

Research Article



Comparitive Study on Antibacterial Activity of Crude and Silver Nanoparticles Synthesized Leaf Extract of *Carica papaya* against Chosen Isolates

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ABSTRACT

The Emergence of Human Pathogens with Antibiotic resistance genes has shifted the focus towards the plant origin antimicrobial drugs with advanced nano-based methods. Screening of phytochemical components is done using various biochemical methods. UV-visible spectrophotometer is utilized to determine the silver Nanoparticle's size, shape, and its stable nature in the aqueous colloidal solution. FT-IR spectral analysis to identify the bio molecules responsible for the reduction of Ag⁺ ions. The extracts of *Carica papaya* leaves were found to be rich in Vitamins, Phenols, Proteolytic enzymes which acts as excellent Antimicrobial agent. Antibacterial activity of crude extracts and silver Nanoparticles synthesized from *Carica papaya* leaves was evaluated against chosen clinical isolates, which shows the effective zone of inhibition lesser side effects. Though crude extract has shown significant results, silver nanoparticles synthesized using specific key phytochemical component would have the better inhibiting capacity.

Keywords: *Carica papaya*; Antibacterial activity; Plant extract; FT-IR study; silver Nanoparticles.

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INTRODUCTION

Caricaceae family is best known for its medicinal remediation, one of the significant medicinal value species is *Carica papaya*¹. Silver Nanoparticles have a long and intriguing history as an antibiotic in human health care². Silver ion is biologically active and readily interacts with proteins, amino acids residue, free anions and receptors on mammalian and eukaryotic cell membrane. Hybrids of silver Nanoparticle with amphiphilic hyper branched macromolecule exhibits effective antimicrobial surface coating agent³. Aqueous extracts of *Carica papaya* leaves rich in phenol, proteolytic enzymes, which act as excellent antimicrobial agent⁴. The *Carica papaya* leaf extract mediated synthesis of silver Nanoparticle was effective and provides additional property such as bactericidal efficient and might act as long searched alternative and could be the answer to antibiotic resistance⁴.

The silver nanoparticles showed efficient biological property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms⁵. The key objective is to prepare the extract of *Carica papaya* leaves by using Percolation method and to Screen for Phytochemical components of

Carica papaya leaves. To characterize silver nanoparticles (AgNPs) of *Carica papaya* leaves by UV-Visible spectrophotometry and FT-IR spectral analysis. Finally, to evaluate the Antibacterial activity of crude extracts and silver nanoparticles (AgNPs) synthesized from *Carica papaya* leaves using the agar well diffusion method.

MATERIALS AND METHODS

Sample collection

The *Carica papaya* plant leaves were collected from the Cumbum Valley, Theni District. Collected leaves were washed with tap water, then rinsed with distilled water, dried, cut into fine pieces and were crushed into fine powder and stored at 37°C.

Preparation of *Carica papaya* solvent extract by percolation method

With the percolation apparatus, 50g of dried *Carica papaya* leave powder was relaxed with ethanol for 40 hours. After transferring, qualitative test was performed to identify the various plant constituents for the stored ethanol extract of *Carica papaya* residue.

Screening of phytochemical components

The leaf extracts of *Carica papaya* were analyzed for the presence of alkaloids, carbohydrates, saponins, phenolic compounds, flavonoids, proteins and amino acids according to the common phytochemical methods such as Mayer's test, Wagner's test, Molisch's test, Benedict's test, Fehling's test, Froth test, Foam test, Ferric chloride test, Alkaline reagent test, Lead acetate test, Xanthoproteic test, Ninhydrin test.



Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered⁶.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown / reddish precipitate indicates the presence of alkaloids.

Detection of carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test

Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Benedict's Test

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test

Filtrates were hydrolyzed with diluted Hydrochloric acid, neutralized with alkali and heated with Fehling's A & B solution. Formation of a red precipitate indicates the presence of reducing sugars.

Detection of saponins**Froth Test**

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. A formation of 1cm layer of foam indicates the presence of saponins.

Foam Test

0.5 gm of the extract was shaken with 2ml of distilled water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols**Ferric Chloride Test**

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Detection of tannins**Gelatin Test**

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids**Alkaline Reagent Test**

Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids.

Lead Acetate Test

Extracts were treated with a few drops of lead acetate solution. Formation of a yellow color precipitate indicates the presence of flavonoids.

Detection of proteins and amino acids**Xanthoproteic Test**

The Extracts were treated with a few drops of concentrated Nitric acid. Formation of yellow color indicates the presence of proteins.

