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Research Article

# EVALUATION OF ANTIBACTERIAL ACTIVITIES OF LATEX OF CARICACEAE (*CARICA PAPAYA* L.)

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## ABSTRACT

**Objective:** *Carica papaya* Linn. belonging to the family of *Caricaceae* is a well-known fruit and medicinal plant that has been widely used for a long time to cure various types of infectious disease especially in South Asian countries. The current study was aimed to evaluate the antibacterial properties of crude latex of *C. papaya.*

**Methods:** The screening of antibacterial activity of latex was evaluated against one Gram-positive bacterium *Bacillus subtilis* and three Gram-negative pathogenic bacterial strains as *Escherichia coli, Agrobacterium sp* and *Rhizobium sp.* Ciprofloxacin was used as a control for investigating the bacterial species. Antibacterial activity was expressed in terms of the radius of zone inhibition. Latex of this plant was tested in seven doses (1, 2, 5, 7, 10, 15 and 20 mg/disk) and it was found that the antibacterial activity was dose dependent and a significant difference was also observed in case of different bacterial stains.

**Results:** The results demonstrated noticeable inhibition of the bacterial growth against the tested organisms. In case of *Agrobacterium* sp. 20 mg of latex showed the average of 20.66 mm zone of inhibition and for *E. coli* this value was 16 mm for the same concentration of latex. The rest of the two bacterial species showed comparative resistance to papaya latex.

**Conclusion:** It is concluded that the latex of *C. papaya* probably contains some valuable antibacterial compounds that are crucial forinhibiting the growth of a wide variety of bacteria, especially Gram-negative bacteria and suggesting this for applying the treatment of a variety of bacterial infections.

**Keywords:** Antibacterial activity, Bacteria, *Escherichia coli*, *Carica papaya*, Disk diffusion, Latex. Zoon inhibition.

## INTRODUCTION

Infectious diseases are the world's major danger to human health and account for approximately 550,000 deaths/day [1]. Most of the severe conditions are claimed due to pathogenic bacteria. Antibacterial drugs are one of our most important tools in combating bacterial infections and saving human life from severe invasion of many infectious diseases. However, from the past few decades, these health opportunities are under risk as many frequently used antibiotics have become less effective against certain illnesses. This not only because of creating toxic reactions, but also due to the emergence of multi drug-resistant strain of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics [2,3]. Consequently, new infections occur, and this rate is gradually increasing in hospitals resulting in high mortality [4]. Therefore, there is the utmost need for searching out newer infection-fighting strategies against a newer worsen strain of pathogenic microbes with less resistance. This exploration of newer strategies is a global challenge taking by many research institutions, pharmaceutical companies and academic institutions, because of resistance of pathogens to commercially available antibiotic [5]. In spite of major systematic advancement in chemistry, antibiotics derived from plants still make a massive contribution to drug discovery and continue to be a main source of bioactive compound for fighting against communicable diseases for a long time especially in developing countries [6]. The antimicrobial drugs derived from plants are the safest, most effective and cheapest alternative sources of drugs against chemically synthesized medicine [7]. The bioactive compounds of secondary metabolites produced by medicinal plants play a key role in antibacterial activity, and their curative potentials are well recognized [8,9]. Between 1981 and 2002 approximately 61% of newly improved drugs were developed based on natural products and they show well efficiency, especially in the areas of infectious disease, and cancer is prevalent. A natural bioactive compound of higher plants may provide a novel basis of antibacterial agents with probable novel mechanisms of action [10]. Out of the 422,127 plant species growing on the whole world about 35,000-70,000 plants species are considered as medicinal plants [11]. Among of them medicinal plants, 20,000 plant species are supposed to be exercised as medicines in the developing countries [12]. Researcher already worked with different medicinal plant parts viz; trunk bark of *Alstonia scholaris* [13], leaf extract (LELC) of *Lantana camara* [14] and seed extracts of *Myristica fragrans* [15] and show significant antimicrobial properties compared with their control. The World Health Organization calculates that plant extracts or their active components are utilized as folk medication in conventional treatment of 80% of the world's population. Due to declining rates of discovery of active novel chemical entities, bioactive components may play a great role in the invention of novel antimicrobial drug.

