Synthesis and Applications of β–cyclodextrin Incorporated Carica Papaya Leaf Extract Electrospun Nanofibers

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ABSTRACT

Electrospinning is an easier and more useful technique that uses electrostatic forces to yield very thin fibers of polymer enriched substances in the nanometer range. So many other techniques are used for the synthesis of nanofibers, yet electrospinning has proven to be an efficient method that has a significant effect on their properties. In the present study, the production of polymer enhanced nanofibers of Carica papaya leaf extracts was done using Electrospinning. The super molecular cyclodextrin incorporated Carica papaya leaf extract nanofibers were also prepared using Electrospinning method. The nanofiber mats were subjected to UV-Visible Spectrophotometer, FT-IR, FESEM and Anti-Bacterial Analysis. The studies showed the supramolecular incorporated extract is having more effect as a drug than the normal extract alone.

Keywords: Antibacterial, Carica papaya, Cyclodextrin, Electrospinning, Nanofiber.

I. INTRODUCTION

Carica papaya belongs to the family caricaeae joined with the passifloracae and it is a distinctive source of possibly useful compounds with diverse constitutions and properties [1]. Papaya (carica papaya linm) is usually known for its food and nutritional values all over the world. The medicinal attributes of papaya fruit and other parts of the plants are also well known in customary system of medicine. Each part of papaya tree has its own economic worth when grown on a business scale. Even though the active components are usually obtained from all parts of the plant, the amount of these components differs. Parts that have a high amount of active components are used for therapeutic purposes. The plant parts are found to have some properties like analgesics, amebicide, antibacterial, cardiolonic, cholagogue, digestive, emenagogue, hypotensive, laxative, antimalarial and vermifuge properties.

Papaya leaves are prepared as a tea for the treatment of malaria. Pappaya leaves contain carpan, a substance that kills microorganisms that frequently interrupt the digestive function. It also contains phenolic compounds, Protocatechuic acid, P-coumaric acid, 5,7-dimethoxy coumarin, caffeic acid, kaemperol, quercetin and chlorogenic acid [2].

The medical society uses the leaves poultice for nervous pains and elephantoid growth [3]. Ayurvedic literature reveals that papaya leaf extract has hemostatic properties [4]. Studies done in dengue patients with leaf juice revealed the effect of leaf juices on elevating white blood cells, platelet count and recovery without hospital admission. This demands the need for the phytochemical profiling of the leaf juice to identify the bioactive constituents attributing significant activity.

The alkaloids, flavonoids, saponins, tannin and glycosides are related with anti-inflammatory activity. Carica papaya leaves extract was also found to have anti-bacterial activity, anti tumour, immune-dulator activities [1]. The leaves also contain cardiac glycosides, anthraquinones, carpane, pseudocarpaine, phenolic compounds. Carica papaya leaves are also used for the treatment of Arthritis, granulomatous tissue formation, sickle cell disease, anemophilic and diabetics. Moreover, juice of carica papaya leaf is used as anti-cancer agent by people in Australia and so many positive results are reported in various publications [5].

Published Online: February 28, 2022
ISSN: 2684-4478
DOI: 10.24018/ejchem.2022.3.1.70

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Nanofibers of *carica papaya* extract can be used for the treatment of various diseases. Fibers with diameters less than or equal to 1000nm (1micron meter) are defined as nanofibers and they can be prepared by several processing techniques, such as drawing, template synthesis, phase separation, self-assembly, electrospinning and so on. In comparison with all the techniques mentioned above; electrospinning (ES) is the only method that can be used for producing nanofibers of different polymers in continuous form and moreover it is a simple technique. Since long lengths, small diameters, and high surface area per unit volume of nanofiber produced by electrospinning, they are of industrial and scientific interest.

A polymer matrix is required for the synthesis of nanofiber. Poly-vinyl alcohol is used as polymer matrix in electrospinning of various nanofibers. The PVA/extract reinforced mats are prepared using electrospinning. The solubility of PVA is improved by heating the polymer and reinforcing it with suitable substances. Many studies are reported about PVA as polymer matrix. Seon Baek Yang in 2018 reported about CC extract incorporated PVA-based electrospun nanofibers and studied in detail the morphologies, antimicrobial properties of PVA/CC extract nanofibers. J. Elliott Sanders reported about Poly (vinyl alcohol) (PVA) and cellulose nano crystals (CNC) random composite mats prepared using the electrospinning method.