Ninhydrin Test

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for a few minutes. Formation of blue color indicates the presence of amino acid.

Detection of glycosides

Extracts were hydrolyzed with diluted Hydrochloric acid, and then subjected to test for glycosides.

Legal's Test

Extracts were treated with Sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red indicates the presence of cardiac glycosides⁷.

Biosynthesis of silver nanoparticles

In 250ml conical flask, 10ml of aqueous extract of *Carica papaya* leaves was added to 90ml of 1mM aqueous AgNO₃ solution and it was incubated with continuous stirring process at 28°C for 24 hours. Silver Nitrate is converted into Silver Nanoparticles and this bio reduction is confirmed by the color change from colorless to dark brown and it also indicates shows the formation of silver nanoparticles by the *Carica papaya leaf extract*. The bio reduction of silver ions in the solution was monitored by sampling the aqueous component after incubation period and Using a UV-Visible Spectrophotometer, absorption maxima was scanned at different wavelengths (420-500 nm).

Characterization of biosynthesized silver nanoparticles

UV-Visible spectrophotometric analysis of silver nanoparticles

An ELICO SL-159 UV- visible spectrophotometer was used to analyses the spectrometry of biosynthesized silver nanoparticles⁵. The bio-reduction of the Ag⁺ ions in solution were checked by periodical sampling of aliquots and the UV-Visible spectra of these aliquots were monitored as a function at time of reaction in 430 & 435 nm range operated at a resolution of 1nm. Distilled water was utilized as a blank¹. A spectrum of silver nanoparticles was plotted with wave length on x-axis and absorbance on y-axis.

Fourier transform infrared (FT-IR) analysis of silver nanoparticles

FT-IR spectral analysis was carried out to identify the possible bio molecules responsible for the reduction of the Ag⁺ ions and the capping of the Ag NPs synthesized by *Carica papaya* extract⁸. The chemical composition of silver nanoparticles was analyzed using a FT-IR spectrometer after bio reduction with silver nitrate. 2ml of bio synthesized liquid silver nanoparticles were scanned using infrared in the range of 400-4000 cm⁻¹.

Determination of antibacterial activity

Antibacterial activity - agar well diffusion method

The agar well diffusion method is widely used to evaluate the antimicrobial activity of plant extracts^{9,10}. Similarly, to the procedure used in disk-diffusion method, the Muller-Hinton agar plate surface is inoculated by spreading a

volume of the microbial inoculum over the entire agar surface. *Carica papaya* crude extract solution (25, 50, 75 & 100 µL) and AgNPs synthesized extract solution at desired concentration is introduced into the hole (diameter 6 to 8mm) of agar. This hole is made aseptically with a sterile cork border¹¹. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested

RESULTS AND DISCUSSION

In our study, Phytochemical activities of Papaya leaves were evaluated under in vitro condition. Alkaloids, Carbohydrates, Saponins, Phenols, Flavonoids, Tannins, Cardiac glycosides, Proteins and Amino acids are the compounds found in the phytochemical analysis of the papaya leaf (**Table 1**). These findings can be attested to the good antimicrobial agent on the different test organism. Comparing to other solvent extracts, the ethanol extracts exhibited a higher inhibitory activity of the test organisms due to its different polarities and different degrees of solubility for the various phytoconstituents¹². The pharmacological properties of *Carica papaya* lie in the various chemical constituents. Few important Phytochemicals found in *Carica papaya* are Lycopene, Betacarotinoïd, Benzylisothiocyanate, Betacryptoxanthin, Benzylglucosinolate, chlorogenic acid, caffeic acid, protocatechuic acid, Quercetin¹³. Papaya rich in tannins possesses antibacterial property which allows them to react with proteins to form stable water-soluble compounds damaging their cell walls and kills bacteria¹⁴.

Table 1: Phytochemical screening of *Carica papaya* leaf extract

S No.	Phytochemicals	Presence of Phytochemical in ethanolic extract of <i>Carica papaya</i>
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Saponins	+
4.	Phenols	+
5.	Tannins	+
6.	Flavonoids	+
7.	Proteins and Amino acids	+
8.	Glycosides (Cardiac glycosides)	+
9.	Steroids	-
10.	Triterpenoids	-

(+): Present; (-): Absent

Karpain, a chemical substance which kills microorganisms that often interfere with the digestive function¹⁵. These

bioactive compounds are used mainly because they are the derivatives of natural plant sources. It would also be



observed that the fresh leaves *Carica papaya* silver nanoparticles exhibited better antimicrobial potentials than the dried leaves *Carica papaya* silver nanoparticles¹⁶. Nano materials had proven to be the most efficient mode of drug delivery in modern Medicine. Other than physical and chemical methods, the Development of green synthesis nanoparticles was cost effective, eco-friendly, and can be easily scaled up for large scale synthesis of nanoparticles¹⁷.

