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Research Article

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Optimization of Caffeic Acid and its Antibacterial Activity Using *Carica papaya* Seed Extract Nanoparticles (CPNPs)

Gumma Jayasri[1*], Akhil Arava[1], Sandeep Sirohi[2], Avinash Singh[3], D. Appala Naidu[1] and Meena Vangalpati[1]

[1]*Department of Chemical Engineering, Andhra University College of Engineering (A), Visakhapatnam, Andhra Pradesh, India*
[2]*Department of Botany, Hariom Saraswati P.G. College, Haridwar, Uttarakhand, India*
[3]*Department of Biotechnology, Meerut Institute of Engineering and Technology, Meerut, Uttar Pradesh, India*

[*]Correspondence to:
Gumma Jayasri
Department of Chemical Engineering,
Andhra University College of Engineering (A),
Visakhapatnam, Andhra Pradesh, India.
E-mail: gummajayasri123@gmail.com

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Abstract

The seeds of the papaya (*Carica papaya*) have long been used for their therapeutic benefits, including possible antibacterial activity. In this study, we used response surface methodology (RSM) to optimize the extraction process and assess the antibacterial activity of *C. papaya* seed nanoparticles (CPNPs) ethanol extract against selected bacterial strains. The papaya seeds were dried and powdered. CPNPs were synthesized using *C. papaya* extract through a green and sustainable approach. The biosynthesized NPs were characterized for their size, morphology, and composition by scanning electron microscope (SEM) analysis. Using the agar well diffusion method, the antibacterial activity was determined against typical bacterial strains, which include *Salmonella* and *Staphylococcus aureus*. To enhance the extract's antibacterial activity, other extraction process parameters including the solid-to-liquid ratio, ethanol concentration, and extraction duration were further adjusted using RSM. Box-Behnken design (BDD) was used to create the experimental design matrix. The purpose of this study was to optimize the extraction procedure using Fourier transform infrared spectroscopy (FTIR) and RSM and to investigate the antibacterial qualities of CPNPs ethanol extract.

Keywords

Carica papaya, Antibacterial activity, Seed extracts, Nanoparticles, Response surface methodology

Introduction

To reduce the prevalence of infectious diseases worldwide, antimicrobial drugs are crucial. Because there are fewer dangerous bacteria, the rise and spread of multi-drug resistant types of bacteria has become identified as a public health concern, or occasionally no, effective antimicrobial medicines available to treat infections caused by pathogenic bacteria [1]. Due to their antibacterial properties, which are the result of phytochemicals produced during the plant's secondary metabolism, many plants have proven useful.

The papaya variety known as *Carica* is a member of the Caricaceaus family. It was first introduced to tropical America and then transported to India in the sixteenth century. The papaya, a popular tropical fruit, is rich in bioactive phytochemicals such benzyl isothiocyanate and dietary antioxidants like vitamin C, tocopherols, beta-carotene, and total phenolic content [2]. Different parts of the *C. papaya* plant, including the leaves, barks, roots, latex, fruit, flowers, and seeds, are used in folk medicine to cure a variety of illnesses. In addition to all of this, papain is a digestive enzyme [1, 3]. *C. papaya* which is used to treat allergies, injuries,

and trauma-related reasons. Overall, the nutrients in papaya promote cardiovascular health, guard against heart conditions, heart attacks, and strokes, as well as guard against colon cancer and an enzyme that is used to treat rheumatoid arthritis.

Various developing diseases and other illnesses characterized by drug resistance exist now. Because it is extremely difficult to treat humans and animals with the currently available medications [4], there is a need for alternative medications. Numerous researchers have shown that herbal medicines can be created as secure, cost-efficient substitutes for the present medications used to treat specific bacterial illnesses. CPNPs derived from papaya seed extracts have demonstrated efficient antimicrobial activity against a wide range of pathogens. Because of the nano-formulation, bioactive compounds can enter microbial cells with greater efficiency, disrupting cell membranes and inhibiting essential biological processes to a greater extent. Knowing the antibacterial qualities of papaya seed extract and the possibilities of biosynthesized CPNPs may help produce antimicrobial treatments that are less harmful to the environment and more affordable, with uses in biotechnology and medicine among other areas.

Consequently, the current study's objective is to examine the antibacterial efficacy of crude papaya (nanosized seed) extracts against harmful bacterial strains.

