Antifungal and antibacterial activities of aqueous and methanolic root extracts of *Carica papaya* linn. (Caricaceae)

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The vast potentialities of plants as a source for anti-microbial drugs with reference to antibacterial agent, motivated the present systematic investigation to screen the aqueous and methanolic root extracts of *Carica papaya* (CPY) for its antimicrobial activity. Eleven microorganism species consisting of seven bacteria and four fungi were tested for their sensitivity to the herbal preparations using the Agar Diffusion method. Ampicillin and tetracycline were used as standard drugs for investigating the bacterial species, while griseofulvin was selected for the fungi, while zones of inhibition were measured to determine the microbicidal property of the test agents. Another set of plates was cultured to estimate the effect of combination therapy using the herbal drug together in varied concentrations with the standard drugs. The results obtained showed both extract to possess good antimicrobial activity against only four of the bacteria and three fungi. However, the organic preparation produced a significant and better efficacy than the water preparation. Combination therapy revealed a synergistic effect between CPY and ampicillin, whereas, antagonism was observed with tetracycline. A wide range of secondary metabolites were identified in both extract with methanolic extract containing a higher amount; Thin Layer Chromatography confirmed the presence of anthraquinones, cardiac glycosides and alkaloids. The study substantiates the folkloric use of CPY in microbial infections. Furthermore, its spectrum of activity has been documented as well as the results of its combination therapy.

Keywords: *Carica papaya*, extract, antibacterial, antifungal, microorganisms.

INTRODUCTION

Infectious diseases constitute the world's major threat to human health and account for almost 50,000 deaths daily (Ahmad and Beg, 2001). However, the general belief that advent of antibiotics would bring an end to the occurrence of infectious diseases was cut short with the advent of resistance to antimicrobial drug. The incidence and increasing frequency of microorganisms that are resistant to common and generally accepted effective first choice drugs is on the increase. The development of resistance to the newer antibiotics by the microbes causing most of the infectious diseases with debilitating effects made the case worse. (Adekunle and Adekunle, 2009). All these make the search for newer sources of antimicrobials a global challenge involving research institutions, pharmaceutical companies and academia (Melendez and Capriles, 2006).

Medicinal plant exemplified by *Carica papaya,* the plant studied in this work, is defined as any substance with one or more of its organs containing substances that can be used for therapeutic purposes or which can be used as precursors for the synthesis of antimicrobial drugs (Sofowora, 1982, 1984). It is estimated that there are about 250, 000 –500, 000 species of plants on earth (Borris, 1996), of which a relatively small percentage (1-10%) of these are used for food by humans and animals (Olowokudejo *et al*., 2008). Medicinal plants contain numerous biologically active compounds such as

carbohydrates, proteins, enzymes, fats and oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols, to mention a few which have medicinal activities.

Carica papaya Linn. (family Caricaceae), commonly called pawpaw (English), Ibepe (Yoruba-Nigeria) or Okroegbe (Igbo-Nigeria), is a tree-like herbaceous plant, widely cultivated for its edible fruits. It originated from Southern Mexico and Costa Rica and was subsequently introduced as a plantation crop in Australia, Hawaii, Phillipines, Sri-Lanka, South Africa, India and in all tropical and subtropical regions (Canini *et al.*, 2007). It is commonly known for its food and nutritional values throughout the world; the leaves fruits and latex obtained from papaya plant are used medicinally and for various purposes (Krishna *et al*., 2008).

It is also employed as a therapeutic remedy, for example, the fruit contains certain immune-stimulating and anti-oxidant agents (Aruoma *et al.*, 2006), immature fruits and roots are used for their abortifacient activity (Cherian, 2000; Sharma and Mahanta, 2000); the seeds are used as a potential post-testicular antifertility drug (Lohiya *et al.*, 2000, 2005 & 2006); the pulp is used by African hospitals for treating wounds and burns (Starley *et al.*, 1999). The latex and the seeds are used in the treatment of gastrointestinal nematode infections and they have shown anthelmintic activity (Stepek *et al*., 2005); and the seeds and immature fruit have shown inhibitory activity against human enteric pathogens (Osato *et al*., 1993; Afolayan, 2003; Krishna *et al*., 2008). The leaves are used to relieve symptoms of asthma and as a vermifuge, in the treatment of gastric problems, fever and amoebic dysentery. However, water, acetone and ethanol extracts of papaya leaves showed no microbicidal activity (Nkuo-Akenji *et al.*, 2001; Dawkins *et al.*, 2003; Leite *et al.*, 2005; Nayak *et al.*, 2007).

