

Antibacterial activity of methanolic extract of unripe *Carica papaya* Linn. fruit

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Abstract

The present study was conducted to evaluate the antibacterial activity of unripe *Carica papaya* fruits. Agar well diffusion method was employed to test antibacterial activity of *C. papaya* unripe fruit's methanolic extract on human pathogens which causes nosocomial infections. The methanolic extract exhibited significant dose dependent antibacterial activity comparable to conventional antibiotic (Streptomycin) used against clinically isolated pathogens. These results suggest that the methanolic fraction of unripe *C. papaya* fruit, as a potential source of natural antibacterial constitute. Further investigations are being carried out in our laboratory to identify the active bio-ingredient(s) of extract and establish the exact mechanism of action in order to explain their therapeutic efficacy.

Millions of people have resorted to the use of natural plant drugs to treat wide range of diseases (nosocomial infections) caused by antibiotic resistant pathogens in hospitals. It has resulted in the global shift towards the use of phytomedicine rather than costly synthetic drug products^{1,4}. *Carica papaya* (Caricaceae family), or Papaya, unbranched tree or shrub, native to Tropical America is a popular traditional medicinal plant. The plant is widely grown for the edible fruit and for the protein-digesting enzyme, papain, which is extracted from the unripe fruit. An unripe

Papaya is a berry developing from syncarpous superior ovary with parietal placentation. It is cooked as a vegetable and used to treat malaria, hypertension, diabetes mellitus, hypercholesterolemia, jaundice and intestinal helminthiasis⁶. The unripe green papaya fruit Juice is applied to treat pimples, eczema and mouth ulcers. It is used as a contraceptive and abortifacient in countries like India, Pakistan, and Sri Lanka. Green unripe papaya contains high concentrations of latex, or the milky sap. Latex consists of papain and chymopapain which has lot of commercial and medicinal importance. Patients

with herniated lumbar intervertebra discs who did not respond to conservative therapy, when given intradiscal injection of Chymopapain showed betterment. In the present study an attempt was made to evaluate *in vitro* antibacterial activity of methanolic extract of *Carica papaya*, against ten nosocomial infection causing microorganisms using the agar well diffusion method.

The dried unripe fruits of *Carica papaya* were grounded into coarse powder. About 200g of powdered was extracted two to three times with methanol at room temperature for 72 h with mass to volume ratio of 1:10 (g/ml). The solvent extracts were evaporated to dryness under vacuum on rotary evaporator between 37°C to 40°C. Extraction yield of the extract was calculated and was stored at 4°C. Phytochemical analysis of the extract was done for the methanolic extract⁵. A collection of ten organisms including three Gram-positive (*Bacillus subtilis*, *Methicillin resistant Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*) and seven Gram-negative organisms (*Escherichia coli*, *Proteus mirabilis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*) used for this study were obtained from Microbiology laboratory of Global Hospital, Hyderabad. Evaluation of antibacterial activity was done following the agar-well diffusion method as reported by Perez *et al.*⁸. The bacterial strains from stock cultures were reactivated by inoculating into Mueller Hinton Broth (MHB) and incubating overnight at 37°C. A final inoculum containing 10⁶ colony forming units (1 x 10⁶ CFU/ml) was spread aseptically using cotton bud on MHA medium in petri dish. About five wells (5mm in

diameter) were punched on agar surface to which different test extracts at a concentration of 10 mg/ml, 20mg/ml and 30mg/ml were added. Antibiotics streptomycin at a concentration of 100µg/well was used as positive reference. Methanol was used as negative controls. Synergistic activity was also analyzed by adding equal concentration of extract and antibiotic (10mg/ml) to the wells in another plate. Later the plates were incubated at 37°C for 18h. Diameter of inhibition zone (DIZ) around each well was measured in mm. Experiments were performed in triplicates.

The methanolic extract was light brown in color and the yield was 40% and the preliminary phytochemical analysis of *Carica papaya* extract reported the presence of flavonoids, phenolic compounds, terpenoids, steroids, carbohydrates, proteins, saponins and glycosides (Table-1). The extract proved to be very effective against both gram negative bacteria and gram positive bacteria taken for the present antibacterial activity test. There are no earlier reports of antibacterial activity of unripe papaya fruit methanolic extract done on hospital collected samples. The plant extract showed remarkable activity against *Escherichia coli*, MRSA and *Enterobacter cloacae* to different concentration of extract (Fig. 1). *Proteus mirabilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermidis* and *Micrococcus luteus* where inhibited at 30 mg/ml and high concentration of extract (crude). *Klebsiella pneumoniae* and *Bacillus subtilis* did not respond even to crude extract concentration, but showed a very good zone of inhibition of 30mm and 35mm respectively in synergistic activity of plant extract with antibiotic (Table 2).

