

Full Length Research Paper

# Antibacterial screening of leaves and stem of *Carica papaya*

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The purpose of the study was to investigate antibacterial activity of ethanolic extracts of leaves and stem of *Carica papaya* on selected microorganisms. Various Gram negative (*Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi A*, *Shigella flexneri*) and Gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Micrococcus luteus*) were used for the screening of antibacterial activities of the extracts of both parts that is leaves and stem. Antibacterial activity was expressed in terms of the radius of zone of inhibition. Both parts were tested in two doses (5 and 10 mgml<sup>-1</sup>) it was found that the antibacterial action was dose dependent and also a significant difference was observed in bacterial sensitivity to leaves and stem extracts, however the leaves extracts were found more active than the stem. The minimum inhibitory concentration (MIC) against test microorganism was studied by turbidity method. The range of MIC observed for leaves and stem was 1250 to 5000 µgml<sup>-1</sup> and 1250 to 10,000 µgml<sup>-1</sup> respectively. *C. papaya* leaves and stem contains some valuable antibacterial compounds that inhibit the growth of wide variety of Gram-positive and Gram-negative organisms. Therefore the leaves and stem of *C. papaya* has antibacterial effects that could be useful in treating variety of bacterial infections.

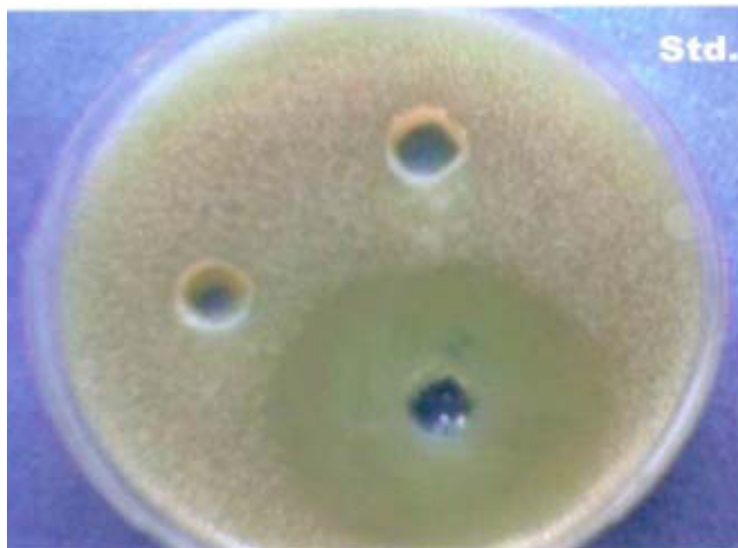
**Key words:** *Carica papaya*, ethanol extract, antibacterial activity, minimum inhibitory concentration.

## INTRODUCTION

According to a research, there are 4, 22,127 plant species growing on earth; among them about 35,000 to 70,000 plants species are used as medicinal plants (Hasan et al., 2007). Out of these medicinal plants, 20,000 plants species are believed to be used medicinally in the third world (Mukherjee, 2004). Approximately 6000 species of flowering plants occur in Pakistan and 700 of them have medicinal value (Shinwari et al., 2006; Stewart, 1972). Of these species, 500 are known for their active constituents, from research conducted in Pakistan about 250 to 300 species known to have entered the herbal market of Pakistan (Williams and Ahmad, 1999). The medicinal value of plants lies in some chemical substances (plant secondary metabolites) that produce a specific biological action on the human body (Hassan et al., 2009). It is important to mention that over

75% of population in Pakistan is cured by using traditional medicines prescribed by more than 50,000 traditional herb practitioners (Gill, 2003) and the folk knowledge of plant curing passes down from family to family of herb practitioners and within communities (Ahmad, 2004). *Carica papaya* (family: Caricaceae) is a large tree-like plant with a single stem growing from 5 to 10 m (16 to 33 ft) tall with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. The leaves are large, 50 to 70 cm diameter, deeply palmately lobed with 7 lobes. Fruits (papaya) appear on the axils of the leaves, maturing into the large 15 to 45 cm long, 10 to 30 cm in diameter fruit (Urasaki et al., 2001; Chen et al., 1987; Kim et al., 2002). Papaya fruits had extensively studied and reported for their anti fungal (Giordani et al., 2009) and antibacterial activities (Rios et al., 1988; Emeruwa, 1982). The plant is native of Tropical America and cultivated all over the tropical and subtropical countries of the world. In Pakistan it is widely

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**Figure 1.** Zone of inhibition by std. drug against *S. aureus* and no zone of inhibition by DMSO.

cultivated in Sind and Punjab (Arshad et al., 2000). It is a threatened plant with potential medicinal value hence, it was considered worthwhile to evaluate the anti microbial activity of leaves and stem.