The present study aimed at investigating the antimicrobial potential and spectra of both aqueous and methanolic extracts of the dried root of *Carica papaya* and also, compared its efficacy with standard antibacterial as well as explored the resultant effect of its combination therapy with each of the standard drugs employed in this study. The latter became imperative because some indigenes always believe that employing both orthodox and traditional medicaments simultaneously will always produce synergy.

Microorganism Isolates

Clinical isolates and standard strains of the following organisms obtained from the Pharmaceutical Microbiology Department of the University of Lagos and Mycology Laboratory, University Teaching Hospital, both institutions in Lagos, Nigeria.

Pseudomonas aeruginosa	ATCC 27853
Salmonella typhi	NCTC 8571
Eschericia coli	NCTC 10418
Staphylococi aureus	ATCC 25923
Bacillus subtilis	Clinical isolate
Shigella dysenterium	Clinical isolate
Klebsiella aerogenes	Clinical isolate
Candida albicans	Clinical isolate
Trichophyton rubrum	Clinical isolate
Epidermophyton floccosum	Clinical isolate
Microsporum audouini	Clinical isolate

Pure isolates were obtained by culturing on the respective selective media and biochemical tests performed to re-identify and confirm the identity of the isolates. Fresh plates of 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 labelled sterile bottles and incubated at 37 ° C prior to antimicrobial susceptibility testing. The fungi species were similarly treated, but cultured on Sabouraud dextrose medium (Oxoid, England).

Plant Collection

Fresh pawpaw root was obtained from Ikotun farm in Lagos- Nigeria and was authenticated in the Department of of Pharmacognosy, Faculty of Pharmacy, University of Lagos, where a voucher specimen number PCG 403 was allotted.

Preparation of Extract

Two extracts of CPY root were prepared for study as described below:

Methanolic Extract

Pawpaw roots were chopped into tiny bits, thoroughly rinsed in water to remove sand particles, drained for a few hours and oven dried at 45 °C for 3 days to obtain a constant weight. The dried plant material was thereafter blended with the aid of an industrial blender, into fine powder. Two hundred grams of the latter was weighed and extracted with 70 % (250 ml) methanol, using Soxhlet extractor.

The extract was dried using Rota-evaporator and the dried extract's weight was thereafter recorded as 41.75 g, giving 20.9 % yield.

Aqueous Extract

The above procedure was repeated with distilled water replacing methanol. A % yield of 29.5 was recorded.

Phytochemical Screening

Both the methanolic and aqueous extracts were screened for presence of secondary metabolites, using the methods of Sofowora (1984) and Farnsworth (1989).

Thin Layer Chromatography (TLC)

Glass plates (20×20 cm) were washed and dried in an oven after which, they were arranged in the TLC board and wiped with acetone.

Thirty grams of silica gel was dissolved in 60 ml distilled water and the slurry poured into the TLC spreader that has been adjusted to 0.25 mm thickness. The spreader was then used to spread the slurry on the plates. The latter were allowed to air-dry before handling and afterwards, they were arranged in a rack and placed in the oven at 100 °C for 1 h.

For experimentation, the plates were kept in the TLC guide and the sample, which was already dissolved in chloroform was spotted on the plate against the reference, using a capillary tube. The solvent font was marked with the aid of a sharp pencil at 15 cm from the spots. Atropine, frangula and cholesterol served as reference samples for identifying alkaloids, anthraquinones and cardiac glycosides respectively.

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

The MIC helps to measure more exactly the concentration of an antibiotic necessary to inhibit growth of standardized inocula under defined conditions (Geo *et al*., 2001).

Serial dilutions of inocula 1×10^{1} -1×10^{8} were made in sterile nutrient broth and the dilution with a visual density equivalent to 10^{6} cfu/ml, which corresponded to 0.5 MacFarland standard was employed in the study (Adebayo *et al.*, 1989).

Appropriate volumes of CPY were added to sterile tubes containing the nutrient broth to give a final concentration of 1 mg/ml to 512 mg/ml, and using a volumetric pipette, an aliquot of the test bacterial and fungal broth cultures were added into each of the tubes. The tubes containing bacterial cultures were incubated at 37°C for 24 h, while fungi tubes were kept at the same temperature but for 48 h. The tubes were read macroscopically to determine the lowest concentration of CPY that did not permit any visible growth when compared with that of the control. Tetracycline and ampicillin served as positive control for the bacterial species, while griseofulvin was selected for the fungi.

Minimum Bactericidal Concentration was determined 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. Control plates containing agar alone were also streaked with the respective organisms. All plates were incubated as before and the concentration at which no visible growth was seen was noted as the MBC.