Electrospinning involves the manufacturing of polymer nanofibers with the help of organic solvents. But organic solvents sometimes cause a detrimental effect on the environment. So, water-soluble polymers can be prepared using water as a solvent instead of organic solvent. The starch-based oligosaccharides cyclodextrins (CDs) are also water-soluble and can be used to prepare nanofibers without polymer matrix [6]. Increased concentration of cyclodextrin produces continuous nanofibers without any breakage. Cyclodextrins can be directly used in various applications due to their cyclic truncated supramolecular structure.

Cyclodextrins are formed by enzymatic degradation process of starch and the arrangement of hydrophobic carbon backbones through the internal part of cyclodextrin creates a hydrophobic cavity, which enables the formation of a host-guest inclusion complex (IC) which is non-covalent in nature. Cyclodextrin being non-toxic in nature can be used in the food industry and cosmetic products.

The combination of cyclodextrins with nanofibers increases the application areas of electrospun nanofibers. Cyclodextrin has a lyophilic outer surface and hydrophobic cavity in which the guest molecules are accommodated. It forms a Host-guest molecule that can be used for potential applications. The incorporations can be directly injected into the bloodstream, which acts on the target area. The inclusion complexes can also be used for drug delivery applications. By using cyclodextrin, continuous nanofibers can be produced even without the use of polymer-matrix.

II. MATERIALS AND METHODS

A. Materials

The fresh leaves of female *Carica papaya* were collected from the native fields of Trivandrum. The plant specimen was identified and authenticated as *Carica papaya* from the Department of botany, Christian college, Kattakada. Poly (vinyl alcohol) (MW=30,000), Dimethyl Formamide (MW=73.09g/mol), Cyclodextrin (MW=1135g/mol) was purchased from sigma. All the reagents used were of laboratory grade.

B. Methods

The fresh leaves of *Carica papaya* were washed with purified water. The washed leaves were drained and dried in an air oven at 60-70 °C for 6 hours. The dried leaves were manually chopped and crushed into a powder form and sieved with a 20 µm mesh. This powder (300 g) was soaked in n-hexane, ethanol, and ethyl acetate with a proportion of 100 g powder in 200 ml solvent each. The solution was kept for three days. After three days, each solution was taken and double filtered separately using Whatmann filter paper.

1) Ultrasound-assisted extraction (USE)

The filtrates obtained by soaking crushed powder in suitable solvents were exposed to 40 KHZ ultrasound waves for 30 minutes at 50°C in a digital ultrasonic bath (GT Sonic- Professional Ultrasonic Cleaner) for extraction. The extracts were obtained by concentration with the help of a BUCHI rotary vacuum R-100 at 75 °C. The ethanol, n-hexane, and ethyl acetate extracts of *Carica papaya* leaves acquired were preserved in an air-tight bottle for remaining studies.

C. Phytochemical Screening of Carica Papaya Leaf Extract

Phytochemical screening of *Carica papaya* extract was performed to test the presence of various phytochemicals.

- Alkaloids- Dragendorff’s Test: The extract is mixed with one or two drops of dragendorff reagent - gives orange red precipitate-Presence of alkaloids.
Carbohydrates-Molisch’s Test: The extract is 1 mixed with α-naphthol in ethanol and added sulphuric acid- purple ring – presence of carbohydrates.
Saponins- Extract is mixed with chloroform and water gives soapy foaming on heating – Presence of saponins.
Glycosides- Extract is mixed with acetic anhydride and sulphuric acid, the solution turns brown colour – absence of glycosides.
Phenolic compounds- Extract is mixed with ferric chloride and lead acetate – presence of phenolic compounds.
Flavanoids– Extract is mixed with 5% NaOH – presence of yellow colour implies the presence of flavonoids.
Tannins– Extract is treated with neutral ferric chloride – presence of green colour confirms the presence of tannins.

