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COMPARATIVE STUDIES OF ANTIMICROBIAL PROPERTIES ON EXTRACTS OF FRESH AND DRIED LEAVES OF *Carica papaya* ON SELECTED CLINICAL BACTERIAL AND FUNGAL ISOLATES

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ABSTRACT

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 ingredient invaluable in the management of many infectious and communicable diseases. The frequency and diversity of life-threatening infection caused by pathogenic micro-organisms has increased steadily worldwide and it is becoming an important cause of morbidity and mortality in immune-compromised patients especially in developing countries. The efficacy of treatments with *Carica papaya* is dependent on the quantity of the different chemical substances present in the preparation. The antibacterial and antifungal activity of both fresh and dried leaves of *Carica papaya* against bacteria and fungus of medical importance was carried out. The aqueous and ethanol extract of both dried and fresh leaves were tested at 25, 50 and 100mg/ml concentration on both bacteria and fungi isolate using the disc diffusion method. Result showed significant broad spectrum antimicrobial activity against Gram positive and Gram negative bacteria and fungus. The aqueous extract was more effective than the ethanol extract for the bacterial activity while for the fungal activity, the ethanol extract was more effective than the aqueous extract. The fresh leaf extract was potent against some of the bacteria which standard antibiotic were not able to inhibit. *Carica papaya* leaves showed a better antifungal activity than the antibacterial activity. Demonstration of antimicrobial activity against the test isolates is an indication that there's possibility of sourcing alternative antibiotic substances in this plant for the development of newer antimicrobial agents.

Keywords: *Carica Papaya*, Antibacterial, Antifungal, Disc Diffusion

INTRODUCTION

The frequency and diversity of life-threatening infections caused by pathogenic micro organisms increased steadily worldwide and it is becoming an important cause of morbidity and mortality in immune-compromised patients especially in

developing countries (Al-Bari, *et al.,* 2008). Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths every day. Emergence of resistant strains of pathogenic micro organism has also continued to post a major health concern about the efficacy of several drugs; most importantly antibiotics in current use (Timothy, 2011). This increasing rate of development of resistance to commonly used antibiotics has lead to the search for newer more effective affordable and readily available sources in particular from medicinal plants (herbs) (Adekunle, 2009). 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 ingredient invaluable in the management of many contractable 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). Medicinal plants are reservoirs of various metabolites and provide unlimited source of important chemicals that have diverse biological properties and represents a rich source from which antimicrobial agents can be obtained. (Timothy, 2011).The antimicrobial properties of plant have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana, *et al,* 2007).

Carica papaya belongs to the family of caricaceae, and several species of caricaceae have been used as remedy against a variety of disease. (Mello, *et al,* 2008). Papaya offers not only the luscious taste but is a rich source of antioxidant nutrient such as carotenes, vitamin c and flavonoids, the B vitamins, foliate and panthothenic acid, and the minerals Potassium and magnesium, and fibre. (Suleiman, 2011).Together, these nutrients promotes the health of the cardiovascular system and also provides protection against colon cancer. The fruit is valued for its proteolytic enzymes including papain, which is used like bromelian, a similar enzyme found in pineapple, to treat sports injuries, other consist of trauma and allergies (Annie, *et al*, 2005). Biochemically, its leaves and fruits are complex, producing several proteins and alkaloids with important pharmaceutical and industrial applications (Elmoussaoui, *et al*, 2001). Carapine, an alkaloids present in papaya can be used as a heart depressant, amoebicidé and diuretic. The fruit and juice are consumed for gastrointestinal ailments; a fresh leaf poultice is used to treat sores. The fresh root with sugarcane alcohol can be taken orally or as a massage to soothe rheumatism. A flower decoction is

taken orally for coughs, bronchitis, asthma, and chest colds. In some countries the seeds are used as abortifacient and vermifuge. Medical research in animals has confirmed the contraceptive and abortifacient capability of papaya (Calzada *et al*, 2007). The seeds of *Carica papaya* have antimicrobial activity against *Trichomonas vaginalis*. It could also be used in urinogenital disorder like trichomoniasis with care to avoid toxicity (Calzada, *et al,* 2007). The seeds irrespective of its maturity stages have bacteriostatic activity on gram positive and negative organisms which could be useful in treating chronic skin ulcer. This research work aimed at investigating the antibacterial and antifungal activity of both fresh and dried leaves of *Carica papaya* against bacteria and fungi of medical importance.