## MATERIALS AND METHODS

### Identification of the plant

The plant *C. Papaya* was collected from Sind, Pakistan. The whole plant was divided into leaves and stem. The plant was identified and authenticated by Mr. Iftikhar Hussain Shah, Department of Pharmacognosy, Riphah Institute of Pharmaceutical Sciences (RIPS-RIU), Islamabad Pakistan. A voucher specimen (1561) was submitted in the Department for future reference.

### Preparation of extracts

Dried leaves (25 g) and stem (25 g) were mechanically pulverized to a coarse powder and extracted with ethanol of 95% in soxhlet extractor for 72 h. After exhaustive extraction, the leaves extract (LE) (Figure 2) and stem extract (SE) were filtered and concentrated with the help of rotary evaporator.

### Experimental microorganism

#### Gram negative strains

*Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi A* and *Shigella flexneri*.

#### Gram positive strains

*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Micrococcus luteus*.

### Preparation of test inoculum

#### Seeded Broth preparation

The various strains of microorganisms were procured from National Institute of Health (NIH), Islamabad and were inoculated in sterile nutrient broth (about 100 ml). This medium was incubated at  $37 \pm 1^\circ\text{C}$  for 24 h and termed as seeded broth.

#### Standardization of seeded broth (viable count)

**Dilution:** In 99 ml of sterile water containing 0.05% Tween 80, 1 ml of seeded broth was added. From this, 1 ml was taken and diluted to 10 ml with sterile water and seeded broth is further diluted up to  $10^{-10}$  dilution.

**Inoculation into nutrient agar Petri dishes:** Seeded broth (0.2 ml) dilutions were inoculated into solidified nutrient agar medium by spread plate method. Number of colonies of microorganisms formed after incubation at  $37 \pm 1^\circ\text{C}$  for 24 h. The seeded broth was suitably diluted to contain  $10^6$  to  $10^7$  colony forming unit/ml (cfu/ml<sup>-1</sup>). It was the working stock and was used for microbiological evaluation.

### Preparation of test compound solution

The test sample/compound that is leaf and stem extract were prepared at the concentration of 5 and 10 mg/ml<sup>-1</sup> each respectively in Dimethyl Sulphoxide (DMSO). Standard drug used in study was Ampicilline trihydrate prepared at concentration of 1 mg/ml<sup>-1</sup> in DMSO (Figure 1)

### Screening of antimicrobial activity

The seeded broth 0.2 ml containing  $10^6$  to  $10^7$  cfu/ml<sup>-1</sup> of the test organism was inoculated in solidified agar plate with the help of micropipette and spreaded. Two or three wells were made in agar



**Figure 2.** Zone of inhibition by ethanol extract of leaf against *M. luteus*.

layer of each Petri dish by a steel borer. To these cavities standard and test sample solution were added. All the work was carried out strictly under aseptic conditions for bacterial assay. The plates for bacterial assay were incubated at  $37 \pm 1^\circ\text{C}$  for 18 h. The antimicrobial potential of test compound was determined on the basis of diameter of zone of inhibition around the wells (Sumitra et al., 2005, 2006).

#### Determination of minimum inhibitory concentration (MIC)

MIC of extracts was determined using turbidity method in nutrient broth medium. The experiment was conducted according to serial dilution method. The suspension of seeded broth was made by transferring 2 ml of the seeded broth to the 100 ml of the 0.9% w/v of the sterilized saline solution. The stock solution of test compounds were prepared at concentration of  $10 \text{ mgml}^{-1}$  in nutrient broth and serially diluted to the 5 assay test tubes (containing 1 ml nutrient broth) to give concentration of 5, 2.5, 1.25, 0.625 and  $0.3125 \text{ mgml}^{-1}$ . Normal saline suspension (0.1 ml) was added to each assay tube. The procedures were conducted under strict aseptic conditions. The inoculated tubes were kept at  $37 \pm 1^\circ\text{C}$  for 24 h for bacterial assay. After incubation period, tubes were removed and observed for any deposits and shaken to suspend bacteria that might have been settle down. MIC values were determined by checking for the absence of visual turbidity (Cappucino et al., 1999).