D. Electrospinning of Polymer Enhanced Carica Papaya Leaf Extracts

The process of electrospinning was done with the help of E-SPIN SUPER ES 1 machine. About 3 g of the extract and the polymer PVA were dissolved in Dimethyl Formamide to make a uniform solution. The PVA concentration maintained as 10% with respect to the extract. When the concentration of the polymer increases beyond 10%, the spinning of nanofiber becomes tough. The prepared polymer solutions were agitated overnight and are filled into a 5 ml standard polypropylene syringe with 27 G blunted stainless steel needle and it is used for electrospinning of nanofibers. For the generation of nanofiber mats through electrospinning, the flow rate was maintained at 1 mL/hour. When a high voltage of about 14 KV was applied, the polymer solution at the tip of the needle is elongated and converted into nanofibers in a continuous form. The collector is enfolded with aluminium foil, and it is placed at a distance of 12 cm from the needle p. Room temperature is maintained for conducting the experiments and a humidity of 10% is ideal for the preparation of electrospun nanofibers. The aluminium foil containing nanofibers was carefully withdrawn from the collector, dried overnight in a vacuum oven, cut down into 10×10 pieces and stored in dry cabinets for further studies. The same method is adopted for the synthesis of cyclodextrin incorporated polymer enhanced Carica papaya leaf extract.

E. UV-Visible Spectroscopy

The sample was placed between a light source and a photo detector. The intensity of beam of light before and after passing through the sample was plotted as extinction as a function of wavelength. Each spectrum background is corrected using a &quot;blank&quot; with a cuvette filled with only the dispersing medium to guarantee that spectral structures from the solvent are not involved in the sample extinction spectrum.

F. Fourier Transform Infrared Spectroscopy

FTIR Spectroscopy is used to analyse the functional groups present in nanofibers. The instrument used for FTIR analysis is Perkin Elmer FTIR spectrometer. The spectrum is obtained over the range of 500-4000 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\).

G. Field Emission Scanning Electron Microscopy

The main difference between FESEM and SEM is that FESEM works on the basis of a field emission electron source. FESEM completely depends on potential gradient that helps to emit the electron beams. In FESEM, a single Tungsten filament with a pointed tip is used as the electron source. An increase in sharpness of the filament helps in the formation of an image with high resolution. Topographic images with higher resolution can be obtained from FESEM. Small squares of dimension 10×10 was cut from the deposited nanofibers and used for the analysis.

H. Anti-Bacterial assay

Procedure (Agar well diffusion method):

15-20 ml of Mueller-Hinton agar was decanted on glass Petriplates of the same size, and it is allowed to undergo solidification. Each plate was punched with a sterile cork. With the help of a cotton swab, the standard inoculums were homogeneously spread on the surface of the prepared plates. 50 µL of the extract solution at preferred concentration was added to each well and Gentamycin is added to one well to make it as a positive control. The plates are then incubated, and a clear zone is found after the incubation process. Mueller Hinton Agar M173 HI media is used for the determination of the activity of microorganisms to the prepared antimicrobial agents. Add 38 grams of Mueller Hinton Agar M173 HI media in 1000 ml distilled water. Heat the solution to dissolve it. After complete dissolution, the solution is sterilized using autoclave at 15 lbs pressure (121 °C) for 15 minutes. Then cool the solution to 45-50 °C. After sufficient cooling, the solutions were again mixed well and transferred into sterile Petri plates. Inoculums were procured from The
Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh: *Staphylococcus aureus* at 37 °C for 24 hours, *Klebsiella pneumonia* at 37 °C for 24 hours, *Pseudomonas aeruginosa* at 37 °C for 24 hours.

III. RESULTS AND DISCUSSION

A. Phytochemical Screening

The phytochemical screening of ethanol, ethyl acetate and aqueous extracts of *Carica papaya* leaf indicates that the aqueous extract has only the presence of alkaloids while ethanolic and ethyl acetate extracts show the presence of major phytochemical components including alkaloids, carbohydrates, glycosides, and flavonoids. Zohaib H.S. et al., Nighat Razvi, Syed Imran Ali and Syed Mohammad S.F.H in 2018 [7] studied about Qualitative phytochemical screening of extract and found that different extracts of *Carica papaya* Linn contains various phytochemicals like alkaloids, flavonoids, tannins, phenolic compounds, glycosides including cardiac glycosides, proteins, and carbohydrates. The phytochemicals are found in greater amount in female plant leaves when compared to male ones.

| Components          | Ethanol extract | Ethyl acetate extract | Water extract |
|---------------------|-----------------|-----------------------|--------------|
| Alkaloids           | +               | +                     | +            |
| Carbohydrates       | +               | +                     | _            |
| Saponins            | +               | +                     | _            |
| Glycosides          | +               | +                     | _            |
| Phenolic Compounds  | -               | +                     | _            |
| Flavonoids          | +               | +                     | _            |

Bamidele V. Owoyele [8] in 2008 reported about the phytochemical screening of *Carica papaya* leaf extracts which is in agreement with the obtained results.