Materials and Method

Preparing plant samples for processing

The *C. papaya* fruit was gathered in the surrounding area. The papaya fruits were rinsed in distilled water after being washed in tap water. The seeds were taken out of the shade after a week and ground with a mortar and pestle. The seeds were processed into a rough powder. Using ethanol as a solvent, bio-active substances were fractionated for 48 h at room temperature while being constantly stirred in the dark. To avoid contamination by seed residue, the appropriate solvent was thoroughly filtered using a centrifuge after fractionation. To create a fine paste, the clear extract was air dried. The extract was measured and kept at 40 °C in the dark for additional examination.

Charecterization of *C. papaya* seed

SEM examination is used to examine the structural and morphological confirmation of synthetic metals. Figure 1 displays the SEM images of a *C. papaya* nanosized seed extract. Particles are undoubtedly responsible for the development of the spherical structure. Particle sizes of metals are smaller, with CPNPs measuring approximately 100 nm. When the particle distribution is homogeneous, we may learn more about morphological research and obtain a better understanding of the size of the particles. A review of the literature indicates that, in some cases, utilizing natural particles as reducing agents might lead to particle agglomeration, making the particles appear slightly larger than they are (Figure 2). This phenomenon is like what happens with some metals during biological synthesis.

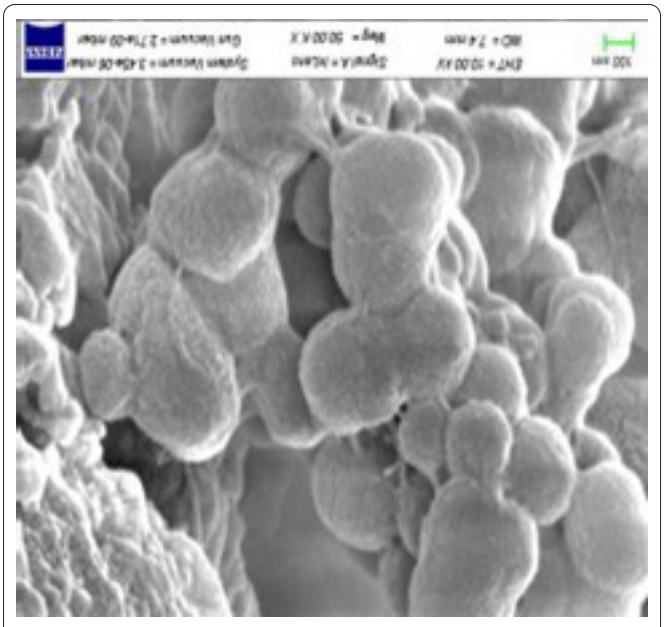

Figure 1: SEM image of CPNPs.

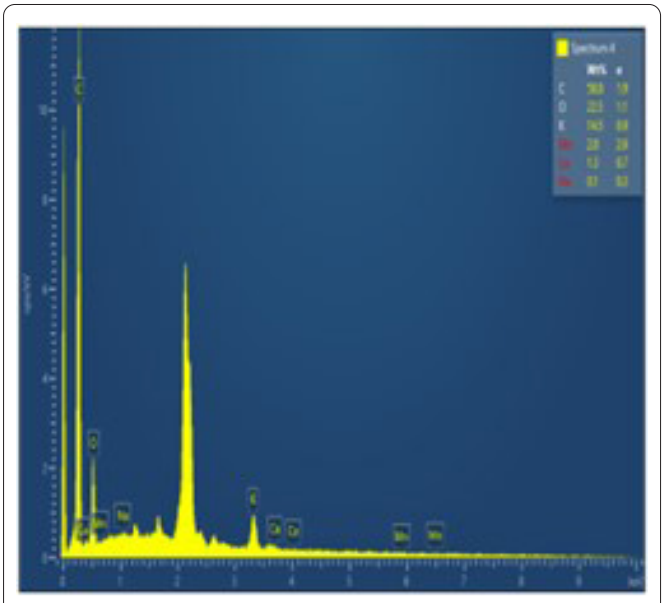

Figure 2: Elemental analysis of CPNPs.

Quantitative determination of caffeic acid in *C. papaya* seed

Utilizing UV-Vis spectroscopy, the amount of caffeic acid in the plant extract was assessed. Using a UV-Vis spectrophotometer, the absorbance of the resultant mixture was measured at 660 nm for the plant extract after 10 ml of ethanol and 1 g of plant extract were combined [5].