## RESULTS AND DISCUSSION

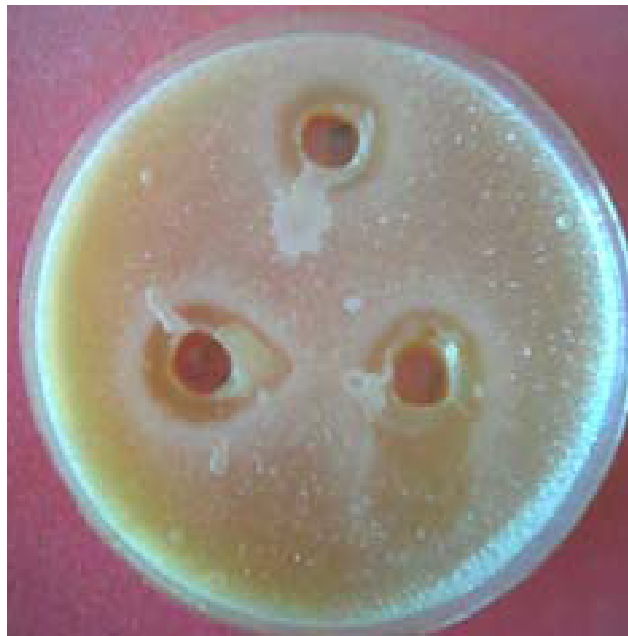
The ethanolic extracts of stem and leaves of *C. papaya* were screened for their antimicrobial activity against different strains of gram negative (*E. coli*, *P. mirabilis*, *P. aeruginosa*, *S. typhi*, *S. paratyphi A*, *S. flexneri*) and gram positive bacteria (*S. aureus*, *S. epidermidis*, *B. subtilis*, *M. luteus*). The antibacterial action was shown in the form of zone of inhibition as given in Table 1. The

antibacterial action of leaves was more than the stem (Figures 2 and 3), moreover both extracts showed dose dependent activities. In addition to having good activity against other bacteria, the leaves exhibited strong activity against *S. typhi* having zone of inhibition 14 and 18 mm at the dose of 5 and  $10 \text{ mgml}^{-1}$  respectively. While the significant activity of the stem was observed against *S. typhi* having zone of inhibition 12 and 14 mm at the dose of 5 and  $10 \text{ mgml}^{-1}$  respectively. The range of MIC for leaves and stem was  $1250$  to  $5000 \text{ } \mu\text{gml}^{-1}$  and  $1250$  to  $10,000 \text{ } \mu\text{gml}^{-1}$  respectively as shown in Table 2. The MIC of leaf extract against *Shigella flexneri*, *S. aureus*, *S. typhi* and *P. aeruginosa* was  $1250 \text{ } \mu\text{gml}^{-1}$ . The MIC of stem extract against *S. flexneri* was  $1250 \text{ } \mu\text{gml}^{-1}$  while against *S. aureus*, *S. typhi* and *P. aeruginosa* was  $5000 \text{ } \mu\text{gml}^{-1}$  respectively. The increase of antibiotic resistance of microorganisms to conventional drugs has necessitated the search for new, efficient and cost effective ways for the control of infectious diseases.

The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents (Kone et al., 2004; Rahman et al., 2011). The use of medicinal plants is part of the Pakistani tradition. Many local regions all over the Pakistan have a great variety of vegetation used by the local population to treat and prevent diseases. From this study we can conclude that this medicinal plant has a wide range of antibacterial activity and supports the traditional use of this plant as medicines. This study demonstrated that the herbal medicine can be as effective as modern medicine to combat pathogenic microorganisms. Using different purification, isolation and characterization methods, antimicrobial principals can be obtained and thus the activity of antimicrobial compounds

**Table 1.** Zone of inhibition in mm of leaves extract (LE) and stem extract (SE) of *C. papaya* against selected bacteria.

Microorganism	Zone of inhibition (mm)				Ampicillin trihydrate	DMSO
	LE		SE			
	5 mgml <sup>-1</sup>	10 mgml <sup>-1</sup>	5 mgml <sup>-1</sup>	10 mgml <sup>-1</sup>		
<i>E. coli</i>	12	15	9	12	22	Nil
<i>S. flexnert</i>	12	16	10	12	25	Nil
<i>S. paratyphi A</i>	10	12	9	11	29	Nil
<i>S. typhi</i>	14	18	12	14	33	Nil
<i>P. aueruginosa</i>	9	12	4	6	29	Nil
<i>M. luteus</i>	11	14	11	13	25	Nil
<i>P. mirabilis</i>	9	11	7	9	28	Nil
<i>B. subtilis</i>	11	15	4	6	34	Nil
<i>S. aureus</i>	12	15	10	12	30	Nil

**Figure 3.** Zone of inhibition by ethanol extract of stem against *S. aureus*.**Table 2.** Minimum inhibitory concentration ( $\mu\text{gml}^{-1}$ ) of LE and SE of *C. papaya*.

Microorganism	Minimum inhibitory concentration ( $\mu\text{gml}^{-1}$ )			
	Ethanol extract (leaves)	Ethanol extract (stem)	Ampicillin trihydrate	DMSO
<i>E. coli</i>	5000	5000	312.5	Nil
<i>S. flexnert</i>	1250	1250	312.5	Nil
<i>S. paratyphi A</i>	1560	6250	312.5	Nil
<i>S. typhi</i>	1250	5000	312.5	Nil
<i>P. aueruginosa</i>	1250	5000	312.5	Nil
<i>M. luteus</i>	1560	6250	312.5	Nil
<i>P. mirabilis</i>	5000	10000	312.5	Nil
<i>B. subtilis</i>	5000	10000	312.5	Nil
<i>S. aureus</i>	1250	5000	312.5	Nil

can be improved for further pharmaceutical uses.

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