

Antibacterial Activity of Seed and Leaf Extract of *Carica Papaya* var. *Pusa dwarf* Linn

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Abstract: The study dealt with the antibacterial activity of aqueous, chloroform extract of leaves and aqueous, methanolic extract of seeds of *Carica papaya* var. *pusa dwarf* through agar well diffusion assay against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and *Salmonella typhi*. It was revealed that the aqueous as well as the methanolic extract of seeds were effective to inhibit the bacterial pathogens while in case of chloroform extract of *Carica papaya* leaves did not show any inhibition against the bacteria and the aqueous leaf extract was potent to inhibit them.

Key words: *Carica papaya* var. *pusa dwarf*, antibacterial activity, seed extract, leaf extract, bacterial pathogens

I. Introduction

Carica papaya belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases (Alabi *et al.*, 2012). *Carica papaya* is a neotropical plant having a wide range of pharmacological activities. The whole plant has its own medicinal value. Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of three powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber (Aravind *et al.*, 2013).

The black seeds of the papaya are edible and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper. Dried papaya seeds actually look quite similar to peppercorns and can be used in just the same way. Grinding a couple over a meal, especially protein rich meals, is a simple way to add extra enzymes to your diet and improve your digestive health. The papaya seeds are very pungent and peppery, making them almost unpalatable. However the seeds seem to have more potent medicinal values than the flesh. Papaya seeds have antibacterial properties and are effective against *E. coli*, *Salmonella* and *Staphylococcus infections*. Papaya seeds may protect the kidneys from toxin induced kidney failure. Papaya can eliminate intestinal parasites. Cure for piles and typhoid and anti-helminthic and anti-amoebic properties (Aravind *et al.*, 2013). The seed of papaya has antimicrobial activity against *Trichomonas vaginalis* trophozoites. It could also be used in urogenital disorder like trichomoniasis with care to avoid toxicity. The seeds, irrespective of its fruit maturity stages have bacteriostatic activity on gram positive and negative organisms which could be useful in treating chronic skin ulcer. However, little information exists on the antimicrobial property of *C. papaya* dried and fresh leaves (Alabi *et al.*, 2012). Recently, antifertility (Lohia *et al.*, 1999) antihelminthic, (Satrija *et al.*, 1995) and anti-inflammatory activity (Oladunmoye, and Osho, 2007) have been reported. CP seeds possess moisture, proteins, fatty acids, and phospholipids, such as phosphatidylcholine and cardiolipin. Other compounds present in seeds are carpapain, benzyl isothiocyanate, benzyl glucosinolate, beta-sitosterol, caricin, enzyme myrosin. The well-studied proteinases from papaya are papain, chymopapain, caricain, and glycyloendopeptidase. Papain occurs in all parts of the tree except the root (Anonymous, 1992). Fruit and seed extracts have antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichiacoli*, and *Pseudomonas aeruginosa* (Tang *et al.*, 1972; Emeruwa, 1982). The juice is used for curing warts, cancer, and tumors. Leaves have been poulticed into nervous pains, elephantoid growths (Asolkaret *et al.*, 1992). The antihyperglycemic effect of unripe mature fruits and seeds of CP have also been reported (Olagunja, et al 1995; Adeneye and Olagunja, 2009).

Papaya leaves are made into tea as a treatment for malaria. Antimalarial and antiplasmodial activity has been noted in some preparations of the plant, the leaves of the papaya plants contain chemical compounds of karpain, Substance which kills microorganisms that often interfere with the digestive function (Udohet *et al.*, 2005). Papaya leaf extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5, 7-dimethoxycoumarin, caffeic acid, kaempferol, quercetin, chlorogenic acid (Romasi *et al.*, 2011). Antimicrobials of plant origin effective in the treatment of infectious diseases and simultaneously mitigating many of the side effects often associated with synthetic antimicrobial agents have been discovered. Medical uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoction from the plants (Iwu *et al.*, 1999).