Pallavi Singh [9] studied about the phytochemicals present in the crude methanolic and aqueous extract of leaves and roots *Carica papaya*, Piper nigrum and Agave americana and found that different phytochemicals alkaloids, terpenoids, flavonoids, sugar, protein, phenols, saponins and quinines are present in the aqueous and methanolic plant extract.

The antioxidant and antibacterial properties of the C. papaya fresh flowers were studied by Manish Kumar Dwivedi [10]. He found that the phytochemicals present in the organic extracts show substantial antibacterial and antioxidant activities. The presence of phenolic compounds or flavonoids increases the antibacterial activity of the organic extracts. Among different plant extracts, n-hexane extracts exhibit strong antioxidant activity.

A. E. Ajiboye [11] studied the qualitative phytochemical screening of acetone and aqueous extract of leaves of *Carica papaya* and specifies the occurrence of saponins, flavonoids, tannins, alkaloids, steroids, and glycosides. The quantitative analysis of phytochemical constituents of *Carica papaya* revealed the percentage yield of flavonoids to be 0.70%, alkaloids 0.480%, tannin 1.02%, Steroids 0.116% and glycosides to be 1.08%.

B. UV Visible Spectroscopy

1) Pure leaf extract

![Fig. 1. UV spectrum of pure leaf extract.](image)

From Table II, the spectral analysis indicates that the maximum absorption is at 241.50 nm which is due to π→π* transitions.
2) Polymer enhanced carica papaya leaf nanofibers

From Table III, the UV spectral analysis of *Carica papaya* leaf extract nanofibers shows maximum absorption at 247 nm due to n→n* transitions.

3) Polymer/Cyclodextrin/Carica papaya Nanofibers

From Table IV, the UV spectral data of PVA/CD/extract nanofiber shows maximum absorption at 235 nm due to n→n* transitions of incorporated polymer.

The three spectral data obtained are almost identical and maximum absorption is obtained in arrange between 230-250 nm. Jyoti Ahlawata, Vinay Kumara, P. Gopinath in 2019[12] studied the UV spectral analysis of *Carica papaya* leaf powder. The maximum absorption was obtained at 255nm. The maximum absorption value is due to the existence of Vitamin C.

Dyah Setyawati1, Sri Andayani, Uun Yanuhar in 2016[13] reported about the UV Visible characterization of ethyl acetate extracts of *Carica papaya*. The UV- Vis analysis of *Carica papaya* was analysed and it confirms the presence of active compounds like flavonoids especially quercetin (at wavelength 249, 240, 221, 219 and 204 nm) based on the standard. It also contains tri-terpenoid which was confirmed by the absorbance of wavelength 436, 261, 223 nm and alkaloid at wavelength 213 nm [11].
TABLE II: UV Spectral Data of Pure Leaf Extract

| No. | P/V | Wavelength | Abs. |
|-----|-----|------------|------|
| 1   | ↑   | 666.00     | 0.054 |
| 2   | ↑   | 607.00     | 0.116 |
| 3   | ↑   | 535.50     | 0.159 |
| 4   | ↑   | 481.50     | 0.444 |
| 5   | ↑   | 413.00     | 1.741 |
| 6   | ↑   | 306.00     | 2.350 |
| 7   | ↑   | 276.00     | 2.793 |
| 8   | ↑   | 241.50     | 3.353 |
| 9   | ↓   | 215.00     | 2.545 |
| 10  | ↓   | 628.00     | 0.072 |
| 11  | ↓   | 580.00     | 0.043 |
| 12  | ↓   | 525.50     | 0.126 |

TABLE III: UV Spectral Data of Polymer Enhanced Leaf Nanofibers

| No. | P/V | Wavelength | Abs. |
|-----|-----|------------|------|
| 1   | ↑   | 727.50     | 0.003 |
| 2   | ↑   | 666.00     | 0.246 |
| 3   | ↑   | 608.00     | 0.056 |
| 4   | ↑   | 536.00     | 0.071 |
| 5   | ↑   | 412.00     | 0.779 |
| 6   | ↑   | 307.00     | 1.248 |
| 7   | ↑   | 276.00     | 1.755 |
| 8   | ↑   | 247.00     | 3.615 |
| 9   | ↑   | 222.00     | 1.979 |
| 10  | ↑   | 210.00     | 2.296 |
| 11  | ↓   | 628.50     | 0.035 |
| 12  | ↓   | 580.00     | 0.022 |