Extraction of plant material

25 g of coarsely powdered papaya seeds were weighed and suspended into a 200 ml conical flask (ethanol, ethyl acetate, and methanol) [6]. The extraction process was place using a shaker incubator at 30 °C and 60 rpm at room temperature. The extracts were filtered using centrifuge and the clear supernant was air dried in glass Petri plates. This powder was sieved and stored in an airtight container until further usage.

RSM

RSM is one such statistical and mathematical methodological combination. By evaluating quantitative data from analytical experiments along with their interaction terms, RSM can be utilized to concurrently create and solve multivariate equations. The polynomial equation that forms the basis of RSM can be used to model the relationship between the independent and dependent factors as well as predict and characterize the optimal circumstances for tests. A well-known RSM design that improves the extraction of bioactive chemicals is the BBD [4, 7-9]. The BBD is used to estimate all two-way, quadratic, and linear interactions. It is composed of rotating lower-dimensional designs. It prohibits reduction. Their domain is devoid of corners. The circular factorial design element's axial points are enclosed outside the box-shaped design area that was developed. This makes it possible to estimate the anticipated response with the same variance regardless of how far one is from the design area's center. The three independent factors examined in the initial BBD extraction trials are listed in table 1. A 2nd order polynomial equation was utilized to forecast the extract yield (response) using Stat-Ease Design Expert Software version 12.

Antibacterial activity

By using the agar-well diffusion method, we investigated the compounds by antimicrobial activity. Using sterile cotton swabs, the standardized cultures of test bacteria were first equally distributed over the Mueller-Hinton Agar plate surface. A sterile corn borer was used to create five wells (6 mm in diameter) in each plate. Chemical and positive controls were introduced to wells in increments of twenty micro-litre. Fluconazole (30 µg/ml) and streptomycin (10 µg) were utilized as reference antibiotics. After one hour of room temperature diffusion, chemicals, antibiotics, and dimethyl sulfoxide were added. All plates are closed with lids and incubated at 37 °C for 24 h. After incubation, plates were observed by zone of inhibition [8, 10]. The average diameter of the inhibition zone is expressed in millilitre, was used to express the antibacterial activity of the compounds based on the measurement of the inhibition zone diameter. Compounds that failed to exhibit an inhibition zone (diameter is greater than 6 mm) were deemed inactive. The inhibitory zone diameter mean values were obtained by testing each chemical in triplicate across two different tests.

Table 1: RSM.

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	0.3187	9	0.0354	660.95	< 0.0001	Significant
A - Time	0.0001	1	0.0001	2.10	0.1906	
B - pH	0.0000	1	0.0000	0.0000	1.0000	
C - Temperature	0.0001	1	0.0001	2.10	0.1906	
AB	0.0002	1	0.0002	4.20	0.0796	
AC	0.0001	1	0.0001	1.87	0.2141	
BC	0.0000	1	0.0000	0.4667	0.5165	
A^2	0.0855	1	0.0855	1596.00	< 0.0001	
B^2	0.0947	1	0.0947	1768.42	< 0.0001	
C^2	0.1044	1	0.1044	1949.68	< 0.0001	
Residual	0.0004	7	0.0001			
Lack of fit	0.0004	3	0.0001			
Pure error	0.0000	4	0.0000			
Cor total	0.3190	16				

Results and Discussion

Extraction studies

To extract the bioactive components, the extraction solvent must be carefully chosen. Three different solvents, namely water, ethanol, and methanol, were used in the experiments. A substantial yield of caffeic acid is produced from the powdered *C. papaya* seed when ethanol is used as the solvent.

Following extraction testing, ethanol was employed as a solvent. One factor at a time, optimization technique was used to conduct the experiments. Using ethanol concentrations ranges from 0% to 100%, caffeic acid was extracted. The experiments' results showed that 60% ethanol produced the greatest extraction of caffeic acid. The greatest caffeic acid extraction was reached at pH = 7, according to research done to find out how pH affected extraction (Figure 3). For several lengths of time 30, 60, 90, and 120 min the seed powder was immersed in the solvent at pH 7.

RSM

The primary and combined effects of time (A), pH (B), and temperature (C) on the extraction of caffeic acid were examined using the BBD, which was implemented based on 17 experimental runs featuring these three variables. To prevent experimental errors, duplicates were employed. The diagnostics test for the appropriateness of the suggested model, which was based on the Fishers F-test, was carried out using the analysis of variance (ANOVA). The regression coefficient (R^2) shows how much of the variance around the mean the model can account for. Table 1 shows the results from 17 tests that correspond to BBD, both in terms of coded and uncoded amounts of independent components. When considering coded factors, the quadratic model is represented.