II. Materials And Methods

Preparation of methanolic and hot aqueous seed extract

Seeds of *Carica papaya* var. *pusa dwarf* Linn were collected from Agriculture Research Farm, SHIATS, Naini, UP. The seed extract was prepared as described in WHO, 1983 with slight modification through hot extraction method. Air dried ripened papaya seeds were powdered using mortar and pestle. For aqueous extract, 5g of dried and powdered seeds were mixed in 100 ml distilled water while for solvent extract preparation 70% methanol was used. The contents were kept in water bath for 1h at 40°C. After cooling both extracts were filtered successively through ordinary cheese cloth and Whatmann filter paper. The residue was then resuspended in same amount of distilled water and 70% methanol and the process was repeated 3-4 times. The extracts were then air dried using a water bath at 40°C and dissolved in DMSO to form different concentrations of viz. 25, 50, 75 and 100% aqueous and methanolic extracts respectively. The hot water seed extract was prepared according to the as described by Oyagade *et al.*, 1999. The hot water extraction was done at 80°C in a water bath for 1/2hours. 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.

Preparation of leaf extract

Healthy, fresh young leaves of *Carica papaya* var. *pusa dwarf* Linn were collected from Agriculture research farm, SHIATS, Naini, UP. The leaf extract was prepared as described by Suresh *et al.*, 2008 with slight modification. Leaves were rinsed under tap water and then by double distilled water then dried at room temperature for 15 days. 20g dried leaves were grounded to powder using a mixer grinder. 20g dried leaves powder was soaked in chloroform for extraction in a rotary shaker for 3-5 days. The extract was then filtered through cheesecloth and the extract was reduced to 10% of the original volume using a water bath at 40°C finally dried as powder. The same process was used for aqueous extract preparation.

Antibacterial activity of aqueous and 70% methanolic extract of *Carica papaya* seeds

The antibacterial activity of aqueous and 70% methanolic extracts of *Carica papaya* seeds was examined using agar well diffusion assay as described by. Known concentrations of aqueous and 70% methanolic extracts of *Carica papaya* seeds i.e. 25, 50, 75, 100 mg/ml DMSO were prepared. To the solidified Nutrient agar medium (composition in g/L: peptone:5; sodium chloride:5; beef extract:3; agar agar:17; pH: 7.2) 5mm wells were cut. An uninoculated plate served as media control and test bacteria swab inoculated was used as a organism control. A plate swab inoculated with test bacteria with a well filled with DMSO was used as treatment control. To the test plates an aliquot of each concentration of aqueous and 70% methanolic extracts of *Carica papaya* seeds was filled after swab inoculation of test bacteria viz. *Staphylococcus aureus* and gram negative bacteria i. e. *Pseudomonas aeruginosa*, *E. coli* and *Salmonella typhi*. All the plates were allowed to incubate at 37°C for 24-48h in bacteriological incubator. After incubation the clear zone around the well was measured in mm scale defined as zone of inhibition of the test bacteria.

III. Results And Discussion

Table 1: Antibacterial activity of 70% methanolic extract of *Carica papaya* seeds on bacterial pathogens

Sample extract	Concentration (mg/ml DMSO)	Zone of Inhibition (mm)			
		<i>S. aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>S typhi</i>
70% methanolic extract of seeds of <i>Carica papaya</i>	25	3.2	3.2	3.0	0.00
	50	4.7	4.5	4.2	0.00
	75	7.3	7.1	6.1	0.00
	100	10.0	8.2	7.4	3.0

Table 2: Antibacterial activity of aqueous extract of *Carica papaya* seeds on bacterial pathogens

Sample extract	Concentration (mg/ml DMSO)	Zone of Inhibition (mm)			
		<i>S. aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>S typhi</i>
aqueous extract of seeds of <i>Carica papaya</i>	25	3.0	2.5	2.5	0.00
	50	4.2	4.1	3.9	0.00
	75	7.1	6.3	5.5	0.00
	100	9.5	7.5	7.0	2.5

Table 3: Antibacterial activity of chloroform extract of *Carica papaya* leaves on bacterial pathogens

Chloroform extract of leaves of <i>Carica papaya</i>	Concentration (mg/mlDMSO)	Zone of Inhibition (mm)			
		<i>S. aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>S typhi</i>
	25	2.5	2.2	2.4	2.7
	50	3.8	4.0	3.7	4.3
	75	4.8	5.9	4.8	6.7
	100	8.4	7.8	6.9	8.8