Antimicrobial Sensitivity Test (Agar Diffusion Method)

The standard dilution of 10^{6} cfu/ml was seeded evenly onto the surface of Mueller Hinton agar (Oxoid, England) plates (15 cm) in triplicates with a sterile swab (Hugo and Russell, 1998). Using a sterile 6 mm diameter cork borer, 4 wells were made in the agar into which appropriate concentrations (10, 20, 40 and 80 mg/ml) of CPY aqueous and organic extracts were added as well as the standard drugs which served as the controls. The plates were incubated as before and the zones of inhibition measured in millimetres to determine antimicrobial activity. For each microorganism species, controls were incubated using pure solvents instead of CPY (Parekh and Chanda, 2007 a, b). The zone diameters of controls were subtracted from the test zones and the resulting diameters presented as ± SEM (Table 2).

A similar procedure was repeated with fungi using standard reagents

Combination Therapy

The study was designed to determine the resultant effect of combination of the extract with each of the standard drugs, as well as determining the effective combination ratio. The extract concentration employed in this work was 80 mg/ml, since it produced the highest inhibition. The various extract:control drug ratio used were 50:50, 60:40, and 70:30. The agar diffusion method earlier described was used and the zone diameter was measured as before. The fourth combination of 0:80 contained only the standard drug (80 mg/ml).

RESULTS

Thin Layer Chromatography

The R_f values recorded for the extract are the same as the reference agents-atropine, frangula and cholesterol, which confirmed the presence of alkaloids,

Table 1: Minimum Inhibitory Concentration (MIC) of CPY extracts

Microorganisms	CPY conc mg/ml											
	8		4		2		1		0.5		0.25	
	AQE	MEE	AQE	MEE	AQE	MEE	AQE	MEE	AQE	MEE	AQE	MEE
Pseudomonas aerogenosa	+	+	+	+	+	+	+	+	+	+	+	+
Salmonella typhi	+	+	+	+	+	+	+	+	+	+	+	+
Eschericia. Coli	-	-	-	-	-	-	-	-	-	-	+	+
Staphylococci aureus	-	-	+	-	+	-	+	+	+	+	+	+
Bacillus subtilis	-	-	+	-	+	-	+	+	+	+	+	+
Klebsiella aerogenes	-	-	+	-	+	-	+	+	+	+	+	+

AQ, ME = Aqueous and Methanolic extract respectively; - no growth, + growth

Table 2: Antibacterial activity of test agents

Microorganism/Drug concentration (mg/ml)	Drugs /Zone of Inhibition (mm)			
	AQE	MEE	AMP	TET
Staphylococcus aureus				
10	24.0±2.88*	26.5±1.67*	26.8±2.13*	13.0±1.13
20	26.0±3.08	28.5±2.49	28.5±2.66	14.7±1.07
40	28.0±2.94	30.7±2.31	30.2±2.58	16.7±1.98
80	30.0±1.81	32.7±1.77	32.5±3.42	18.8±2.17
Bacillus subtilis				
10	21.5±1.45*	23.0±2.33*	30.0±3.19*	16.5±2.56
20	23.5±1.80	25.0±2.11	32.0±2.38	18.5±2.66
40	25.1±1.87	26.5±2.34	34.0±3.62	20.0±2.39
80	27.1±2.22	28.5±3.48	36.0±3.08	22.0±2.90
Klebsiella aerogenes				
10	12.5±1.60	13.8±1.07	16.7±1.63*	9.5±1.15
20	14.7±2.30	16.0±2.04	18.0±1.47*	11.8±1.25
40	16.8±1.21	17.8±2.04	20.1±2.92	13.5±2.10
80	18.5±3.06	20.0±1.70	22.5±3.38	15.2±1.52
Escherichia coli				
10	13.1±1.11	14.0±1.90	15.1±1.59	10.1±0.27
20	14.8±1.17	16.2±1.42	17.5±2.28	12.6±1.65
40	17.0±3.19	18.1±2.69	19.0±1.41	14.2±0.70
80	19.0±1.54	20.0±2.05	21.2±2.24	16.1±1.13

AQE, MEE, AMP, TET = Aqueous extract, Methanolic extract, Ampicillin, Tetracycline
*p<0.05when compared with tetracycline

anthraquinones and cholesterol similar to the reference in the extract. These phytoconstituents were also inferred in the phytochemical screening carried out.

