

## COMPARATIVE STUDY ON THE EFFECT OF DIFFERENT SOLVENT EXTRACTS OF *CALOTROPIS GIGANTEA* AND *CARICA PAPAYA* LATEX AGAINST NEW BACTERIAL ISOLATES – AN *IN VITRO* STUDY

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### ABSTRACT

**Objective:** To evaluate the comparative preliminary study on antibacterial activity of different solvent extracts of *Calotropis gigantea* and *Carica papaya* latex against two new strains of *Bacillus licheniformis* isolated from poultry farm which may cause Septicemia.

**Methods:** Antibacterial effects of aqueous and ethanolic extracts of *Calotropis gigantea* and *Carica papaya* latex was carried out using disc diffusion method. Fourier Transform Infrared (FT-IR) Spectroscopic analysis of the plants latex was done to find out the presence of different functional groups. Multiple antibiotic resistances (MAR) index of the strains was determined against six antibiotics using disc diffusion method. Fluorescence analysis of latex extracts was analyzed under short and long wavelength. Statistical analysis was expressed as mean  $\pm$  standard deviation using Microsoft Excel 2007.

**Results:** Antibacterial activity of the aqueous and ethanolic extract of both the plants latex showed concentration-dependent activity against both the tested bacteria with the zone of inhibition at various concentrations. FT-IR Spectroscopic analysis of the plants latex showed the presence of functional groups in the sample. MAR index of the strains was found to be zero. Fluorescence of the latex extracts was visualized at different wavelengths due to the presence of fluorescent compounds.

**Conclusion:** The present investigation clearly demonstrated that latex of *C.gigantea* was more effective than that of *C. papaya* against both the strains and established a good support for the use of *C.gigantea* latex as a potential agent for the prevention of septicemia which may be caused by new strains of bacteria.

**Key words:** *C. gigantea*, *C. papaya*, Disc diffusion, FT-IR Spectroscopy, Latex, MAR index.

### INTRODUCTION

Plants have been a major source of medicine and the presences of plant secondary metabolites have been implicated for most plants therapeutic activities [1]. Herbal plants are one of the effective and potential sources of traditional and modern medicines. Medicinal plants are gifts of nature to cure number of diseases among human beings [2]. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases [3]. Compounds produced from herbal plants are safe and more effective compared to the synthetically produced antimicrobial agents because plant based drugs cause less or no side effects when compared with synthetic antibiotics. *Calotropis* is one of such medicinal plants reported to be used in distinct ways to treat infections [4]. *Calotropis* has two species., *Calotropis gigantea* and *Calotropis procera*.

The species of *Calotropis gigantea* has flowers ranging from purple to white colour. *C. gigantea* is a traditional medicinal plant. It belongs to the family of *Asclepiadaceae*, habitat of India, Indonesia, Sri Lanka and China. It is commonly known as milk weed or crown flower weed. *C. gigantea* is medicinally used to treat disease such as fever, rheumatism, indigestion, cough, cold, nausea, vomiting, leprosy, diarrhea and eczema [5]. It is scientifically reported for its cytotoxic, antipyretic and wound healing activity also [6]. *C. gigantea* is latex bearing plant and release the latex after a tissue injury. Leaves, roots, stem, flowers and latex of *C. gigantea* are used to cure several diseases in traditional medicinal system. Latex of *C. gigantea* is a mixture of alkaloids, tannins, gum, sugars, starch, resins and proteins [7].