Table 4: Antibacterial activity of aqueous extract of *Carica papaya* leaves on bacterial pathogens

Aqueous extract of leaves of <i>Carica papaya</i>	Concentration (mg/ml DMSO)	Zone of Inhibition (mm)			
		<i>S. aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>S.typhi</i>
	25	3.2	3.0	3.0	0.00
	50	5.1	4.8	3.5	0.00
	75	7.1	5.4	4.9	0.00
	100	9.8	8.5	6.2	2.5

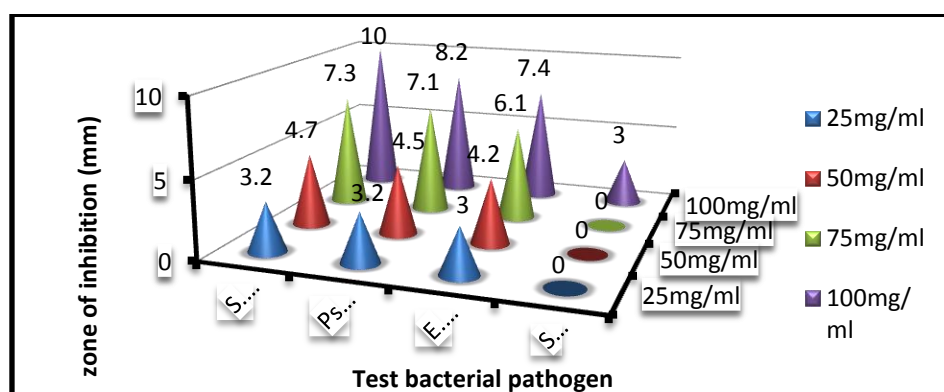


Fig 1: Antibacterial activity of 70% methanolic extract of seeds of *Carica papaya* leaves on bacterial pathogens

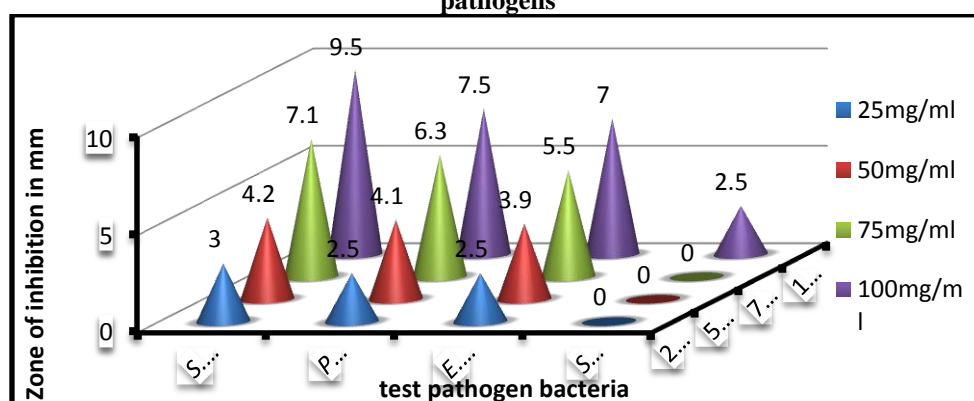


Fig 2: Antibacterial activity of aqueous extract of seeds of *Carica papaya* leaves on bacterial pathogens

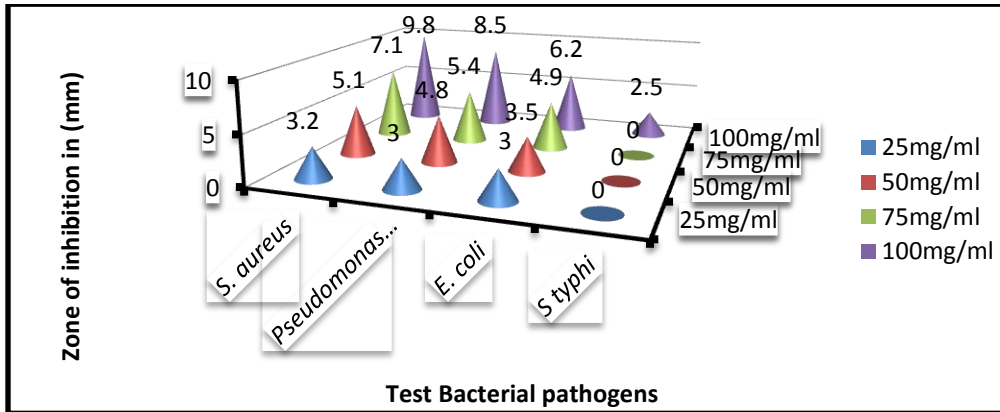


Fig 3: Antibacterial activity of chloroform extract of *Carica papaya* leaves on bacterial pathogens