Minimum Inhibitory Concentration

Out of the seven bacterial species explored, only *E. coli, Staph, Bacillus and Klebsiella* were sensitive to CPY, with *E. coli* recording 0.5 mg/ml MIC for the two extract preparations. The remaining sensitive bacteria recorded 8.0 mg/ml and 2.0 mg/ml for aqueous and methanolic extract respectively (Table 1). All the bacterial species were sensitive to the standard drugs with MIC recorded as ranging from 1.56-100 µg/ml and 18-1250 µg/ml (not shown) respectively for ampicillin and tetracycline.

Antimicrobial sensitivity tests

Bacterial species

Trend of antimicrobial activity recorded in all the four bacterial species that were sensitive to CPY was tetracycline < aqueous extract < ethanolic ≤ ampicillin (Table 2); both the extract of CPY produced significant

Table 3: Antifungal activity of test agents

Microorganism/Drug concentration mg/ml	Drugs /Zone of Inhibition (mm)		
	AQE	MEE	GRS
Trichophyton rubrum			
12.5	11.5±0.31	11.5±0.33	16.0±1.67
25	14.0±1.92	15.0±1.40	21.5±3.08
50	16.0±2.48*	18.7±3.75*	25.5±4.19
100	18.0±2.15*	22.0±3.56*	30.5±4.86
200	21.5±2.74*	26.0±3.37*	35.0±2.33
400	23.7±3.05*	29.8±3.53*	39.5±4.20
Epidermophyton floccosum			
12.5	-	10.8±1.26	13.7±1.40
25	-	12.5±1.83	16.5±2.06
50	3.8±0.05	14.2±1.52	20.0±3.35
100	6.5±0.91	16.0±2.29*	23.0±4.07
200	8.7±0.46	17.5±3.02*	26.0±4.81
400	11.5±1.22*	19.6±3.30*	29.0±3.28
Microsporum audouinii			
12.5	-	5.0±0.13*	12.0±1.11
25	2.8±0.05	8.0±0.29*	15.0±1.60
50	7.5±0.46	12.5±1.56	17.7±2.48
100	11.6±1.37*	16.5±2.55	21.0±3.08
200	15.5±2.30*	20.0±3.85	24.0±4.92
400	19.8±2.03*	24.6±3.46	26.8±4.71

AQE, MEE, GRS = Aqueous extract, Methanolic extract, Griseofulvin
*p<0.05 when compared with control

($p < 0.05$) effect when compared with tetracycline.

Fungal species

While griseofulvin was significantly more effective than CPY ($p<0.05$), sensitivity of the three fungi to herbal drug was *T. rubrum* > *E. floccosum* > *M. audouini* (Table 3).

DISCUSSION

Papaya contains many biologically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion. The level of the compounds varies in the fruit, latex, leaves and roots.

It has been used for treating digestive problems and intestinal worms. The softening qualities of papain have been taken advantage of, in the treatment of warts, corns, sinuses, and chronic forms of scaly eczema, cutaneous tubercles, and other hardness of the skin, produced by irritation. Papain also is used to treat arthritis (Krishna *et al.,* 2008).

The presence of bioactive substances has also been reported to confer resistance to plants against bacteria, fungi and pests (Srinivasan *et al.*, 2001), and therefore explains the demonstration of antibacterial activity of CPY studied in the present work.

The aqueous and methanolic extracts investigated revealed the presence of alkaloids, flavonoids, anthraquinones, tannins, cardiac glycosides, and reducing sugars; however, all the phytoconstituents were more in the alcoholic extraction than the aqueous, as indicated by the intensity of the different confirmatory colours. It has been earlier reported by Tona *et al.*, 1998 and Topuriya *et al.*, 1978 that the active ingredients vary from one extract to another, which could be due to difference in solubility of the active components in the various solvents (de Boer *et al.*, 2005).

The thin layer chromatography further confirmed the presence of alkaloids, anthraquinones and cardiac glycosides.

The efficacy of treatments with *Carica papaya* is dependent on the quantity of the different compounds in the preparation. The quantity of the compounds differ in the fruit, latex, leaves, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree (Wagh *et al*., 1993). Latex (with a minimum protein concentration of 138 µg/ml) and root extracts inhibited *Candida albicans*. However, aqueous