TABLE IV: UV Spectral Data of Polymer/Cyclodextrin/Carica Papaya Nanofibers

| P/V | Wavelength | Abs. |
|-----|------------|------|
| 1   | 666.00     | 0.222 |
| 2   | 607.50     | 0.052 |
| 3   | 535.50     | 0.067 |
| 4   | 411.50     | 0.692 |
| 5   | 306.00     | 1.009 |
| 6   | 276.00     | 1.431 |
| 7   | 255.00     | 3.736 |
| 8   | 224.00     | 3.273 |
| 9   | 214.00     | 2.639 |
| 10  | 628.00     | 0.034 |
| 11  | 580.50     | 0.024 |
| 12  | 524.00     | 0.053 |

C. Fourier Transform Infrared Spectroscopy

1) Pure leaf extract

![IR spectrum of pure leaf extract](image-url)
The IR spectra indicates strong peaks at 3321 cm\(^{-1}\) (OH), 2854 cm\(^{-1}\) (CH), 1723 cm\(^{-1}\) and 1628 cm\(^{-1}\) (C=O), 1366 cm\(^{-1}\) (CH\(_2\)), 1278 cm\(^{-1}\) and 1040 cm\(^{-1}\) (C-C), 741 cm\(^{-1}\) and 704 cm\(^{-1}\) (CH). The peak at 3321 cm\(^{-1}\) indicates the OH stretching frequency. The values 2926 and 2854 cm\(^{-1}\) indicate C-H stretching. The presence of carbonyl or ketone group C=O can be confirmed by the absorption wavenumber 1723 cm\(^{-1}\) and 1628 cm\(^{-1}\).1366 cm\(^{-1}\) indicates CH\(_2\) vibrations.1121,1278 and 1040 shows C-C. The values near 700 cm\(^{-1}\)show C-H out of plane vibrations.

2) Polymer enhanced Carica papaya leaf nanofibers

![Fig.5. IR spectrum of polymer enhanced leaf nanofibers.](image)

The IR spectra indicates strong peaks at 3327 cm\(^{-1}\)(OH), 2925 and 2854 cm\(^{-1}\) (CH), 1736 cm\(^{-1}\) and 1608 cm\(^{-1}\) (C=O), 1457 cm\(^{-1}\) (CH\(_2\)), 1278 cm\(^{-1}\) and 1093 cm\(^{-1}\) (C-O), 843 cm\(^{-1}\) (CH) and 662 cm\(^{-1}\) (CN). The peak at 3327 cm\(^{-1}\) indicates strong OH stretching vibrations.

2925 and 2854 cm\(^{-1}\) indicate C-H stretching. The presence of C=O is shown by value in 1736 and 1608 cm\(^{-1}\).1457 indicates CH\(_2\) stretching.1093 cm\(^{-1}\) confirms the existence of C-O bond 843 cm\(^{-1}\) shows an aromatic C-H, while 662 cm\(^{-1}\) indicates CN.

3) Polymer/Cyclodextrin/Carica papaya nanofibers

![Fig.6. IR spectrum of polymer/cyclodextrin/ leaf nanofibers.](image)
The IR spectra indicates strong peaks at 3413 cm\(^{-1}\) (OH), 1736 cm\(^{-1}\) (C=O), 1413 cm\(^{-1}\) (CH\(_2\)), 1065 cm\(^{-1}\) (C-O), 511 cm\(^{-1}\) (CN). The IR spectra show the presence of OH stretching at 3413 cm\(^{-1}\). The values at 1595 cm\(^{-1}\) and 1515 cm\(^{-1}\) cofirms C-N vibrations present in the extract. The CH\(_2\) deformations are shown by 1413 and 1065 indicates the C-O stretching frequency.

The IR spectrum of pure *Carica papaya* leaf extract, Polymer enhanced nanofibers and Polymer/cyclodextrin/leaf extract nanofibers showed almost similar values. The values designated the existence of OH stretching, C-O, C-N, C-H vibrations.

The values obtained in FTIR Spectra show good agreement with the work reported by Ahlawata et al. “*Carica papaya* loaded poly (vinyl alcohol)- gelatin nanofibrous scaffold for potential application in wound dressing” [12]. He used the FTIR Spectral values to study the conformational studies.