Due to the small size of nanoparticles, which may confer the ability to penetrate the cells/microbes and execute the bactericidal property. Surface Plasmon Resonance (SPR) caused the color change in bio-reduction of silver Nanoparticles⁵. SPR is the resonant oscillation of conduction electrons at the interface between a negative

and positive permittivity materials stimulated by incident light. Sometimes the color changed by metallic nanoparticles is due to the coherent excitation of all free electrons which are released by phenolic compounds present in the extract¹⁸.

Carica papaya leaf extract's FTIR spectrum displays the presence of AgNPs with transmission peaks at 536.21, 1639.49 and 3456.44 cm^{-1} respectively (**Figure 1**). The absorption peak at 3456.44 cm^{-1} corresponded to OH groups present in alcohols and phenolics; band appearing at 1639.49 cm^{-1} could be due to amide bond coming from the carbonyl group of a protein, absorption peak at 536.21 cm^{-1} appear from C-H stretch and aromatic (**Figure 1**).

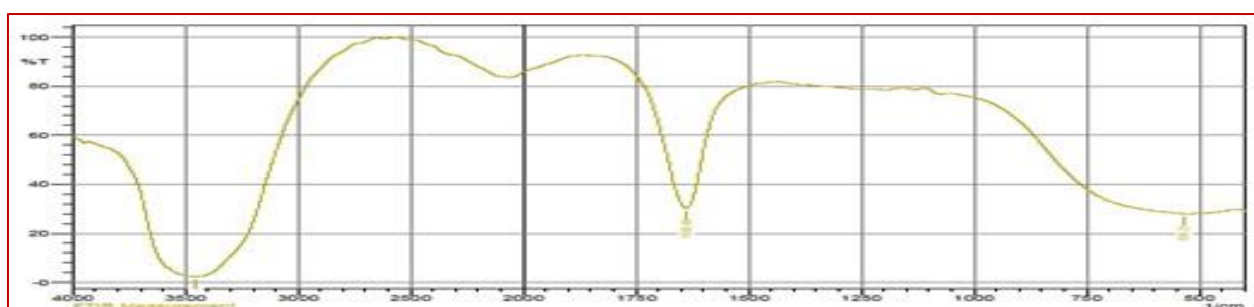


Figure 1: FT-IR spectra of silver nanoparticles synthesized from *Carica papaya* leaf extract

The antibacterial activity of crude extract was studied against the pathogenic microbial strain such as *Escherichia coli*, *Enterococcus spp*, *Klebsiella spp*, *Staphylococcus spp* using the agar well diffusion method.

The effective zone of inhibition was observed in crude extract against *Staphylococcus spp*, *Klebsiella spp*, *Escherichia coli*, *Enterococcus spp* while increasing the concentration (25, 50, 75 and 100 μl) (**Figure 2-5**). The highest activity of 22mm diameter of zone of inhibition was obtained against *Staphylococcus spp*, and 20mm diameter of zone of inhibition was observed against *Klebsiella spp* and *Escherichia coli*, and 19mm diameter of zone of inhibition was observed against *Enterococcus spp*. The synthesized silver nanoparticles derived from the papaya leaf extract exhibited an excellent antibacterial activity against both gram positive and gram-negative bacteria. The results suggest that silver nanoparticles undergo an interaction with bacterial cell and displayed the strong bactericidal action against *Escherichia coli*, *Enterococcus spp*, *Klebsiella spp*,

Staphylococcus spp. The highest activity of 18mm diameter of zone of inhibition was obtained against *Staphylococcus spp*, and 17mm diameter of zone of inhibition against *Klebsiella spp*, and 15mm diameter of zone of inhibition was obtained against *Escherichia coli*, and 13mm diameter of zone of inhibition against *Enterococcus spp*. Among the evaluation of Gram positive and Gram-negative bacteria, Gram negative bacteria were more susceptible to this extract, however, is at disparity with an earlier report indicating that plant extracts are more active against Gram positive bacteria. The Zone of inhibition produced by the synthesized silver nanoparticles by destructing the cell wall and ceasing the cell permeability, formation of free radicals, inactivating important enzymes, interaction of silver nanoparticles with DNA and interruption of DNA replication and translation and by dephosphorylating the tyrosine residues on peptides inhibiting the signal transduction and growth in bacteria¹⁹.