Latex is reported to contain purgative properties, procoagulant activity and wound healing activity [8,9]. The latex of *C. gigantea* is a mixture of cardiac glycosides, calotropin, gigantol, flavanoids, alkaloids and proteolytic enzymes [10]. *Carica papaya* (Papaya), belongs to the family of *Caricaceae*, and several species of *Caricaceae* have been used as remedy against a variety of disease. *C. papaya*

offers not only the taste but also it is a rich source of antioxidant nutrients such as carotenes, Vitamin C, flavanoids, folate and pantothenic acid, minerals and fiber [11]. Its leaves and fruits produce several proteins and alkaloids with important pharmaceutical and industrial applications. The latex (milky sap) of an unripe *C. papaya* contains Papain and Chymopapain. Vitamins and traces of an alkaloid called Carpaine have also been found in the latex. The concentration of latex reduces upon ripening and once it is completely ripen then there is almost no latex left. Seed and pulp of *C. papaya* were reported to show bacteriostatic properties against several pathogens such as *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* [12]. Infectious diseases are a leading cause of death worldwide and antibiotic resistance has become a global concern [13]. Septicemia is blood poisoning disease, and is classified as having a large amount of bacteria or their toxins in the blood. It is a fatal whole body inflammation caused by severe infection. The disease can continue even after the infection that caused it is gone. Severe septicemia results in dysfunction of organs. The infectious agents of Septicemia are usually bacteria but can also be fungi and viruses. *B. licheniformis* is one of the Gram (+) bacterial agent of Septicemia. *B. licheniformis* is an aerobic, spore forming and rod shaped bacteria in the soil environment. Birds that tend to stay on the ground more than the air and on the water are common carriers of this bacterium. It is mostly found around the bird's chest area and back plumage. This bacterium was isolated in case with Bacteremia or Septicemia [14] as a human pathogen and cause serious infections. There is continuous need of the development of new effective antimicrobial drugs because of the emergence of new infectious diseases and drug resistance. Antibiotic resistance of microorganisms can be prevented by using new and potential natural compounds that are not based upon synthetic antimicrobial agents. As there is less research study on the effect of plant latex as antimicrobial agent so in view of this, the focus of this present context was to determine the comparative analysis for antibacterial activity of different solvent extracts of *C. gigantea* and *C. papaya* latex against two new strains of

*B. licheniformis* isolated from poultry farm which may be the causative agents of life threatening condition called Septicemia. The present investigation was also focused on the FT-IR analysis of the latex samples and the comparative inhibitory effects of both the plants latex to the antibiotics against new strains.

## MATERIALS AND METHODS

### Sample collection and isolation

Samples (surface soil) were collected from poultry farm and were brought to the laboratory in aseptic condition. 1 gram of surface soil sample was suspended in 9 ml of saline and mixed vigorously to make uniform suspension. After that soil samples were serially diluted up to  $10^{-5}$  and 0.1ml of aliquots were spread over nutrient agar plates from  $10^{-4}$  and  $10^{-5}$  dilution. The plates were incubated at 37°C for 24 hours. Pure strains were picked out and purified by repeated streaking on nutrient agar slants. The culture was streaked on slants and kept in incubator at 37°C for 24 hours and were preserved in slants at  $4 \pm 2^\circ\text{C}$ .

### Biochemical and morphological characterization

Purified isolates were characterized by Biochemical analysis using Indole test, Methyl Red test, Voges Proskauer test, Citrate utilization test, Catalase test and Urease test. Gram staining and Motility test were performed under Morphological test.

### Genomic DNA isolation

2 ml of bacterial culture were centrifuged at 6000 rpm for 5 minutes. The supernatant was discarded. 1 ml of UniFlex™ Buffer 1 and 10 µl of RNase were added to the pellet obtained. Mixed well by pipetting and incubated for 30 minutes at 37°C in a water bath. To the lysed samples 1 ml of 1:1 phenol:chloroform were added and mixed well. The samples were centrifuged at 10,000 rpm for 15 minutes at room temperature. The aqueous layers were separated in a fresh 1.5 ml vial. To the aqueous layer 1 ml of UniFlex™ Buffer 2 were added and mixed well by pipetting. The mixture was centrifuged at 12,000 rpm for 15 minutes at room temperature. The supernatant was discarded. To the pellet 500 µl of 70% ethanol were mixed. Again it was centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was discarded. The pellet was air dried for about 10-15 minutes till the ethanol evaporate. The pellet was resuspended in 50-100 µl of UniFlex™ Elution Buffer. DNA was stored at  $-20^\circ\text{C}$ .