*C. papaya* belongs to the family Caricaceae and commonly known as papaya, papayer, tinti, pepol, chich put, fan kua, wan shou kuo, kavunagaci, kepaya etc. The plant is narrated as a fast growing, erect, typically unbranched tree or shrub, trunk of about 20 cm in diameter, hollow with prominent leaf scars and spongy-fibrous tissue, having extensive rooting system and 7-8 m tall containing copious latex in all part of the plant. It is commonly familiar for its food and nutritional values throughout the world; the leaves, fruits, roots and latex obtained from papaya plant are medicinally usable parts for treatment [16]. The fruits juicy in taste enriched with antioxidant nutrients like carotene, vitamin C, vitamin B, flavonoids, folate, panthotenic acids and minerals such as potassium and magnesium, and also a good source of fiber, playing an important role to maintain the functions of cardiovascular system and provide protection against colon cancer [17,18]. Plant parts act as analgesic, amoebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, and febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge. *C. papaya* contains many bioactive components, including chymopapain and papain (important proteolytic enzymes found in the milky white latex) that are broadly known as being functional for treatment of digestive disorders and turbulence of the gastrointestinal tract. Nevertheless, water, acetone and ethanol extracts of papaya demonstrate no antimicrobicidal activity [19,20]. Additionally the plant also contains terpenoids, eugenol, thymol, saponins and alkaloids [21]. Papaya latex is effectively useful for therapeutic purpose against dyspepsia and is externally functional to burns and scalds. This milky white substance antagonistic to fungal growth, especially for *Candida albicans* [22] and act as folk medicine to treat skin eczema caused by this fungus. A leaves and young fruit extract are inimical to intestinal worms and successfully use for the treatment of boils [23]. Mauritius people uses smoke from dried papaya leaves for the purpose of alleviating from asthma attacks. Ripen fruit extracts are used for a variety of therapeutic purposes including the treatment of ringworm, malaria and hypertension. Whereas unripe fruit extracts alleviate the condition of diabetes [24]. The latex and the seeds are used in the treatment of gastrointestinal nematode infections and showing anthelmintic activity [25,26]. Evidently the bioactive component of papaya has been used for a long time against a wide variety of microorganisms and contributing a major role of curing various types of infectious disease. Due to failure of chemically synthesized antibiotic to protect the emergence of multi drug resistant bacteria, so there is a principle need to evaluate the antibacterial activity of the latex of papaya for the purpose of searching out newer, safer and more sustainable antibacterial drug. Considering all views of points, the present study evaluates the efficiency of antibacterial activity of *C. papaya* latex can be used new tools for novel drug development.

## METHODS

### Collection of plant materials

Fresh latex was collected from locally grown *C. papaya* initially by 4-6 longitudinal incisions were made on the young fruit using a stainless steel knife. The exuded fresh latex was allowed running down the fruit and dripping into collecting devices attached around the trunk. With the help of micropipette 500 µl collected latex was transferred immediately to eppendorf tube. Then 500 µl methanol was added into this eppendorf tube and shaken for 5 minutes to dissolve the latex into methanol uniformly.

### Collection of microorganisms

Bacterial cultures of Gram-negative bacteria *Escherichia coli, Agrobacterium sp. Rhizobium sp.* and Gram-positive bacteria *Bacillus subtilis* were collected from the microbiology laboratory, Genetic Engineering and Biotechnology Department of the University of Rajshahi. All the test strains were maintained on nutrient agar slopes and were sub cultured once in every 2-week in LB broth medium.

### Preparation of culture medium

For preparing 1 L broth media, all the ingredients (10 g/L of peptone, 5 g/L of yeast extract, 5 g/L NaCl except agar) were suspended in 1 L of double distilled water and were mixed thoroughly. For preparing agar plates, 15 g/L of agar was added to the desired medium for solidification. The pH of the medium was adjusted at 7.0 using 1 N NaOH and autoclaved at 121°C for 20 minutes.

### Disk diffusion assays

The screening of antimicrobial activity of the investigated extracts was determined by the disk-diffusion method. The 100 µl microbial cell suspension was inoculated in 20 ml sterile agar medium and poured into sterile petri dishes. The disks were cut at 6 diameters by punching the Whatman No.1 filter paper with the help of punch machine. The paper disks were soaked with different concentrations (1, 2, 5, 7, 10, 15 and 20 mg/disk) of papaya latex with micropipette and kept for 5-10 minutes in laminar air flow hood for drying. The test organisms (100 µl) were inoculated with a sterile spreader on the surface of solid medium in plates. The agar plates inoculated with test organisms were incubated for 1 hr before placing the latex impregnated paper disks on the plates. The bacterial plates impregnated with different concentration of latex disk were incubated at 37±0.1°C for 24 hrs. Antimicrobial activity was determined by measuring the zone of inhibition around each well (excluding the diameter of the wall), and the diameters of these zones were measured in millimeters.