MATERIALS AND METHODS

Clinical bacterial isolates used in this study was obtained from the department of medical microbiology and parasitology, Sacred Heart Hospital, Lantoro Aboekuta. Fresh and dried leaves of Carica papaya were dried ground into powdery form; this was kept for various extractions.

Phytochemical Screening

Test for Alkaloid

5g of the powdered leaf of *Carica papaya* was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. 1ml of filtrate was treated with few drops of dragend off's reagent. Blue–black turbidity served as preliminary evidence of alkaloids.

Test for Saponin

5g each of the powdered leaf of *Carica papaya* was shaken with distilled water in a test tube .Frothing which persisted on warming was taken as preliminary evidence.

Test for Tannin

5g of powdered leaf was stirred with 100ml distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black or blue-green precipitate determined the presence of Tannins (Trease and Evans, 1989).

Test for Flavonoids

5ml of diluted ammonia solution was added to 5g of powdered leaf of *Carica papaya* followed by the addition of concentrated H_2SO_4.A yellow colouration determined the presence of flavonoids.

Preparation of Disc

Paper puncher was used to punch to punch Whitman no 1 filter paper to produce 5mm disc. The discs were sterilized by hot air, the funded filter paper placed in an oven at 180 ^{0}C for 10minutes. They were stored in a sterile container until ready for use.

Agar Diffusion Test

Nutrient agar was inoculated with the test organism, adjusted to 10^6 using 0.5 McFarland standards. The discs were soaked in the extract, the socked discs were applied over the seeded nutrient agar plates at equidistance and the control placed at their middle. The plates were incubated at 37°C for 24hrs. After the incubation period the inhibition zone diameter around the disc was measured to the nearest ml using a transparent ruler, this indicates a positive antibacterial activity of the respective extracts. Each experiment was carried out in triplicates.

Minimum Inhibition Concentration (MIC)

The MIC was determined according to Ochei and Kolhatkar (2008) using, microtubes dilution method. Twelve sterile test tubes were set up the 0.5ml diluent was introduced into the tubes with a micropipette starting from tube 2 to tube 12, then 0.5ml of the extract was added to tube 2 to tube 11, after which one drop each of the organisms were added to each of the tube to 11. The micro-titer plate was incubated at 37°C for 24hrs and observed for visible growth. The lowest concentration at which no detectable bacterial growth occurred was considered as Minimum Inhibitory Concentration (MIC).

Minimum Bactericidal Concentration (MBC)

MBC was determined as described by Ochei and Kolhatar (2008).Subculture was made from the last tube showing no turbidity which indicates no growth, to the previous tubes showing no growth on blood agar. The plate was incubated at 37^{0}C for 18 – 24 hours and observed for growth. The tube showing no growth is indicated as minimum bactericidal concentration.

Determination of Minimum Bactericidal and Fungicidal Concentration (MBC& MFC)

The MBC and MFC were determined by Ochei and kolhatar (2008). Subculture was made from the last tube showing no turbidity which indicates no growth, to the previous tubes showing no growth on blood agar. The plate was incubated at

37^0c for 18 – 24hrs and observed for bacteria and fungal growth. The tubes showing no growth were indicated as minimum bacteria concentration and minimum fungicidal concentration respectively.

STATISTICAL ANALYSIS

The data obtained were subjected to analysis of variance (ANOVA)

RESULTS AND DISCUSSION

Table 1: Phytochemical Screening of *Carica papaya*

Parameter	Fresh Ethanol	Fresh Aqueous	Dried Ethanol	Dried Aqueous
Alkaloid	+	+	++	+
Flavonoid	+	-	++	+
Saponin	+	+	+	+
Tannins	+	-	++	+
Steroid	++	+	+	+
Glycoside	+	+	+	+
Anthraquinone	+	+	+	-

Legend: + /++ means present, - means absent

Table 2: Zone of Inhibition (MM) of extracts of Fresh and Dried Leaves of *Carica papaya* on Bacteria Isolates