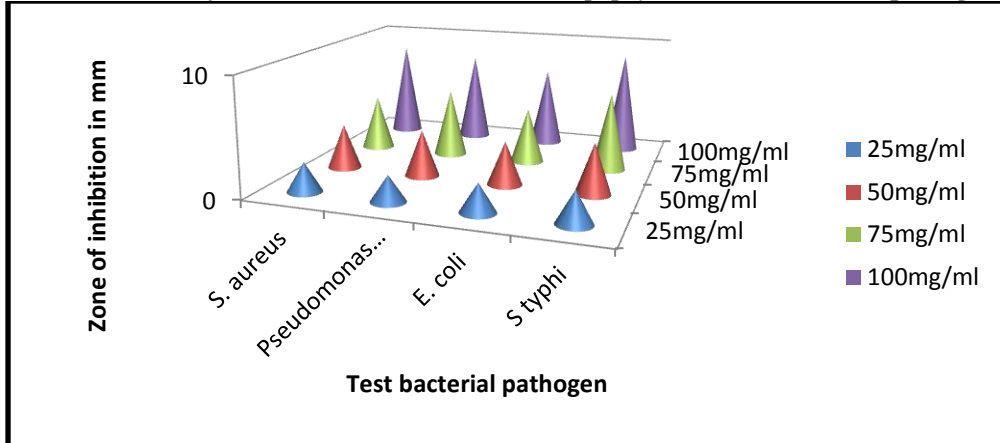


Fig 4: Antibacterial activity of aqueous extract of *Carica papaya* leaves on bacterial pathogens

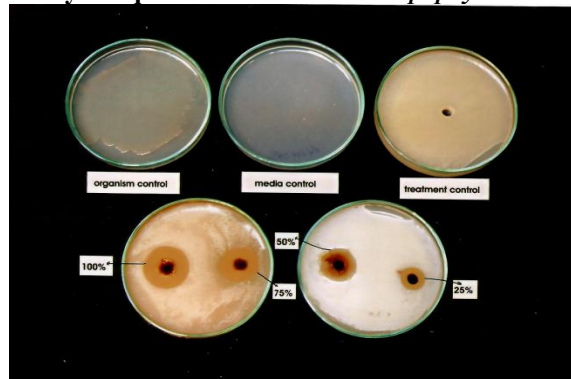


Plate1: Antibacterial activity of aqueous seed extract of *Carica papaya* var. *pusa dwarf* on *E. coli*

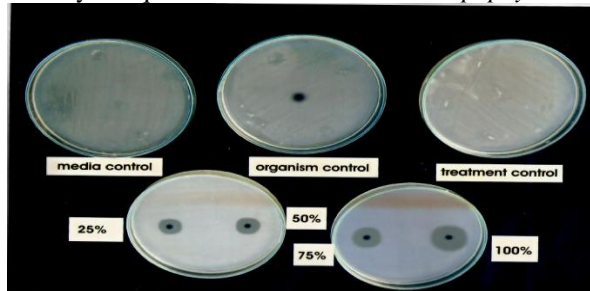


Plate 2: Antibacterial activity of aqueous leaf extract of *Carica papaya* var. *pusa dwarf* on *E. coli*