## RESULTS

The antibacterial activity of latex of *C. papaya* against different strains of Gram-negative (*E. coli, Agrobacterium sp., Rhizobium sp.)* and Gram-positive bacteria (*B. subtilis)* was screened by the agar disk diffusion method. The antibacterial activity was shown in the form of zone of inhibition. The inhibitory action of latex showed dose-dependent activities as well as having good inimical response on the basis of strain of bacteria. The paper disks contain different concentrations (1, 2, 5, 7, 10, 15 and 20 mg/disk) of papaya latex. Among all of the doses 10, 15 and 20 mg/disk showed remarkable inhibition performances. This prospected bioactive compound exhibited strong inimical activity against *E. coli* and having a zone of inhibition of average 14.66, 15.66 and 16 mm at the dose of 10, 15 and 20 mg/disk respectively (Fig. 1). While the significant antibacterial activity of the latex was also observed against *Agrobacterium sp* and having zone of inhibition of average 15.83, 16.33 and 20.66 mm at the dose of 10, 15 and 20 mg/disk respectively (Fig. 2). On the other hand, *B. subtilis* and *Rhizobium sp.* did not show susceptibility to this plant latex (Figs. 3 and 4). Zone of inhibition of this potential antibacterial compound were compared with one standard commercial antibiotic (ciprofloxacin 50 µg). The results demonstrated that the significant inhibition of the bacterial growth against both strains of the tested organisms has especially been Gram-negative.