Organisms	Extraction method: (dried) mg/ml: Mean, mm						Extraction Method (Fresh) mg/ml: mean, mm						
	Ethanol			Aqueous			Ethanol			Aqueous			
	100	50	25	100	50	25	100	50	25	100	50	25	Control
Staphylococcus aureus	Nil	Nil	Nil	8	Nil	Nil	8	Nil	Nil	8	Nil	Nil	21
Shi gella spp	Nil	Nil	Nil	7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	21
Escherichia coli	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	7	Nil	Nil	16
Salmonella sp	Nil	Nil	Nil	Nil	Nil	Nil	7	Nil	Nil	7	Nil	Nil	19

Anti-bacterial activities of dried extract of *Carica papaya* leaves are shown in table 2. Aqueous extract of dried leaves of *Carica papaya* recorded zone diameter of 8mm and 7mm against *Staphylococus aureus* and *Shigella sp*, respectively, while ethanol extract recorded zero zone diameter against all the test organisms. The control antibiotic (ciprofloxacin) recorded 21mm, 21mm, 16mm and 19mm on *Staphylococus aureus*, *Shigella sp*, *Escherichia coli* and

Salmonella sp respectively. Aqueous extract of fresh leaves of *Carica papaya* recorded zone diameter of 8mm, 7mm and 7mm against *Staphylococus aureus*, *Escherichia coli* and *Salmonella sp* respectively while Ethanol extract recorded zone diameter of 8mm and 7mm against *Staphylococus aureus* and *Salmonella sp* respectively.

Table 3: Zone of Inhibition (MM) of Fresh and Dried Leaves of *Carica Papaya* on Fungal Isolates.

Organism	Extraction Method Dried extract mg/ml, mean, mm						Extraction Method Fresh extract mg/ml; mean, mm						
	Ethanol			Aqueous			Ethanol			Aqueous			
	100	50	25	100	50	25	100	50	25	100	50	25	Control
Candida albican	12	8	Nil	9	6	Nil	8	6	Nil	Nil	Nil	Nil	21

The antifungal activity of dried extract of *Carica papaya* leaves is shown in Table 3. The aqueous extract of dried leaves of *Carica papaya* recorded zone diameter of 9mm and 6mm at 100 and 50% concentration respectively against *Candida albicans* while the Ethanol extract recorded zone diameter of 12mm and 8mm at 100 and 50% concentration against *Candida albicans*. The antifungal activity of fresh extract of *Carica papaya* leaves is shown in table3. The ethanol extract of fresh leaves of papaya recorded zone diameter of 8mm and 6mm at 100 and 50% concentration respectively against *Carica albicans*, while the aqueous extract recorded zero zone diameter against *Carica albicans*. The control antibiotic (fluconazole) recorded 21mm.

Table 4: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Dried and Fresh Leaves of *Carica Papaya*.

Organism	Extraction Method Dried extract, mg/ml;				Extraction Method Fresh Extract, mg/ml					
	Ethanol		Aqueous		Ethanol		Aqueous		Control	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	100	100	50	100	50	100	50	100	12.5	12.5
Shigella	100	100	50	100	100	100	100	100	12.5	12.5
Escherichia coli	100	100	100	100	100	100	50	100	6.25	12.5
Salmonella sp	100	100	100	100	50	100	100	100	12.5	12.5

Control – Ciprofloxacin

Minimum inhibitory and minimum bactericidal concentration of dried leaves of *Carica papaya* is shown in table 4. The aqueous extract of *Carica papaya* recorded MIC and MBC of 50mg/ml and 100mg/ml against *S.aureus and shigella sp* respectively while the ethanol extract recorded MIC and MBC of 100 against all the test organisms. The aqueous extract of fresh leaves of C.papaya shown in table 4 recorded MIC of 50 mg/ml against *S.aureus, E.coli and salmonella sp* while MBC recorded of 100mg/ml. The ethanol extract of fresh leaves recorded MIC of 50mg/ml against *S.aureus* and Salmonella sp while other test organisms recorded MIC of 100mg/ml. All the test organisms recorded MBC of 100mg/ml

Table 5: Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of Dried and Fresh Leaves of *Carica Papaya*