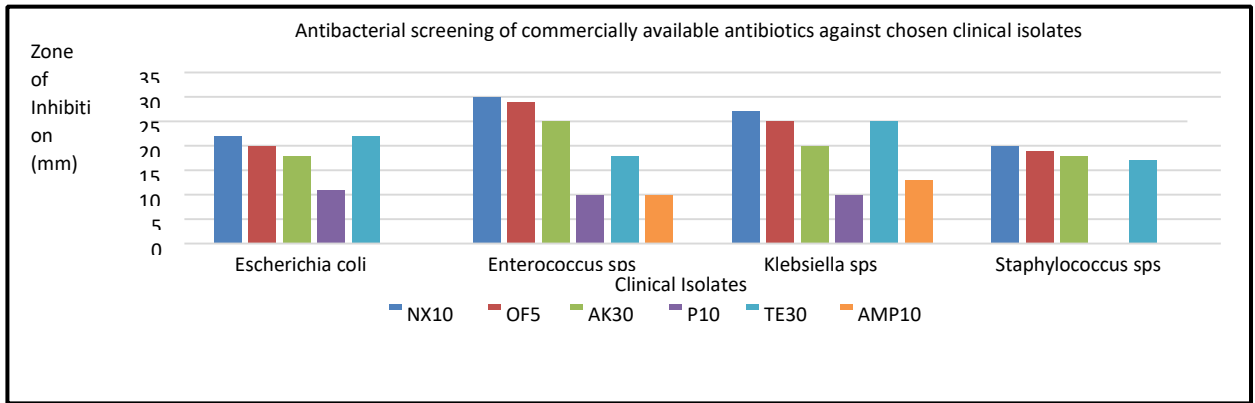


Figure 2: Antibacterial screening of commercially available antibiotics against chosen clinical isolates

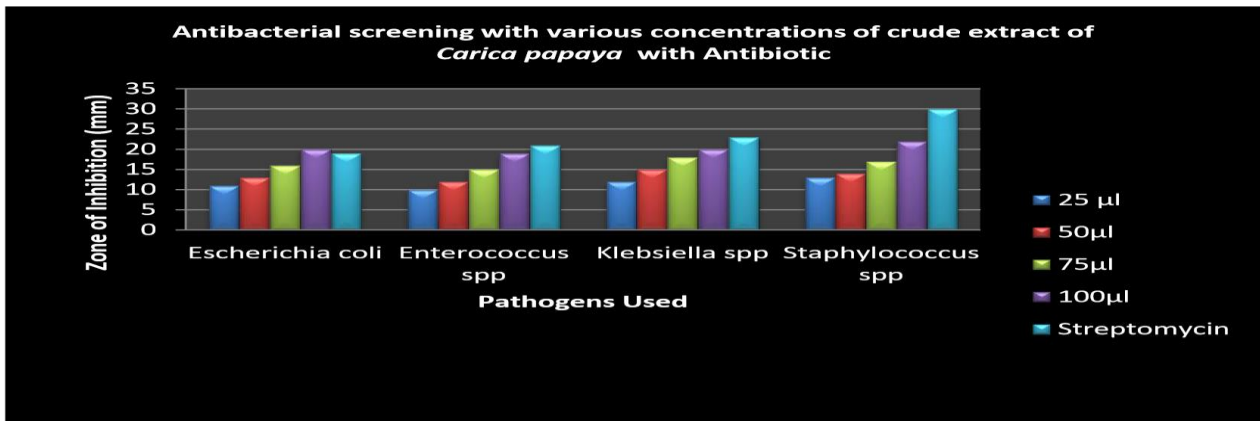


Figure 3: Antibacterial screening of crude Carica papaya leaf extract against chosen clinical isolates with antibiotic

