



Research Article

Green synthesis of silver nanoparticles from *Psidium guajava* leaves and its antibacterial activity

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Abstract: In the present study, *Psidium guajava* leaves were taken for synthesizing silver nanoparticles and checked their antibacterial activity against *E.coli*, *Klebsiella*, *Pseudomonas*, *Staphylococcus* and *Acinetobacter*. The plant extract was analysed for the detection of the presence of protein, carbohydrate, flavonoids, terpenoids, glycosides, steroids, saponins, phenols and tannins. In this present study, the antibacterial activity of green synthesized silver nanoparticles from guava leaf shows the zone of inhibition against all the five pathogens.

Keywords: Guava Leaves, extract, silver nanoparticles, antibacterial activity.

Introduction

Traditional medicine, which has compounds derived from medicinal plants are used nowadays. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Plants of various origins have been exploited effectively over many generations for therapeutic purposes.

Guava leaves have several chemical constituents such as comarins, essential oils, flavonoids, triterpenes and ellagitannins which are known to have antimicrobial properties. The leaves of *Psidium guajava* tree have a long history of medicinal uses that are still employed today. Guava leaves and fruit juice has also been tested in treatment of infantile diarrhoea and the results showed that, those who were treated with guava recovered at 3 days which was shorter than the controls and the study concluded that guava had good curative effect on infantile Rotoviral enteritis (Wei *et al.*, 2000). *Psidium guajava* or guava is a plant in the family Myrtaceae along with clove, allspice and eucalyptus. Native to tropical America, it is now cultivated in many tropical and subtropical countries for its edible fruit (Perez *et al.*, 2008). Guava leaves, roots, and fruits have been used for the prevention and treatment of diarrhea (Almeida *et al.*, 1995) and a high level of antibacterial activity was detected in guava leaves (Hidetoshi and Darnto, 2002). In several studies, guava showed significant antibacterial activity against common food-borne diarrhoea-causing bacteria such as *Staphylococcus species*, *Shigella species*, *Salmonella species*, *Bacillus species*, *E. coli*, *Clostridium species* and food spoilage bacteria

such as *Pseudomonas* species (Baby Joseph, 2011). In this paper, an attempt was made to study antibacterial properties of Silver nanoparticles synthesized from leaves of *Psidium guajava* against human bacterial pathogens. From the results, it was concluded that leaves extract of *Psidium guajava* plant was effective and efficient against bacterial pathogen used. *Psidium guajava* leaves could serve as good source of antibacterial agents.

Material and Methods

Leaves of *Psidium guajava* were collected and washed thoroughly in water to remove mud and dust particles and dried in the shade for about 10 days. The shade dried leaves were then powdered coarsely in mixer and stored in separate air tight containers at room temperature for further use. For acetone extraction, 20gms of coarsely powdered leaves were taken and 100 ml of the acetone was added. The solvent was then evaporated to dryness under reduced pressure and the extracted compound was used for the antibacterial assay. Phytochemical tests of plant extract was also done and for this 1gm of the extract of *Psidium guajava* was mixed in 1ml of dimethyl sulfoxide solution.

The bacterial strains used for the test were *E.coli*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Acinetobacter*. Muller Hinton agar and Nutrient broth was used as the media for the culturing of bacterial strains. Loopful of bacterial cultures were inoculated in the nutrient broth and incubated at 37°C for 24 hours. For Green synthesis of silver nanoparticles, about 40gms of the crude leaf

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powder was mixed with 200 ml sterile distilled water and kept in the shaker at 120 rpm for overnight. After overnight incubation, the flasks were allowed to stand still for about 30 minutes at room temp to settle. After settling the supernatant was filtered to get the filtrate which is the aqueous plant extract.

To 9 ml of the aqueous plant extract of *Psidium guajava* 45 ml of 1 mM silver nitrate solution was added. The flasks were covered with aluminum foil and incubated at room temperature in shaking condition in dark for 48 hours. After incubation, the mixture was changed in colour from orange brown to dark brown.

Elemental analysis and chemical characterization of green synthesized silver nanoparticles from *Psidium guajava* was analysed by Energy-dispersive X-ray spectroscopy. Silver nanoparticle synthesized was sent to SITRA Coimbatore for the analysis of the synthesized silver nanoparticles by Energy-dispersive x-ray spectroscopy. EDX can be used to confirm the composition and distribution of the nanoparticles through spectrum and elemental mapping by using an EDX spectrometer. Anti-bacterial activities of AgNPs were tested separately using disc diffusion method (Bauer *et al.*, 1966). The suspensions of the bacterial strains were prepared corresponding to 0.5McFarland scale and swabbed on to the surface of sterile Mueller–Hinton agar plates. The different concentrations (5, 10, 15, 20 μ l) of green synthesized silver nanoparticles were transferred to the wells. Then the plates were incubated at 37°C for overnight in upright position. The assessment of antibacterial activity was done based on the measurement of the diameter of inhibition zone formed around the disc.