### PCR amplification and sequencing

The 16S ribosomal RNA was amplified by using the PCR (ependorfep.Gradient) with *Taq* DNA polymerase and primers 27F (5' AGTTTGATCCTGGCTCAG 3') and 1492R (5'ACGGCTACC TTGTTACGACTT 3'). The conditions for thermal cycling were as follows: denaturation of the target DNA at 94°C for 4 min followed by 30 cycles at 94°C for 1 min, primer annealing at 52°C for 1 min and primer extension at 72°C for 1 min. At the end of the cycling, the reaction mixture was held at 72°C for 10 min and then cooled to 4°C. PCR amplification was detected by agarose gel electrophoresis and visualized by alpha image gel doc after ethidium bromide staining. The PCR product obtained was sequenced by an automated sequencer (Genetic Analyzer 3130, Applied Biosystems, and USA). The same primers as above were used for sequencing. The sequence was compared for similarity with the reference species of bacteria contained in genomic database banks, using the NCBI BLAST available at <http://www.ncbi.nlm.nih.gov/>. 16S rRNA sequence was then submitted to the GenBank, NCBI, USA.

### Collection of plant samples

*C.gigantea* latex and *C. papaya* latex were collected from Egmore, Tamil Nadu, India and Loyola college campus, Nungambakkam, Tamil Nadu, India respectively. The latex was collected in the morning time. The apical shoot part and the fruit (unripe fruit) were used for the collection of latex from *C. gigantea* (white flower plant) and *C. papaya* respectively. The apical shoot part and the fruit were first washed with sterilized distilled water and then wiped with 95%

ethanol. The latex was collected using syringe in the centrifuge tubes and was stored in sealed and labeled containers for further use.

### Plants extract preparation (Aqueous and ethanolic extract of latex)

*C. gigantea* latex and *C. papaya* latex were separately extracted with two solvents (water and ethanol). The latex obtained from both the plants was filtered using sterile mesh cloth. These filtered extracts were centrifuged at 6000 rpm for 15 minutes. Supernatants were collected as 100% extracts. Latex extracts of these two plants were diluted to make different concentrations such as 80%, 60%, 40%, 20% and 10% by mixing with appropriate volume of sterile double distilled water. The ethanolic extract of latex was prepared following the same procedure with the exception of solvent which was 95% ethanol instead of sterilized double distilled water.

### Microorganisms used

*Bacillus licheniformis* strain 018 (Accession no.-KC342225) and *Bacillus licheniformis* strain BIHPUR 0104 (Accession no.-KC424492) isolated from poultry farm were used.

### Antibacterial sensitivity test using disc diffusion method

The microorganisms (*Bacillus licheniformis* strain 018 and *Bacillus licheniformis* strain BIHPUR 0104) grown in Nutrient broth were transferred to Mueller Hinton Agar plates with the help of sterile cotton swabs. 25 µl of pure extracts, aqueous and ethanolic latex extracts of *C. gigantea* and *C. papaya* were aseptically transferred to each discs (6mm) at all dilutions that were made in triplicate. The discs were also loaded with 25 µl of combination of both the plant's latex (1:1) at 100% concentration. 25 µl of sterilized double distilled water and 95% ethanol were added in sterile discs as negative control in aqueous and ethanolic extract plates respectively. The soaked discs were transferred aseptically to the plates seeded with the microorganisms with the help of ethanol dipped and flamed forceps. The petriplates were incubated in upright position at 37°C for 24 hours. After 24 hours zone of inhibition formed by different solvent extracts of latex at different concentrations against the tested microbes were measured. The mean and standard deviation of the diameter of zone of inhibition were calculated.