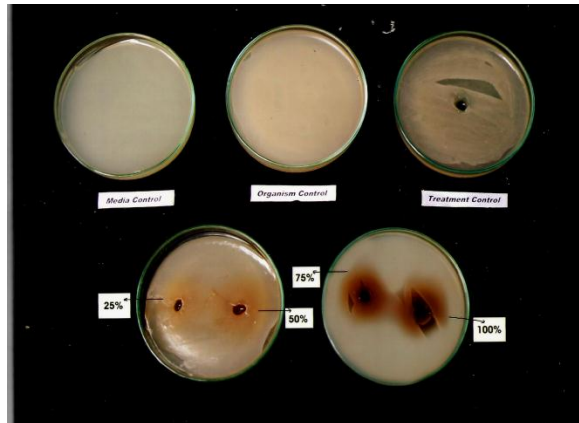


Plate: 3 Antibacterial activity of chloroform leaf extract of *Carica papaya* var. *pusa dwarf* on *Pseudomonas auruginosa*

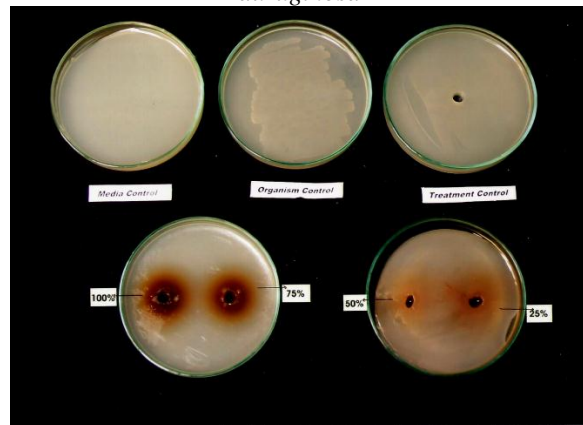


Plate: 4 Antibacterial activity of chloroform leaf extract of *Carica papaya* var. *pusa dwarf* on *S. aureus*

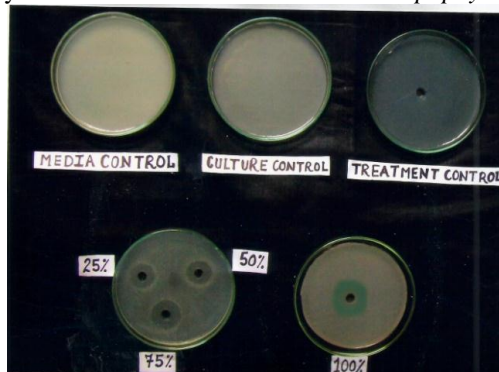


Plate: 5 Antibacterial activity of aqueous leaf extract of *Carica papaya* var. *pusa dwarf* on *Pseudomonas auruginosa*

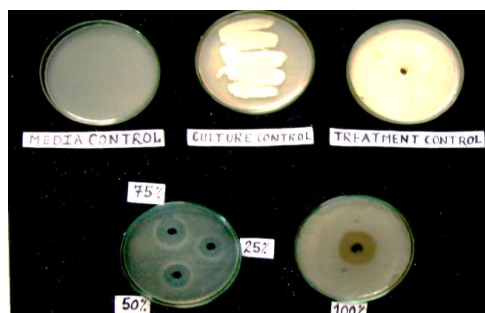


Plate: 6 Antibacterial activity of aqueous seed extract of *Carica papaya* var. *pusa dwarf* on *Salmonella typhi*



Plate: 7 Antibacterial activity of aqueous seed extract of *Carica papaya* var. *pusa dwarf* on *Pseudomonas auruginosa*



Plate: 8 Antibacterial activity of methanolic seed extract of *Carica papaya* var. *pusa dwarf* on *S. aureus*

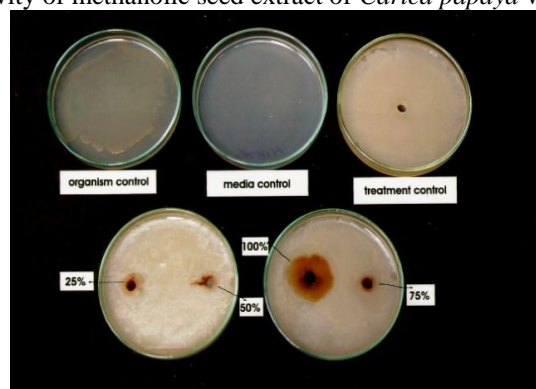


Plate: 9 Antibacterial activity of aqueous leaf extract of *Carica papaya* var. *pusa dwarf* on *Salmonella typhi*