Table 4: Effect of drug-combination on microorganisms

Microorganisms/Drug Concentrations (mg/ml)	Drug/Zone of Inhibition (mm) MEE:AMP	MEE:TET
Staphylococcus aureus		
ext 0:control 80	39.5±8.32	29.5±6.74
ext 50:control 50	40.2±10.51	25.5±5.60
ext 60:control 40	42.5±7.16	20.5±5.11
ext 70:control 30	38.0±6.33	18.5±3.08*
Bacillus subtilis		
ext 0:control 80	30.5±7.98	19.3±3.50
ext 50:control 50	31.5±8.24	16.0±2.47
ext 60:control 40	32.0±9.55	14.0±2.26
ext 70:control 30	29.5±6.81	12.0±1.38*
Klebsiella aerogenes		
ext 0:control 80	22.8±3.16	19.0±2.70
ext 50:control 50	23.8±4.49	15.0±2.63
ext 60:control 40	28.0±5.96	13.0±2.48*
ext 70:control 30	22.0±3.07	11.7±1.59*
Escherichia coli		
ext 0:control 80	26.0±4.18	17.8±3.11
ext 50:control 50	26.2±5.36	17.0±2.99
ext 60:control 40	30.2±6.62	15.9±2.31
ext 70:control 30	21.5±3.87	10.2±2.10*

MEE:AMP, MEE:TET = Methanolic extract:Ampicillin and tetracycline combination respectively.
*p<0.05 when compared with control

extracts were not active. Extracts of pulp and seeds showed inhibitory properties when tested against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus subtilis*, and other bacteria *in vitro* (Osato *et al.*, 1993), and Alpha-D-mannosidase and N-acetyl-beta-D-glucosaminidase (isolated from latex) acted synergistically to inhibit yeast growth (Grand 1989).

On the other hand, both aqueous and organic extracts of CPY leaves did not show any antibacterial property (Leite *et al*., 2005).

Both the root extracts of CPY were found to possess antibacterial property, which is in congruence with the report of Doughari *et al*., (2007). The study also featured a similar spectrum of activity with the two different extracts; however, the alcoholic extract displayed superiority in all the tests conducted. MIC and MBC values were found to be equal for the sensitive bacteria; thus, suggesting that evaluation of MIC is sufficient for measuring bactericidal activity (Natrajan *et al.*, 2003; Pundir *et al.*, 2010). Furthermore, significant ($p<0.05$) zones of inhibition were recorded as ranging from 21.5mm to 30.0 mm for the two gram positive organisms at concentrations of 10-80 mg/ml explored whereas, gram negative bacteria ranged between 12.5mm-19.0mm at the same concentrations (Table 2). The extract was found to demonstrate a good antibacterial activity zone of inhibition with a measurement of greater than or equal to 10 mm indicating good antibacterial activity (Kujumgiev *et al*., 1999). The study showed a better sensitivity to CPY by gram positive organisms. The latter finding is in consonance with the report of Jigna and Sumitra, (2006), Palombo and Semple, (2001) Matu and van Staden (2003). It is noteworthy that the antimicrobial activity of the alcoholic extract compared well with the standard drug ampicillin and produced a greater efficacy than tetracycline (Table 2).

A similar trend of activity was observed in the evaluation of antifungal property of CPY ($p<0.05$) (Table 3). However, CPY root extract as demonstrated in the study is only effective in combating fungal infections of the skin and keratinized tissues and not against those of the mucous membrane or systemic infections due to Candida spp.

It has already been established that combining a bacteriostatic agent with a bactericidal will produce antagonism, and since tetracycline is bacteriostatic in its activity, it could be inferred that CPY is bactericidal, the property, which could also account for its synergism with ampicillin. The latter speculation is in consonance with the report of Giordiani *et al.*, (1997), who reported that, the latex of papaya and fluconazole possess synergistic effect on *C. albicans* destruction, which is as a result of

partial cell wall degradation, a bactericidal effect.

The scope of the present study has not directly evaluated the mechanism of antimicrobial activity of CPY, but a few deductions could be made from the fore-going.

Papaya preparations have proved to possess antioxidant property (Aruoma *et al.*, 2006; Mehdipour *et al.*, 2006; Marotta *et al.*, 2007). Antibacterial activity of papaya could be correlated to its scavenging action on superoxide and hydroxyl radical, which could be part of cellular metabolism of the enteropathogens (Osato *et al.*, 1993). The latter mechanism could be attributed to flavonoids, which are free radical scavengers, and antioxidation could be one of the mechanisms for antibiotic activity of CPY. Alkaloids and tannins were also reported by Cowan (1999) and Draughon (2004) to produce antibacterial action.

The immune-stimulating property of the fruit (Aruoma *et al*., 2006) may also be shared by the root, in which case, increased levels of immune cells would stimulate immune response to infection (Agbaje *et al.,* 2006).

CONCLUSION

The demonstration of activity against the test microorganisms 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, the property which is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms.

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