### Multiple antibiotic resistances (MAR) index determination

The antibiotic susceptibility pattern of the test organisms were performed as per standard procedure. A homogeneous bacterial lawn was prepared on Mueller Hinton Agar plates using sterile cotton swabs. The sterile discs of 6mm diameter were soaked with 25 µl of antibiotics. Using an ethanol dipped and flamed forceps the standard antibiotic and soaked discs were aseptically placed over the agar plates sufficiently separated to avoid overlapping of zone of inhibition. Plates were incubated at 37°C for 24 hours. After 24 hours, diameter of zone of inhibition was measured in mm and results were recorded. MAR index was calculated by the ratio of number of antibiotics ineffective over the organisms to the number of antibiotics exposed [15]. The antibiotics used in this study were Ampicillin (AMP-10 µg), Kanamycin (K-30 µg), Nalidixic acid (NA-30 µg), Streptomycin (S-10 µg), Cephotaxime (CTX- 30µg) and Penicillin G (P-10 µg)

### Fluorescence analysis of latex extracts

The aqueous and ethanolic extracts of *C. gigantea* and *C. papaya* latex was analyzed in normal light. After that the same solvent extracts were seen under short wavelength (302 nm) and long wavelength (365 nm) of UV light. The presence of fluorescent compounds in the latex extracts were visualized in the form of different colors of the extracts and the results were compared with the color appeared in the normal light.

### Fourier Transform Infrared (FT-IR) spectroscopy analysis of the plant latex samples

The crude latex of *C.gigantea* and *C.papaya* was ground into fine powder using mortar and pestle. About 3 mg of the samples were mixed with 300 mg of KBr and pressed into a pellet. The samples pellet were placed into the sample holder and FT-IR spectra were

recorded in the range of 4000-450  $\text{cm}^{-1}$  in FT-IR spectroscopy [ Model no.- IRAffinity- 1( SHIMADZU ) ].

### Statistical analysis

The results of the antibacterial activity of different solvent extracts of *C. gigantea* and *C. papaya* latex were expressed as mean  $\pm$  standard deviation of the response of three replicates determination per sample. Results were determined by Microsoft Excel 2007.

## RESULTS

### Antibacterial activity test

In this study the microorganisms isolated from poultry farm were identified as new strains of *Bacillus* species according to Morphological, Biochemical characteristics and 16S rRNA gene sequencing. Antibacterial activity of different solvent extracts of *C. gigantea* and *C. papaya* latex at different concentrations were determined by Agar disc diffusion method against new strains of *Bacillus* species isolated from poultry farm. The latex of *C. gigantea* was found to be more effective compared to the latex of *C. papaya* against both the new strains.

Aqueous extracts of *C. gigantea* latex were found to be more effective against *Bacillus licheniformis* strain BIHPUR 0104 compared to *Bacillus licheniformis* strain 018 with maximum zone of inhibition of 14.5 mm at 100% concentration. *Bacillus licheniformis* strain BIHPUR 0104 was found to be resistant to ethanolic extracts of *C. gigantea* latex. Both the solvent extracts of latex at 10% concentration were found to be ineffective against both the strains.

Aqueous extracts of latex were found to be effective even at 40% concentration compared to ethanolic extracts of latex against *Bacillus licheniformis* strain 018 with minimum zone of inhibition of 9 mm. Aqueous extracts of *C. gigantea* latex were effective against strain BIHPUR 0104 even at 20% concentration. Aqueous extracts of *C. papaya* latex were found to be more effective compared to ethanolic extracts against strain 018 with maximum zone of inhibition of 12 mm. On the other hand ethanolic extracts of *C. papaya* latex were more active than aqueous extracts against strain BIHPUR 0104 with maximum zone of inhibition of 12.5 mm. Strain BIHPUR 0104 was resistant to aqueous extract of *C. papaya* latex at all dilutions except 100% concentration. Aqueous extract of latex were also found to be effective against strain 018 compared to the strain BIHPUR 0104 (Table 1 and 2). In another study antibacterial activity of the combined latex extracts (1:1) were found to be reduced against both the strains.

The combined extract of *C. gigantea* and *C. papaya* were showing zone of inhibition of 10.5 mm against both the strains (Table 3).