Kokila et al. [14] reported about the FTIR analysis *Carica papaya* extract. The work relates to the green synthesis of silver nanoparticles using *carica papaya* peel extract and FTIR technique is used to confirm the presence of silver nanoparticles on the *carica papaya* peel extract by comparing the FTIR spectra of pure *carica papaya* extract and silver nanoparticles incorporated *carica papaya* peel extract.

Bashaa, S Z.V.P. Murthy b, B. Jhaa in 2009 [15] reported about the use of FTIR analysis in confirming various functional groups like hydroxyl, carboxyl, sulfhydryl, sulfonate, etc. in *Carica papaya* extract. He revealed that the presence of functional groups in *Carica papaya* leaves is responsible for the adsorption activity of different bio sorbents. Using FT-IR analysis, he confirms the presence of various functional groups in *C. papaya*. The nature of the bio sorbent – Hg (II) ions interaction was explained on the basis of FT-IR.

D. Field Emission Scanning Electron Microscopy

1) FESEM of Pure Leaf Extract

![Fig. 7. FESEM images of polymer enhanced *carica papaya* leaf extract nanofibers.](image)

Field emission scanning electron microscopy (FESEM) is used to analyse the surface morphology of prepared extract-PVA nanofibers. The nanofiber contains various randomly interconnected structures. The diameter of the nanofibers was calculated using image J analysis software. The results clearly disclose that the addition of extract to PVA create an impact on the morphology of the sample. The diameter calculated is about 5 µm. The diameter of the plant extract nanofiber is decreased when incorporated into a suitable polymer matrix. Change in viscosity and conductivity of the spinning solution causes a decrease in the diameter of the synthesized nanofiber.

2) PVA/Cyclodextrin/Carica papaya nanofibers

![Fig. 8. FESEM images of cyclodextrin incorporated *carica papaya* leaf nanofiber.](image)
The surface morphology of prepared Cyclodextrin incorporated Carica papaya nanofiber mats was analysed using field emission scanning electron microscopy (FESEM). The micrograph images depict randomly interconnected continuous fibers. The diameter of the nanofibers was calculated using image J analysis software. The result clearly reveals that the supramolecular incorporation of nanofibers is possible. It is noted that the diameter is about 5000 nm (25 µm). Analyzing the result, we can observe that a very few number of beads are present in the electrospun nanofiber which might be caused due to the concentration conditions.

Ahlawata et al. in 2019 [12] synthesized PVA/Gelatin/Carica papaya nanofibrous scaffolds by electrospinning method. Various experimental techniques including FTIR, XRD, TEM, FESEM, AFM, and TGA, etc., are used for the characterization of synthesized nanofiber scaffolds. The successful electrospinning of nanofibrous scaffold of PVA, PVA/Gelatin and PVA/Gelatin/ Carica papaya can be established from the FESEM images. The synthesized scaffolds are bead free, continuous and the surface is smooth and homogenous. The average diameter of these nanofibers was calculated as 140±20 nm.

Radhakrishnan Sridhar in 2013 [16] reported about the SEM analysis of polymer enhanced nanofibers. His study confirms that the surface morphology of the synthesized nanofiber can be analysed using FESEM technique. By suitably analyzing the surface, the nanofibers can be used as a drug carrier that can efficiently deliver the drug to the targeted site.

Banala [17] studies about the morphological changes of carica papaya extract with the incorporation of silver nanoparticles. The FESEM technique is used to confirm the presence of synthesized silver nanoparticles in the papaya leaf extract.

E. Anti-Bacterial Assay

1) Antibacterial assay of ethanol extract

The values from Table V confirmed that the ethanol extract does not show any antimicrobial activity against Staphylococcus aureus for a given sample for certain concentration with respect to a standard Gentamycin (80 mcg). But it shows moderate antibacterial activity against Pseudomonas aeruginosa at different concentrations of the sample.

2) Antibacterial assay of ethyl acetate extract

The values from Table VI confirmed that the ethyl acetate extract does not show any antimicrobial activity against Staphylococcus aureus for a given sample for a certain concentration with respect to a standard Gentamycin (80 mcg). But it shows slight antibacterial activity against Pseudomonas aeruginosa at different concentrations of the sample.
3) Antibacterial assay of nanofiber extract

The values from Table VII confirmed that the nanofiber extract shows moderate antibacterial activity against *Staphylococcus aureus* for a given sample for a certain concentration with respect to a standard Gentamycin (80 mcg). But it does not show any antibacterial activity against *Pseudomonas aeruginosa* at different concentrations of the sample.