Final equation with coded factors

$$\%\text{Caffeic acid extraction} = +2.13 - 0.0038A + 0.0000B - 0.0037C - 0.0075AB - 0.0050AC - 0.0025BC - 0.1425A^2 - 0.1500B^2 - 0.1575C^2$$

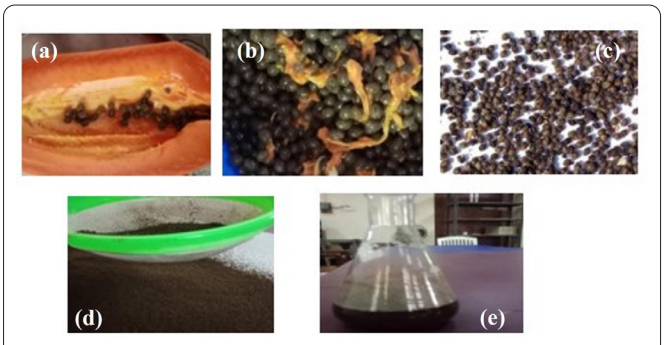

Figure 3: (a) *C. papaya* fruit, **(b)** *C. papaya* seeds, **(c)** *C. papaya* seeds air dried, **(d)** Seed powder extract, and **(e)** Ethanolic extract of seed.

Quadratic model ANOVA

Response 1: %Extraction of caffeic acid. Factor coding is coded. Sum of squares is Type III – Partial. A model's significance is indicated by its F-value of 660.95. A huge F-value like this could only happen by chance (0.01%).

Model terms are significant if the p-value is less than 0.0500. In this instance, important model terms are A^2, B^2, and C^2. The model terms are deemed not significant if their values exceed 0.1000. Model reduction could help your model if it has a lot of unnecessary terms (aside from those needed to maintain hierarchy).

Fit statistics

The adjusted R^2 of 0.9973 and the predicted R^2 of 0.9812 are reasonably in agreement with each other; that is, the difference is less than 0.2 (Table 2). The surface contour plots that are produced will make it easier to see the statistical impact of the independent factors on the dependent variables. The experimental data was transferred to a 3D response plot, and the effects of temperature, time, and pH were studied to calculate the extract yield. In this investigation, the extraction duration was modified to maximize extraction effectiveness and energy savings (Figure 4 and figure 5) [10].

FTIR

The active groups and biomolecules of papaya seed extract were identified using FTIR measurements. The *C. papaya* seed extract was shown in figure 6.

Table 2: Fit statistics.

Std. dev.	0.0073
Mean	1.92
C.V. %	0.3816
R^2	0.9988
Adjusted R^2	0.9973
Predicted R^2	0.9812
Adeq precision	55.8910

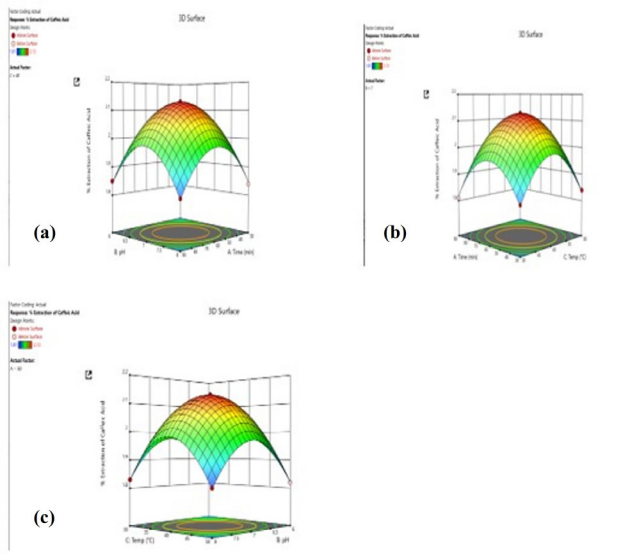

Figure 4: 3D response plot of **(a)** time vs pH, **(b)** time vs temperature, and **(c)** temperature vs pH.

Optimization

Maximizing the response across several criteria is the goal of the optimisation study. The design expert software determines which elements are required to achieve the best results for this goal. Using the result from the experiment that was acquired in the optimal circumstances, the expected extraction percentage was validated. Ideal parameters for the maximal caffeic acid extraction were 60% ethanol, 60 min extraction time, and 40 °C.