### MAR Index Determination

The MAR index value of the test organisms were reported in Table 4. The MAR value is a ratio of the number of ineffective antibiotics to the number of antibiotics exposed. The MAR value of the test organisms was found to be zero.

### Fluorescence analysis of latex extracts

Fluorescence of aqueous and ethanolic extract of *C. gigantea* and *C. papaya* latex were visualized under normal light and UV light (short wavelength and long wavelength). Aqueous extract of *C. gigantea* were visualized as milky white, light blue and light blue in normal light, 302 nm and 365 nm of wavelength. Ethanolic extracts of *C. gigantea* latex was visualized as yellowish brown, light blue and light blue at the same above respective wavelength. In the same way aqueous extract of *C. papaya* were visualized as brown, light blue and light blue in normal light, short wavelength and long wavelength respectively. Ethanolic extract was visible as milky white in normal light but it was visualized as light blue in the wavelength of 302 nm and 365 nm (Table 5).

### Fourier Transform Infrared (FT-IR) spectroscopy analysis of the plant latex samples

The results for FT-IR analysis for the functional groups present in the latex of *C. gigantea* are listed in Figure (a). In the spectra of *C. gigantea* latex, absorption peaks were observed at 3745.76  $\text{cm}^{-1}$  and 3387.00  $\text{cm}^{-1}$  due to N-H and -OH stretching of acid. Strong peaks at 2931.80  $\text{cm}^{-1}$ , 2872.01  $\text{cm}^{-1}$  and 2856.58  $\text{cm}^{-1}$  are due to -CH<sub>3</sub> and -CH<sub>2</sub> aliphatic stretching. Peaks from 2393.66  $\text{cm}^{-1}$  to 2073.48  $\text{cm}^{-1}$  are representative for asymmetric N-H and torsional stretching. Strong absorption band at 1734.01  $\text{cm}^{-1}$  and 1649.14  $\text{cm}^{-1}$  are representative for C=O carbonyl stretching of acids. Peaks at 1543.05  $\text{cm}^{-1}$  and 1519.91  $\text{cm}^{-1}$  are due to NH<sub>2</sub> or N-H bonding. Peaks from 1454.33  $\text{cm}^{-1}$  to 1323.17  $\text{cm}^{-1}$  are representative for C=O stretching. The absorption bands from 1244.09  $\text{cm}^{-1}$  to 983.70  $\text{cm}^{-1}$  are due to C-N stretching. Weak absorption band at 677.01  $\text{cm}^{-1}$  and 628.79  $\text{cm}^{-1}$  are representative for N-H out of plane bending. Figure (b) demonstrates the FT-IR spectra of *C. papaya* latex. Strong absorption bands from 3552.88  $\text{cm}^{-1}$  to 3232.70  $\text{cm}^{-1}$  are representative for -OH intermolecular H-bonding. Strong peak at 2970.38  $\text{cm}^{-1}$  are due to deformations of -CH<sub>3</sub>, -CH<sub>2</sub> groups for aliphatic. Peak values at 1635.64  $\text{cm}^{-1}$  and 1554.63  $\text{cm}^{-1}$  are due to C-O coupled with N-H bonding. Absorption bands from 669.30  $\text{cm}^{-1}$  to 514.99  $\text{cm}^{-1}$  are representative for CH out of plane bending

**Table 1: Shows the antibacterial activity of different solvent extracts of *C. gigantea* and *C. papaya* latex at different concentrations against *Bacillus licheniformis* strain 018 by disc diffusion method (in mm).**

Concentration	<i>C. gigantea</i>		<i>C. papaya</i>	
	Aqueous	Ethanolic	Aqueous	Ethanolic
10%	NZI	NZI	NZI	NZI
20%	NZI	NZI	NZI	NZI
40%	9.0 $\pm$ 0	NZI	NZI	NZI
60%	10.5 $\pm$ 0.7	11.5 $\pm$ 0.7	10.0 $\pm$ 0	8.5 $\pm$ 0.7
80%	11.0 $\pm$ 0	12.5 $\pm$ 0.7	10.5 $\pm$ 0.7	9.0 $\pm$ 0
100%	13.0 $\pm$ 0	13.0 $\pm$ 0	12.0 $\pm$ 0	12.0 $\pm$ 0

\*NZI= NO zone of inhibition.