T. Kokila et al. [14] reported about the antimicrobial activity of *carica papaya* peel extracts against gram-positive and gram-negative bacteria. Ahlawata *et al.* [42] reported about the antibacterial effects of *carica papaya* leaf nanofibers against pathogenic bacteria. From the above Anti-bacterial assay, it is seen that the Ethanolic extract of *Carica papaya* leaves shows anti-bacterial activity against *Pseudomonas aeruginosa* while the ethyl acetate extract shows active against *Klebsiella Pneumoniae* bacteria. Since the ethanolic extract is used to spin nanofiber mats, the nanofibers of *Carica papaya* leaves also show activity against *Pseudomonas aeruginosa*. From the studies it is clear that the extract as well as the nanofiber shows anti-bacterial activity.

Augustin I Airodion [18] confirmed that both ethanolic and aqueous extracts of *C. papaya* leaves possess antibacterial activities against *C. bacillus*, *S. epidemidis*, *S. viridans* and *E. coli* and also inhibited their growth. But the Ethanolic extract has a greater effect than aqueous extract. However, antibacterial activity was not observed for *S. typhi*.

A study conducted by Suresh *et al.* found that among five different plant extracts, the *Carica papaya* leaf has the highest antibacterial activity against gram-positive bacteria (*Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*) and had lesser results on gram-negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacteria [19]. The gram-negative bacteria have a thick murein layer present on their outer membrane which prevents the entry of plant extract inhibitor substances inside the cell.

In another study, ethanol, methanol, ethyl acetate, Aqueous, n-hexane and ethanol extract of *Carica papaya* leaves were analysed for antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *Pseudomonas aeruginosa* by Chandra *et al.* [23]. She found that the three extracts show significant antibacterial activity. Among the three extracts, n-hexane and ethanol had the highest zone of inhibition against *S. aureus* and while aqueous extract had a high zone of inhibition against *B. subtilis*, *E. coli*, and *Pseudomonas aeruginosa*.

Mangalanayaki and Nirosha [22] observed the antibacterial activity of ethanol and ethyl acetate of *Carica papaya* leaf extracts against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* using Agar well diffusion method. All the extracts show high antibacterial activity against all the gram-negative bacteria than gram-positive. The Ethanolic extract leaves and roots have moderate ability to kill all the bacterial pathogens than aqueous extract of leaves and root.

Ogunjobi and Ogunjobi [24] confirms the antimicrobial activity of *carica papaya* leaf extract through cold extraction precipitation on eight bacterial strains *Staphylococcus aureus*, *Salmonella typhi* B; *Shigelladysenteria*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Pseudomonas fluorescens*; *Proteus vulgaris*; and *Bacillus subtilis*.

Romasiset *et al.* [25] studied the antibacterial activity of ethanol, ethyl acetate, and hexane extract of papaya leaf against *Bacillus stearo-thermophilus*, *Listeria mono cytogenes*, *Pseudomonas sp.*, and *Escherichia coli* by agar diffusion method. The study shows that papaya leaves contain potential natural antibacterial components.

The results of the study conducted by Hema *et al.* [26] showed that the propanolic extracts of *Carica
papaya have highest activity than the ethanol extract. Among the gram-positive and gram-negative bacteria tested against the leaf extract of C. papaya, the gram-negative bacteria were more exposed especially Proteus vulgaris to the extracts.

acetone, chloroform, and hot water extracts of papaya leaves act as an antibacterial agent against Bacillus cereus, Klebsiella pneumonia, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus [20]. Ethanol, methanol, ethyl acetate and acetone plant extracts were more operative for B. cereus. Methanol, ethyl acetate and chloroform extracts were more active for E. coli. Ethyl acetate and acetone extracts were more effective against P. aeruginosa. Chloroform extract was more effective against Micrococcus luteus. Petroleum ether and Hexane extract was no activity against any bacteria. Hot water extract was found to be more effective for S. aureus. [21].

The resistance of plants against bacteria, fungi and pests is due to the presence of bioactive substances. Since these phytochemicals are present in carica papaya plant extract, they also demonstrate antibacterial activity [22]. Organic extracts have more antibacterial activity than aqueous extracts. The greater solubility of active phytochemicals in organic solvents accounts for its antimicrobial activity. Among the gram-positive and gram-negative bacteria gram-negative bacteria were more exposed to the extracts.