Organism	**Extraction Method Dried extract, mg/ml**				**Extraction Method Fresh extract, mg/ml**					
	Ethanol		**Aqueous**		**Ethanol**		**Aqueous**		**Control**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Candida albican	*25*	*50*	*50*	*100*	*50*	*100*	*100*	*100*	*6.25*	*12.5*

The minimum inhibitory and minimum fungicidal concentration of dried leaves of *C.papaya* is shown in table 5. The aqueous extract of fresh leaves recorded 100mg/ml for MIC and MBC against *C.albican* and ethanol extract recorded 50mg/ml for MIC and 100mg/ml for MBC. The control antifungal (fluconazole) recorded MIC of 6.25mg/ml and MBC of 12.5mg/ml. Plant products particularly extract of various plant parts have been used extensively as natural antimicrobial. The presence of bioactive substances been reported to confer resistance to plant against bacteria and fungus and therefore explains the demonstration of antimicrobial activity by the plant extract used in this study (Srinivasan, *et al.*, 2001). Result of this study revealed significant antimicrobial activity with the extract demonstrating broad spectrum of activity against both bacteria (*Staphylococcus aureus* – gram-positive, *Shigella sp*, *Escherichia coli*, *Salmonella sp*, gram-negative) and fungus (*Candida albicans*). The organisms used in this study are associated with various form of infections in humans. The bacteria are associated with skin infections such as boils, impetigo cellulitis (s.aureus), dysentery that results in the destruction of the epithelial cells of the intestinal mucosa in the caecum and rectum (*Shigella sp*), gastro-intestinal infections, dysentery and urinary tract infections (*Escherichia coli*), typhoid fever, paratyphoid fever, botulism (*Salmonella sp*). However the fungus (*C.albican*) is associated with candidiasis (Prescott, *et al.*, 2002). The

demonstration of activity against all these micro-organisms had shown that *C.papaya* can be used to produce raw materials/substance for further development of diverse antibiotics with broad spectrum activity.

The bacterial test of this study demonstrated that the aqueous extract was more effective than the ethanolic extract. This may be due to the fact that the organic extract (ethanol) has inhibitory activity against the antibacterial component of the extract (Boer, *et al.,* 2005). The result further showed that the fresh and dried samples were effective against gram-positive and gram-negative bacteria, but the fresh sample inhibited more bacteria than the dried sample. The fact that the fresh and dried samples extracts were active against both gram-positive and gram-negative bacteria tested may indicate a broad spectrum of activity. This result is very significant because of the possibility of developing therapeutic substances that may be more active against multidrug resistant organisms. This observation is in accordance with the reports of Doughari *et al* (Doughari, *et al.,* 2007) and Alo *et al.,* 2012. In the fungal test, the study demonstrated that the organic extract was more effective than the aqueous extract. This may be due to better solubility of active component in the organic solvent (Boer, *et al.,* 2005). Furthermore, *Carica papaya* leaves showed a better antifungal activity than the antibacterial activity. The zone of inhibition of the fungus was the highest of the various zones of inhibition recorded in this study. This therefore suggests that this plant part is better used for the treatment of the studied bacteria. The efficacy of treatments with Carica papaya is dependent on the quantity of the different chemical substances varies in the fruit, latex, leaves and roots and varies with the extraction method, age of the plant part and the cultivator and sex of the tree (Wagh, *et al.,* 1993). The low minimum inhibitory concentration (MIC) value observed for C.albican is a good indication of high efficacy against this fungus (Doughari, *et al.,* 2006).

Although the mechanism of action is not understood, it has been proposed that its action against the bacteria and fungi may be due to the inhibition of cell wall formation in the cell resulting in leakage of cytoplasmic constituents by the bioactive components of the extract (Bias, *et al*, 2002). While phytochemical compounds such as tannin coagulate the wall proteins, saponins facilitate the entry of toxic material or leakage of vital constituents from cell (Musa *et al.,* 2011). Flavonoids inhibit the activity of enzymes (Musa, *et al.*, 2011) by forming complexes with bacterial cell walls.

CONCLUSION

In conclusion, plant-based antimicrobials have enormous therapeutic and preferential potential. They can serve the desired purpose with lesser side effect that is associated with synthetic antimicrobials. The antimicrobial activity of *Carica papaya* leaves was demonstrated in this study. Demonstration of antimicrobial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents.

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