**Table 2: Shows the antibacterial activity of different solvent extracts of *C. gigantea* and *C. papaya* latex at different concentrations against *Bacillus licheniformis* strain BIHPUR 0104 by disc diffusion method (in mm).**

Concentration	<i>C. gigantea</i>		<i>C. papaya</i>	
	Aqueous	Ethanolic	Aqueous	Ethanolic
10%	NZI	NZI	NZI	NZI
20%	10.0 $\pm$ 0	NZI	NZI	NZI
40%	12.5 $\pm$ 0.7	NZI	NZI	NZI
60%	13.0 $\pm$ 0	NZI	NZI	10.5 $\pm$ 0.7
80%	13.5 $\pm$ 0.7	NZI	NZI	12.5 $\pm$ 0.7
100%	14.5 $\pm$ 0.7	14.5 $\pm$ 0.7	12.0 $\pm$ 0	12.5 $\pm$ 0.8

\*NZI= NO zone of inhibition.

Table 3: Shows the combined effect of *C. gigantea* and *C. papaya* latex against both strains (in mm)

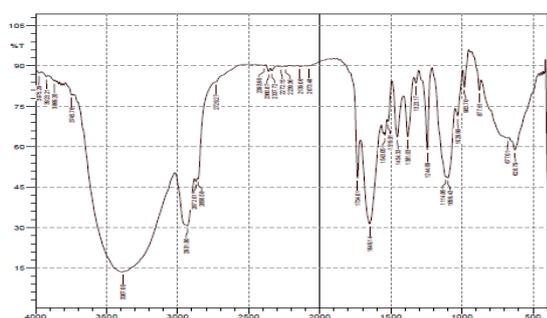
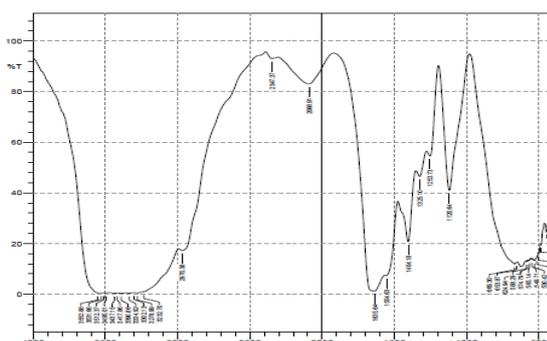
Bacteria	<i>C. gigantea</i>	<i>C. papaya</i>	Combination (1:1)
<i>B. licheniformis</i> strain 018	13.0±0	12.0±0	10.5±0.7
<i>B. licheniformis</i> strain BIHPUR 0104	14.5±0.7	12.5±0.7	10.5±0.7

Table 4: Shows the MAR index determination of the bacterial isolates

Bacteria	MAR value	No. of ineffective antibiotics	Name of ineffective antibiotics
<i>B. licheniformis</i> strain 018	0 (Zero)	None	None
<i>B. licheniformis</i> strain BIHPUR 0104	0 (Zero)	None	None

Table 5: Shows the Fluorescence analysis of *C. gigantea* and *C. papaya* latex extracts

Extracts	<i>C. gigantea</i>			<i>C. papaya</i>		
	Normal light	Short wavelength	Long wavelength	Normal light	Short wavelength	Long wavelength
Aqueous	Milky white	Light blue	Light blue	Light yellow	Light blue	Light blue
Ethanollic	Yellowish brown	Light blue	Light blue	Milky white	Light blue	Light blue

Fig. a: Shows FT-IR spectrum of *C. gigantea* latexFig. b: Shows FT-IR spectrum of *C. papaya* latex

## DISCUSSION

The growing population concern about health problems has led to the development of natural antimicrobials to control a lot of diseases. Plant products, particularly extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants.