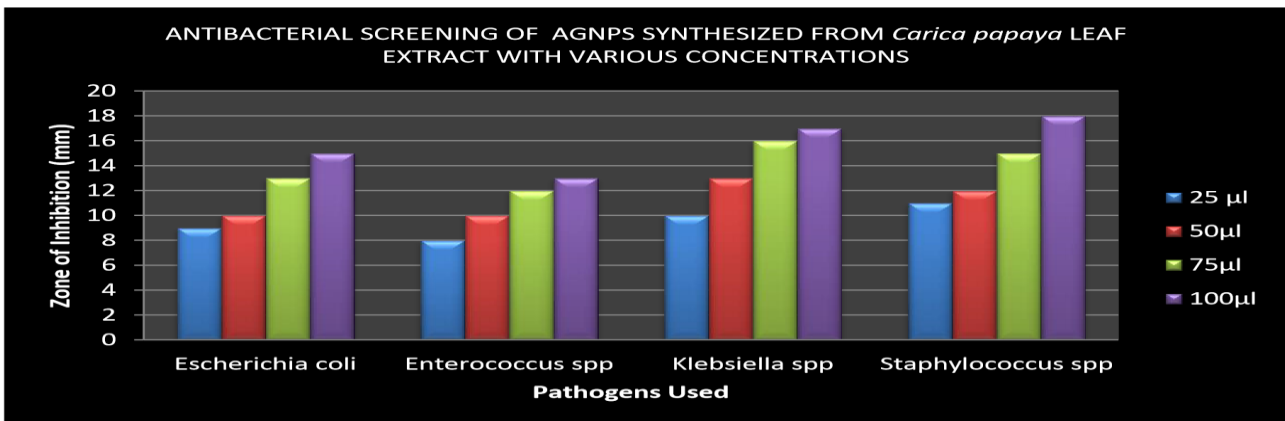


Figure 4: Antibacterial screening of AgNPs synthesized Carica papaya leaf extract (Ethanol)

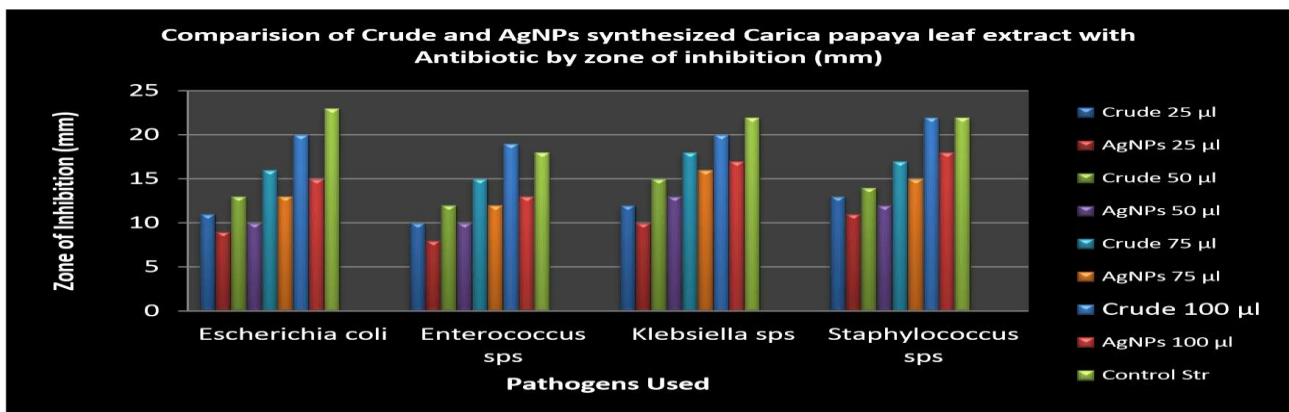


Figure 5: Antibacterial activity-comparison of crude and AgNPs synthesized Carica papaya leaf extract (Ethanol) with antibiotic

CONCLUSION

Even though this study found that crude extract is more potent than biosynthesized silver nanoparticles. Silver nanoparticles synthesized with identified Phytochemical constituent will show best results. Further research, exploration is needed to identify the particular constituent's biosynthesis to produce novel and cost-effective Nano based antimicrobial agents. *Carica papaya* leaves can be developed into a promising drug candidate for biomedical applications. Large surface area of Silver nanoparticles is one of the major factors for its efficient biological property, which establishes better contact with microorganisms than any other salts.

The silver nanoparticles get attached to the cell membrane and also penetrated inside the bacteria. The bacterial membrane contains sulfur containing proteins and the silver nanoparticles get attached to these proteins in the cell as well as and the phosphorus containing compounds like DNA²⁰.

Great Push toward silver nanoparticles for delivering drugs will be seen in future due to the ease delivery in drug delivery²¹. The usage of medicinal plant materials for the synthesis of nanoparticles has been revolutionized in recent years and could serve as an alternative for antibiotics²².

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