection. However, it is recommended to investigate the therapeutic potentials of plants from the traditional African system of medicine as it could give a better insight on how best these plants can be used in the treatment of diseases and subsequently bridge the gap between the failing commonly used antibiotics and it is also important to study its pharmacology and encourage combination studies.

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