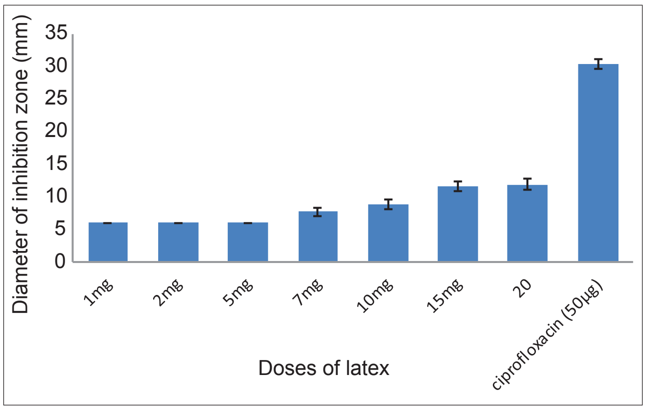


**Fig. 1: Antibacterial activity of papaya latex against *Escherichia coli***

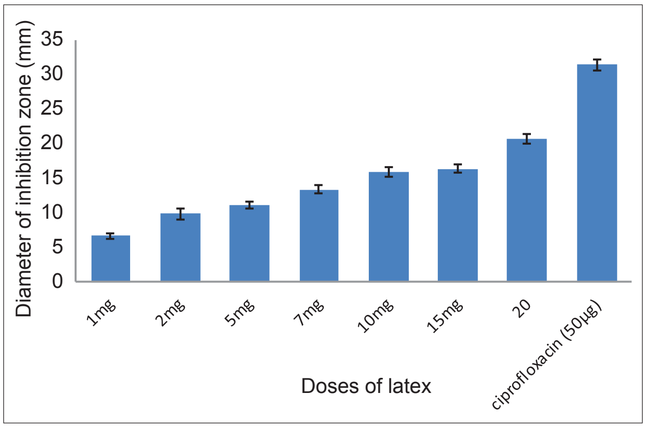


**Fig. 2: Antibacterial activity of papaya latex against *Agrobacterium sp***

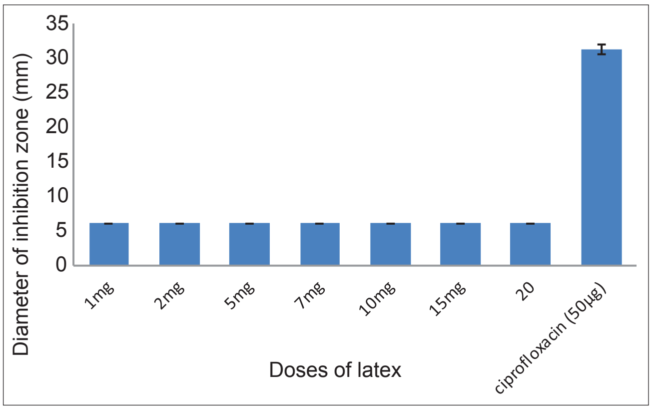


**Fig. 3: Antibacterial activity of papaya latex against *Bacillus subtilis***

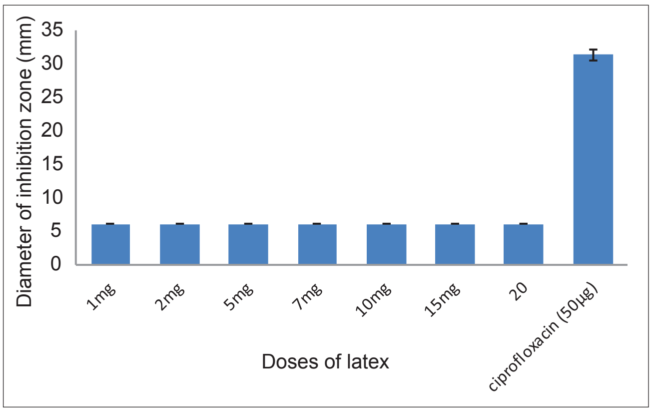


**Fig. 4: Antibacterial activity of papaya latex against *Rhizobium sp***

## DISCUSSION

Many biologically active phytochemicals have been isolated from papaya, and their medicinal uses of different part of the papaya are well documented in the early literature. Kurian (2001) described that the fruit, leaf and latex of *C. papaya* is used for the treatment of typhoid fever, wound infection, asthma, fever, diarrhea, boils and hypertension [27]. It also used for therapeutic purpose in patients with inflammatory disorders of intestine, liver and eye [28]. Mature seed of this valuable plant showed inhibitory action against human intestinal parasites due to the presence of important anthelmintic chemical agents such as benzyl isothiocyanate and papain [29]. According to Giordani *et al.* (1991) the extract from this plant showed inhibitory action against fungal growth for the presence of fungal cell wall hydrolyzing enzyme [22]. According to an ample investigation by various researchers demonstrated that the latex of papaya plant showed antiamoebic [30], antitrichomonal [31], antimalarial [32], leishmanicidal [33], antimicrobial [34]. Anti-inflammatory [35], antihypertensive [36], antihyperlipidemic [37], antioxidant [38] and antidiabetic activity [39]. An aqueous extract of *C. papaya* was showed antagonistic effect on the growth of various tumor cell lines and also on human lymphocytes [40]. In the present study, the disk for antibacterial screening contains following dose of latex extract 1, 2, 5, 7, 10, 15 and 20 mg/disk. This investigation clearly demonstrated that dose of 10, 15 and 20 mg/disk was enough to show inhibitory activity of two Gram-negative bacteria such as *E. coli* and *Agrobacterium sp* and expressing zone of inhibition of average 14.66, 15.66, 16 mm and 15.83, 16.33, 20.66 mm respectively. While two other bacterial species such as *B. subtilis* and *Rhizobium sp.* expressing resistance to this plant extract. Though latex from fruit and seed extracts showed antibacterial activity against *Staphylococcus aureus, Bacillus cereus*, and *Pseudomonas aeuroginosa* [41,42]. In the present study, the latex was isolated from the raw fruits and applied directly as an antibacterial agent. From the above mention results, the current study clearly indicated that the latex extract of *C. papaya* has antibacterial properties to some bacterial species and the prospected dose (10, 15 and 20 mg/disk) is sufficient to inhibit for bacterial growth. Further study needed to carry out for discovering the lead compound and proceeding for the development of new pharmaceutical drug.

## CONCLUSION

The latex of fruits of *C. Papaya* plant has shown antagonistic activity against some bacterial species probably due to the presence of some important inimical secondary metabolites. Therefore, these plants can be used to discover natural bioactive products that potentially serve as leads in the development of novel pharmaceutical research activities and suggesting for using as potential antibacterial agent to cure bacterial infection.

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