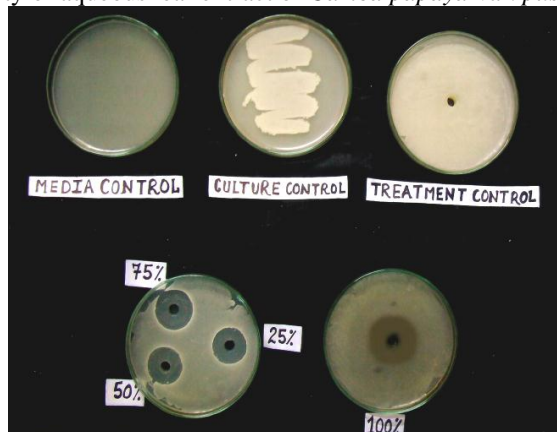


Plate: 10 Antibacterial activity of aqueous leaf extract of *Carica papaya* var. *pusa dwarf* on *S. aureus*



Plate: 11 Antibacterial activity of aqueous seed extract of *Carica papaya* var. *pusa dwarf* on *S. aureus*

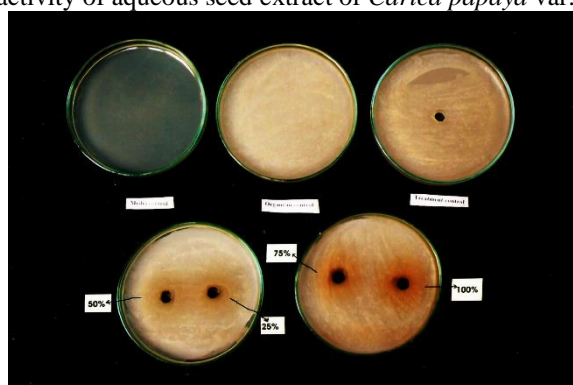


Plate: 12 Antibacterial activity of chloroform leaf extract of *Carica papaya* var. *pusa dwarf* on *Salmonella typhi*

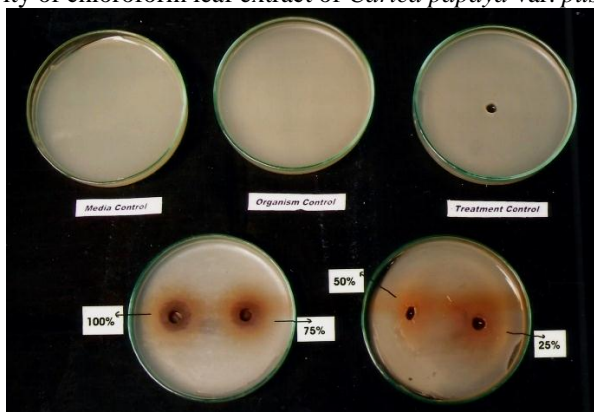


Plate: 13 Antibacterial activity of chloroform leaf extract of *Carica papaya* var. *pusa dwarf* on *E. coli*

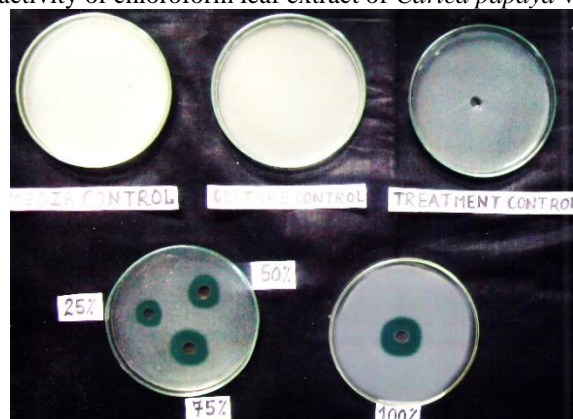


Plate: 14 Antibacterial activity of methanolic seed extract of *Carica papaya* var. *pusa dwarf* on *Pseudomonas auruginos*



Plate: 15 Antibacterial activity of methanolic seed extract of *Carica papaya* var. *pusa dwarf* on *E. coli*



Plate: 16 Antibacterial activity of methanolic seed extract of *Carica papaya* var. *pusa dwarf* on *Salmonella typhi*

Aqueous extract of *Carica papaya* seeds showed increasing zone of inhibition of *S. aureus*, *P. aeruginosa* and *E. coli* with increasing seed extract concentration as 25, 50, 75 and 100mg/ml while the extract concentration of 25, 50, and 75mg/ml were unable to show zone of inhibition against *S. typhi* and the pathogen was inhibited at 100mg/ml concentration of the extract with a zone of inhibition of 2.5mm (Table: 1; Fig: 1).