The presence of active compounds has been reported to confer resistance to plants against bacteria and therefore explains the demonstration of antibacterial activity by the plant extracts. Vaibhav *et al* [16] reported that aqueous extract of *C. gigantea* showed high inhibitory activity than ethanolic extract against *B. subtilis*, *E. coli*, *S. aureus* etc. In our study, aqueous extracts of *C. gigantea* latex were also found to be effective against new strains of bacteria that had been isolated from poultry farm. But *B. licheniformis* strain 018 were found to be more susceptible to ethanolic extracts of latex than aqueous extract based on zone of

inhibition. The variation in the susceptibility of these two strains against different solvents in this study favors the finding of Subramanian *et al* [17] who demonstrated that antimicrobial activity of different solvent extracts of *C. gigantea* showed varying degrees of antibacterial activity against all the microorganisms tested. The aqueous and ethanolic extracts were obtained from the latex of *Calotropis* species and were tested against different bacterial pathogens. Ethanolic latex extract showed wider zone of inhibition compared to aqueous extract [18]. But in this study *B. licheniformis* strain 018 and *B. licheniformis* strain BIHPUR 0104 were found to be more susceptible to aqueous extract compared to ethanolic extract of latex. Aqueous extract of *C. gigantea* latex were found to be more effective compared to ethanolic extract against *B. licheniformis* strain 018 even at 40% concentration. The broad antibacterial activity of aqueous extract may be due to the presence of biologically active ingredients with antimicrobial activity in the aqueous extract of *C. gigantea* latex. Alabi *et al* [19] demonstrated that the organic extracts of *C. papaya* were more effective than aqueous extracts. This finding was almost similar to our investigation. *B. licheniformis* strain BIHPUR 0104 was more susceptible to ethanolic extract of *C. papaya* latex than aqueous extract. This may be due to the better solubility of the active components in organic solvents. The better efficacy of the ethanolic extract against the aqueous extract may be because different solvents have different polarities, hence different degrees of solubility for the various phytoconstituents. On the other hand, aqueous extracts of *C. papaya* latex were found to be more effective than ethanolic extract against *B. licheniformis* strain 018. This may be due to the reason that the active compound responsible for inhibiting the growth of *B. licheniformis* strain 018 is more soluble in water than that of ethanol. The efficacy of treatments with *C. papaya* is dependent on the quantity of the different chemical substances present in the preparation. The quantity of chemical substances varies in the latex and varies with the extraction method, age of plant part and the sex of the tree [20]. According to Kumar *et al* [21] the aqueous extract of *C. gigantea* latex possess significant amount of antimicrobial activity against both Gram (+) and Gram (-) bacteria. In our investigation aqueous extract of *C. gigantea* latex was also effective against *B. licheniformis* strains i.e., Gram (+) bacteria. Aqueous extract of *C. papaya* latex was also showing inhibitory effect on *B. licheniformis* strain 018. But ethanolic extract of *C. papaya* latex was more effective compared to aqueous extract against both the strains. The susceptibility of microorganisms against the ethanolic extract of *C. papaya* latex favors the finding of Kumar *et al* [22] who demonstrated that ethanolic extract of plant had shown better activity against the pathogenic microorganisms. Seed and pulp of *C. papaya* was

reported to say bacteriostatic properties against several pathogens (Osato *et al.*, 1993). Our study provides further confirmation about the potential activity of *C. papaya* latex by showing moderate level of bactericidal properties. The latex of *C. papaya* showed a potential role as an anthelmintic [23] and antifungal activity [24]. One more step in the effectiveness of *C. papaya* latex can be seen from our study by showing its moderate level of antibacterial activity against new strains of pathogenic bacteria. The aqueous and ethanolic extracts of plants latex were showing significant increase in zone of inhibition as the concentration increased. This finding agrees with the work of Khusro *et al* [25] who showed that the higher concentration of ethanolic and aqueous extracts of the plant, the more bacteria were inhibited to grow, as indicated by the larger zone of inhibition. The combined 100% concentration of *C. gigantea* and *C. papaya* latex were showing less effectiveness on the strains compared to the same concentration of latex tested alone.