Result and Discussion

Plant leaves is used as medicines against gastroenteritis, diarrhoea and toothache for replace antibiotics. The present study screened the antibacterial effects of silver nanoparticles of Guava. The major active compounds present in these extracts. The results obtained in various tests are tabulated in Table: 1.

The color change seen in the mixtures due to the reaction between the silver nitrate and the leaves indicating the synthesis of silver nanoparticles was recorded by visual observation. With regard to *Psidium guajava*, the leaves extract and silver nitrate mixture before and after incubation showed a color change from orange brown to dark brown (figure 1) indicating synthesis of silver nanoparticles. The synthesized silver nanoparticles (AgNPs) exhibited different colors in aqueous medium as a result of surface plasmon vibrations. In this present study, the antibacterial activity of green synthesized silver

nanoparticles from guava leaf shows the zone of inhibition against all the five pathogens under study (Figure2).

Table 1. Phytochemical analysis of acetone extract of *Psidium guajava*

S.No.	Test	Result
1.	Test for protein:	
	• Ninhydrin test	+ve
2.	Test for carbohydrates:	
	• Fehling's test	-ve
	• Benedict's test	+ve
	• Molisch's test	-ve
3.	• Iodin test	+ve
	Test for phenols & tannins	+ve
4.	Test for flavonoids:	
	• Alkaline reagent test	+ve
5.	Test for saponins	+ve
	Test for glycosides:	
6.	• Liebermann's test	-ve
	• Salkowski's test	+ve
	• Keller-kilani test	+ve
7.	Test for steroids	+ve
8.	Test for terpenoids	-ve

+ ve = positive; -ve = negative

Figure 1. Synthesis of Silver nanoparticles

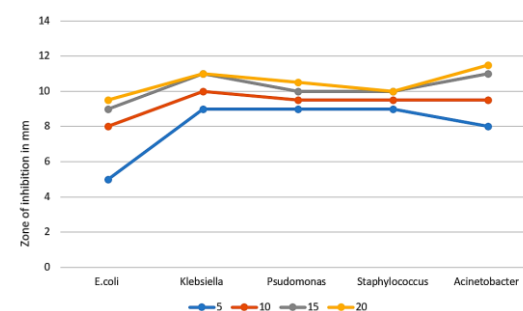


Figure 2. Antibacterial activity of silver nanoparticles in different concentration

Energy dispersive X-ray (EDX) spectrometer established the existence of elemental sign of the silver and homogenous allocation of silver nanoparticle. Examination of AgNPs by Energy Dispersive X-ray (EDX) spectrometer established the existence of elemental indication of the Ag and homogenous distribution of AgNPs. The pointed sign peak of Ag powerfully established the reduction of AgNO_3 to AgNPs. The upright axis expresses the number of X-ray counts while the parallel axis shows energy in KeV. Detection lines

for the main release energy for Ag were clarified and these communicate with peaks in the spectrum, thus giving affirmation that Ag has been properly recognized and present in the solution (figure 3)

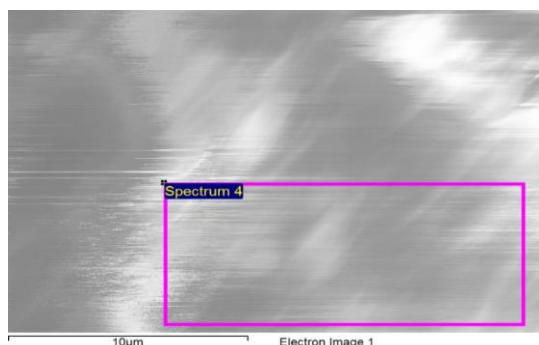


Figure 3: EDX study of the AgNP

Element	Weight %	Atomic %	Compd %	Formula
C K	25.57	32.52	93.71	CO ₂
Si K	0.75	0.41	1.61	SiO ₂
K K	0.86	0.34	1.04	K ₂ O
Ag L	3.40	0.48	3.65	Ag ₂ O
O	69.42	66.26		
Totals	100.00			

Conclusion

Green synthesis of silver nanoparticles was done by using *Psidium guajava* leaf extract and 1mM AgNO₃ solution. The synthesized silver nanoparticles was analysed and confirmed by Energy-dispersive x-ray spectroscopy. The antibacterial activity of synthesized silver nanoparticles on *E.coli*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Acinetobacter* in different concentration was assessed by well diffusion method. From the results, it was concluded that leaves extract of *Psidium guajava* Linn. plant has shown effective and efficient result against bacterial pathogen used. *Psidium guajava*

leaves could serve as good source of antibacterial agents.

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