70% methanolic extract of *Carica papaya* seeds inhibited *S. aureus*, *P. aeruginosa* and *E. coli* with increasing seed extract concentration as 25, 50, 75 and 100mg/ml while the extract concentration of 25, 50, and 75mg/ml were unable to show zone of inhibition against *S. typhi* and the pathogen was inhibited at 100mg/ml concentration of the extract with a zone of inhibition of 3.0mm. Among the four concentrations 100mg/ml of extract concentration of seeds of *Carica papaya* had highest zone of inhibition against all pathogenic bacteria tested (Table: 2; Fig: 2).

Aqueous, n-hexane and ethanol extract of *Carica papaya* leaves was investigated by Chandra *et al.*, 2011 for antibacterial activity of *S. aureus*, *B. subtilis*, *E. coli*, and *Pseudomonas aeruginosa* and it was observed that the three extracts were able to inhibit all the bacteria tested. Among the three extract n-hexane and ethanol had highest inhibition of *S. aureus* 17.33 and 15.67(30mg/disc) while aqueous extract had inhibition of 9(30mg/disc) also in case of *B. subtilis*, *E. coli*, and *Pseudomonas aeruginosa*. Mangalanayaki and Nirosha, 2013 examined the antibacterial activity of the leaves of the *Carica papaya* using solvents ethanol and ethyl acetate against *Staphylococcus aureus*, *Streptococcus pneumonia*, *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* by well diffusion method. The extract demonstrated higher activities against all the Gram negative bacteria than Gram positive bacteria tested, with the highest activity (16 mm zone of inhibition) demonstrated against *Salmonella typhi*. The ethanolic extract leaves and roots moderately to kill all the bacterial pathogens than aqueous extract of leaves and root. Ogunjobi and Ogunjobi (2011) through cold extraction precipitation on eight bacterial strains *Staphylococcus aureus*; *Salmonella typhi* B; *Shigella dysenteria*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Pseudomonas fluorescens*; *Proteus vulgaris*; and *Bacillus subtilis*. Romasi *et al.*, 2011 studied the ethanol, ethyl acetate, and hexane extract of papaya leaf against *Bacillus stearothermophilus*, *Listeria monocytogenes*, *Pseudomonas sp.*, and *Escherichia coli* by agar diffusion method. The results of the study conducted by Hema *et al.*, 2013 showed that the propanolic extracts of *Carica papaya* were more effective than the ethanol extracts demonstrated the highest activity. Among the Gram-positive and Gram-negative bacteria tested against the leaf extract of *C. papaya*, the Gram-negative bacteria were more susceptible especially *Proteus vulgaris* 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). The fact that the extracts were active against both Gram negative and Gram positive bacteria tested may indicate a broad spectrum of activity and the phytochemical analysis revealed the presence many phyto constituents. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms. Zakira *et al.*, 2006 analyzed the antimicrobial activity of *Carica papaya* flowers against bacterial pathogens. Romasi *et al.*, 2011 reported that the extracts of papaya leaves could inhibit the growth of *Rhizopus stolonifer*. Antibacterial activity of *Carica papaya* leaf extracts on pathogenic bacteria was observed in this study. Papaya leaves were extracted by using maceration method and three kinds of solvents: ethanol, ethyl acetate, and hexane. Papaya leaf extracts were tested against *Bacillus stearothermophilus*, *Listeria monocytogenes*, *Pseudomonas* sp., and *Escherichia coli* by agar diffusion method. This research indicated that papaya leaves have potential natural antibacterial compounds. Sherwani *et al.*, 2013; Omojasola and Awe, 2004 also examined the leaf extract of *Carica papaya* against plant and human pathogenic bacteria.

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