This may be due to the reason that the active components in the plants latex would be inactivated to some extent when they were combined together in 1:1 ratio which resulted the strains to become resistant. This finding is against the work of Sunita *et al* [26] who demonstrated that zone of inhibition was the largest in combination of the plants in the same ratio. But our investigation favors the work of Onyeagba *et al* [27] who reported that when two or three plant extracts were combined then their activity was reduced against the some of the tested pathogenic organisms. MAR index determination of the bacterial isolates was found to be zero because the new strains were susceptible to all antibiotics that had been tested. But the effectiveness of some of the antibiotics such as Penicillin G, Ampicillin, Cephalexin and Nalidixic acid were found to be less compared to the latex against strain 018. On the other hand all the antibiotics compared to latex were more effective against strain BIHPUR 0104 (Data not shown).

The above results clearly demonstrated the emergence of the antibiotic resistant bacteria and provide a strong reason for the quest of new antimicrobial agents other than antibiotics. The presence of fluorescent compounds in the latex of *C. gigantea* and *C. papaya* were visualized at two different wavelengths. Further confirmation of the presence of fluorescent compounds was analyzed by UV- spectrophotometer. Both the plants latex showed the peaks with different absorbance at different wavelengths (Data not shown). From this investigation, it is clear that latex of *C. gigantea* is more effective than that of *C. papaya* against these two new strains of *B. licheniformis* which may be the causative agents of septicemia. *C. papaya* latex had shown moderate level of activity against these two new strains.

Although the mechanism of action of these extracts is not understood. The inhibitory action of latex against these two strains may be due to the inhibition of cell wall formation resulting in the secretion of cytoplasmic constituents from the cell. The phytochemical compounds may also inhibit the activity of enzymes by forming complexes with bacterial cell walls or their membranes. Commercially available antimicrobial agents such as antibiotics are now used to treat infectious diseases. The major problem encountered with antibiotics in the clinical research is resistance to drug, toxicity, high cost and low efficiency. Drugs derived from herbal plants are susceptible to microbes, non-toxic, cheap and highly effective. Medicinal plants have always remained the major sources of traditional medicine. Our results conclude the effectiveness of *C. gigantea* latex over to the *C. papaya* latex for the inhibition of new strains of *B. licheniformis* which may cause septicemia especially to the poultry farm workers. People working in the poultry farm are more prone to get this disease because this bacterium is present mostly on the surface and near the chest region of birds and therefore workers are continuously exposed to these bacteria. So from this investigation it is clear that *C. gigantea* latex can be a potential agent to inhibit the growth of these bacteria which may cause septicemia to the poultry farm workers. The most active and non-toxic compound from this plant latex can be isolated and new drugs can be synthesized for the prevention and treatments of this disease. Isolation of pharmaceutically important bioactive

compounds from *C. gigantea* latex may prove its importance in therapeutic applications.

## CONCLUSION

Plant based antimicrobials have enormous therapeutics potential with lesser side effects. Current study clearly concludes that *C. gigantea* latex possesses significant amount of antibacterial activity compared to *C. papaya* latex against new strains of bacteria. The observed antibacterial activities of latex extracts in both the solvents justify the traditional use of this plant against new strains of *B. licheniformis* which may be the causative agents of septicemia. Thus the findings revealed the medicinal potential of *C. gigantea* against this disease to develop a drug. The effective and non-toxic biomolecules from this plant latex and virulent genes from these strains need to be isolated and identified. Overall studies indicating that the plants latex can be considered for the medicinal purposes which are less studied so far. A further extensive investigation on its pharmacokinetics, dynamics and clinical trials are needed to know their therapeutic utility against this disease.

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