Antibacterial activity

Using agar well diffusion technique, the seed extract antibacterial activity was determined *in vitro*. *Salmonella* and *S. aureus* were the germs under investigation. These were incubated at 37 °C for 24 h. We evaluated antibacterial activity using the zone of inhibition (Figure 7 and table 3).

Conclusion

The results of an ethanolic solvent-based antibacterial assay on crude papaya (CPNPs) reveal inhibition zone for

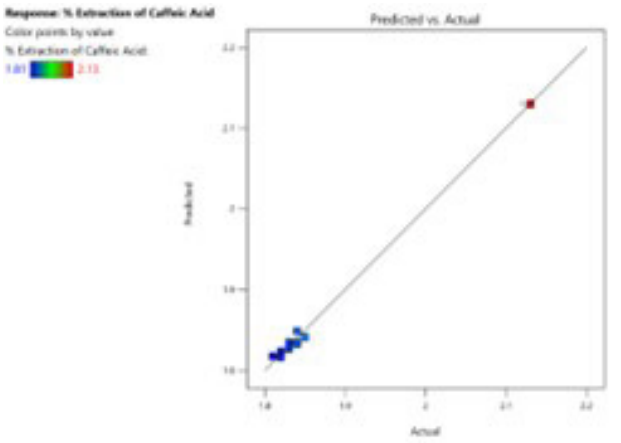

Figure 5: Experimentation and predicted value (RSM).

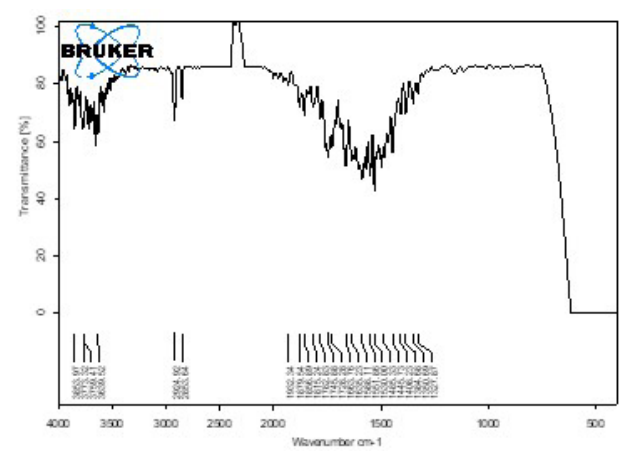

Figure 6: FTIR of *C. papaya* seed extract.

Table 3: % of inhibition zone by agar well diffusion method.

S. No.	Plant part	Zone of inhibition (mm)							
		S. aureus				*Salmonella*			
		100 µg	250 µg	500 µg	1000 µg	100 µg	250 µg	500 µg	1000 µg
1	*C. papaya*	0	8	10	12	14	22	28	38
2	Streptomycin (30 µg)	34				32			

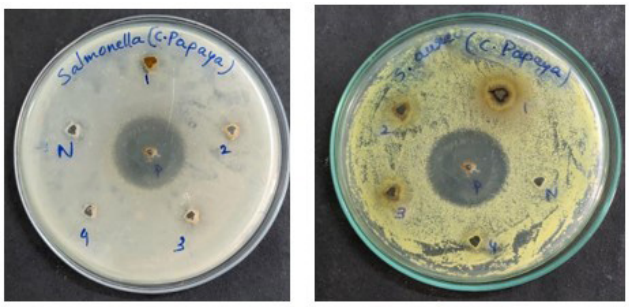

Figure 7: Inhibition zone by agar well diffusion method.

all microorganisms examined. Plant-based medications have significantly improved human health, and they are a source of inspiration for the creation of novel drug molecules. According to the results of the current investigation, caffeic acid produced from *C. papaya* nano sized seed extract might be useful in pharmacology. When compared to a positive control, the ethanol crude extract from *C. papaya* nanosized seed extract showed similar antimicrobial efficacy against *S. aureus* and *salmonella*. This demonstrates the plant's potential for use in the creation of medications to treat a range of human ailments. The plant portion and the extraction solvent used seemed to have an impact on the susceptibility of the two types of bacteria. This outcome demonstrated that medicinal plant extract performs similarly to conventional antibiotics.

Acknowledgments

None.

Conflict of Interest

None.

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