Table-1. Extract yield and phytochemical composition of Methanolic extract of *Carica papaya*

Phytochemical tests for compounds	Methanol extract
Yield* (%)	40
Colour and physical nature of extract	Light brown
Alkaloids	--
Flavonoids	+
Phenolic compounds	+
Terpenoids	+
Steroids	+
Carbohydrates	+
Protein	+
Reducing sugars	-
Saponins	+
Tannins	-
Lignins	-
Glycosides	+

* yield calculated as the ratio of the mass of the obtained methanol extract/mass of the plant powder

+ Presence of constituent

- Absence of constituent

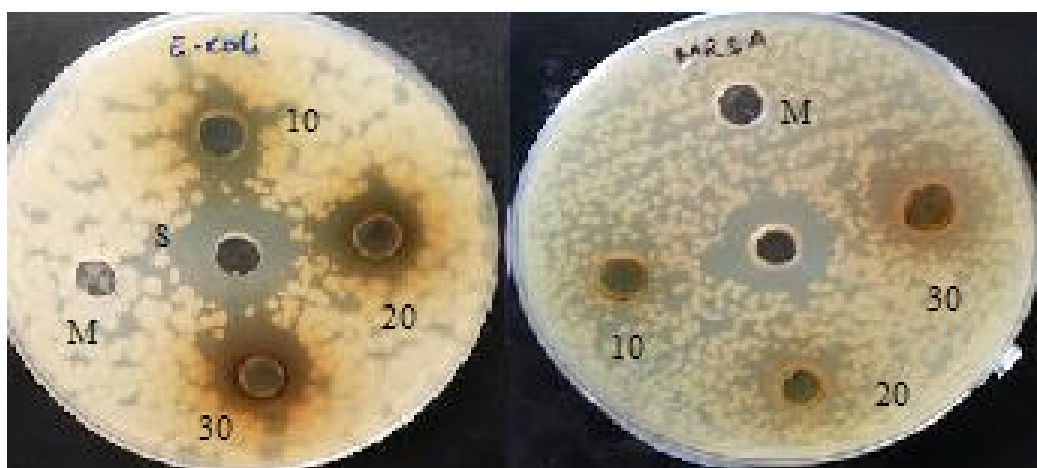


Fig 1. Antibacterial activity of methanolic plant extracts
S: Antibiotic standard; M: Methanol Control

Table-2. Antibacterial activity of methanolic plant extracts

S. No.	Micro-organism	Property	Zone of inhibition (diameter in mm)						Antibiotic standard (10mg/ml)	Solvent
			<i>Carica papaya</i> unripe fruit methanolic plant extract (mg/ml)							
			Syn	Crude	10	20	30			
1.	<i>Proteus mirabilis</i>	Gram negative bacteria	24±1.23	13±0.59	-	-	10±1.92	20±0.50	-	
2.	<i>Salmonella typhi</i>		27±1.45	22±0.53	-	-	-	12±0.40	-	
3.	<i>Enterobacter cloacae</i>		18±0.86	16±0.68	-	12±0.56	15±0.92	13±0.50	-	
4.	<i>Klebsiella pneumoniae</i>		30±1.52	-	-	-	-	21±0.50	-	
5.	<i>Pseudomonas aeruginosa</i>		20±1.34	25±1.62	-	-	22±1.51	17±0.40	-	
6.	<i>Escherichia coli</i>		28±1.87	23±1.04	12±0.86	16±0.53	22±1.29	25±0.50	-	
7.	<i>Micrococcus luteus</i>		23±2.63	22±0.58	-	-	-	18±0.50	-	
8.	MRSA	Gram positive bacteria	20±4.75	20±0.51	-	14±1.86	20±0.57	16±0.50	-	
9.	<i>Bacillus subtilis</i>		35±0.98	-	-	-	-	28±0.50	-	
10.	<i>Staphylococcus epidermidis</i>		25±4.32	15±1.82	-	-	-	10±0.50	-	

*Standard drug used: Streptomycin

Syn: Synergistic activity of plant extract and antibiotic streptomycin

Osato⁷ and other researchers reported the bacteriostatic property of supernatant extract of blended unripe papaya against pathogenic bacteria^{2,3,7}. Recently antibacterial activity of unripe endocarp acetone extracts was reported to have high activities against all the gram-negative bacteria⁹. The present study proves that unripe *Carica papaya* fruit methanolic extracts showed good antibacterial activity against nosocomial infection causing organisms. Further chemical and biological characterization of active fraction of the extract against pathogenic microorganisms is under study.

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