Zakira et al. [27] analyzed the antimicrobial activity of Carica papaya flowers against bacterial pathogens. Sherwani et al. [28], Omojasola and Awe [29] also examined the leaf extract of Carica papaya against plant and human pathogenic bacteria.

### TABLE V: ANTIBACTERIAL ASSAY OF ETHANOL EXTRACT

| Sample          | Concentrations of sample | Staphylococcus aureus | Pseudomonas aeruginosa |
|-----------------|--------------------------|-----------------------|------------------------|
| Standard Gentamycin (80mcg) | 24                        | 25                    |
| Ethanol         | Negative control         | -                     | -                      |
| T1 (400mcg)     | -                        | -                     | -                      |
| T2 (800mcg)     | -                        | -                     | -                      |

### TABLE VI: ANTIBACTERIAL ASSAY OF ETHYL ACETATE EXTRACT

| Sample          | Concentrations of sample | Staphylococcus aureus | Pseudomonas aeruginosa |
|-----------------|--------------------------|-----------------------|------------------------|
| Standard Gentamycin (80mcg) | 23                        | 30                    |
| Ethyl acetate   | Negative control         | -                     | -                      |
| T1 (400mcg)     | -                        | -                     | -                      |
| T2 (800mcg)     | -                        | -                     | -                      |

### TABLE VII: ANTIBACTERIAL ASSAY OF NANOFIBER EXTRACT

| Sample          | Concentrations of sample | Pseudomonas aeruginosa | Klebsiella pneumoniae |
|-----------------|--------------------------|------------------------|-----------------------|
| Standard Gentamycin (80mcg) | 24                        | 29                    |
| Ethanol         | Negative control         | -                      | -                     |
| T1 (400mcg)     | 11                       | -                      | -                     |
| T2 (800mcg)     | 13                       | -                      | -                     |

**IV. SUMMARY AND CONCLUSION**

Carica papaya leaf extract was incorporated into PVA by electrospinning. This novel technique was effective for the encapsulation of the natural extract to generate a continuous and protective film of nanofibers. The phytochemical components of the extract were found out by different methods. Polymer cyclodextrin extract nanofibers were also prepared by electrospinning. The encapsulation was observed by FESEM and, thereafter, confirmed by chemical analysis using ATR-FTIR and UV spectroscopy.

The UV spectral analysis of pure extract, extract nanofibers and cyclodextrin inclusion complex were observed at 240 nm, 247 nm, and 231 nm, respectively. The IR graphs of three samples showed similar peaks in the range 3400 cm⁻¹ due to OH stretching, 1400 cm⁻¹ due to CH2 deformations, 1700-1600 cm⁻¹ due to C=O vibrations and peaks below 700 cm⁻¹ indicates C-H out of plane deformations.

The FESEM analysis shows the structural features of the nanofibers. With the increase in concentration, the size of the nanofiber got increasing were observed through this method. It is noted that the diameter of the sample obtained has been 5 µm. The addition of USE extracts to PVA influence the morphology of the sample. The FESEM analysis of supramolecular incorporated nanofibers showed that the incorporation does not affect the morphology of electrospun nanofibers, but a very few number of beads were observed due to the concentration factor. The presence of the inhibition zone confirmed the presence of antibacterial
activity against bacteria namely against *Klebsiella Pneumoniae* and *Pseudomonas aeruginosa*, while it is inactive against *Salmonella typhi*.

The phytochemical studies, chemical analysis, surface studies and antibacterial assay reveal that *Carica papaya* leaves have active components present in them when it is converted into nanofiber. The properties of leaf extract were retained when it was converted into nanofiber also. The IR and UV studies show that the peaks were almost similar in leaf extract, leaf nanofibers and cyclodextrin incorporated nanofibers. These conclusions lead to the fact that *Carica papaya* nanofibers have potential applications as that of the leaf extract and can be used for various medicinal purposes. Active cyclodextrin incorporation paves the way to a broad range of applications in medical fields.

**ACKNOWLEDGMENT**

I acknowledge the Department of Science and Technology (DST), for providing funds under the Fund for Improvement of S&T infrastructure in universities & higher educational institutions (FIST) for providing funds to purchase equipment in the instrumentation lab and also for computer facilities in Computer and Networking lab. I also acknowledge Kerala State Council for Science, Technology and Environment (KSCSTE) for providing funds under the Selective Augmentation of Research and Development (SARD) scheme for the procurement of equipment. I acknowledge CLIF, University of Kerala, for helping the project by analyzing a sample.

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