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Research Article

Piper betle L. Silver Nanoparticle-Synthesis, Characterization and Antibacterial Study

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ABSTRACT

Background: Development of nano-based drugs has gained huge attention globally and is widely preferred in healthcare industry specifically due to its smaller size and high loading efficiency for targeted drug delivery. Apart from health care, the application of nanoparticle has expanded in various sectors for different purposes. The synthesis of nanoparticle from natural sources is in huge demand since the process is convenient, no harsh chemicals are used, highly efficient and utilizes natural bioactive compounds as reducing agents. **Aim:** The present study aims to characterize silver nanoparticle synthesized using aqueous extracts from *Piper betle* L. leaves and determining their antibacterial potential. **Materials and Methods:** Silver nanoparticle synthesis was carried out employing aqueous extracts from *Piper betle* L. leaves with 1 mM concentration of silver nitrate and characterized by means of UV-visible analysis, Particle size analysis, FTIR analysis, SEM with EDAX analysis and Zeta potential analysis. It was determined for its antibacterial activity against *Streptococcus mutans, Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter* spp. and *Salmonella typhi.* **Results:** Silver nanoparticle was synthesized and confirmed by the formation of dark brown colour. Characterization studies revealed peak formation at the wavelength of 392.6 nm, presence of functional groups, spherical shaped and monodispersed silver nanoparticle with polydispersity index of 0.288 and 70.60% silver content confirming the stability of nanoparticle and its ability in inhibiting the bacterial pathogens. **Conclusion:** This study highlights silver nanoparticle biosynthesis using aqueous extracts from *Piper betle* L. leaves as well as its antibacterial activity against different pathogenic organisms.

Keywords: Antibacterial activity, Characterization, *Piper betle* L., Silver nanoparticle.

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INTRODUCTION

Nanotechnology relates to the process of nanoparticle synthesis with diverse role in healthcare, agriculture, in food and packaging industry, coating fabrics having repellent and antimicrobial properties, in cosmetic products, electronics and so on. This can be attributed specifically to their nano size, increased surface area to volume ratio, mechanical and optical properties.[1,2] Compared to the bulk metals, metallic nanoparticles are known to exhibit better properties. Metals such as silver, gold, copper, platinum, aluminum, lead, zinc, palladium are used for nanoparticle synthesis.[3] Process of nanoparticle synthesis involves Chemical reduction, biological synthesis, UV irradiation, Microwave irradiation, Photochemical method, Thermal decomposition. Apart from biological synthesis, all the other methods require use of harsh chemicals, high pressure and temperature conditions which could have a negative effect on the environment and on human health.[4,5] Biological synthesis of nanoparticles involves the usage of natural sources like plants, algae, microorganisms as reducing or capping agents. However, among these, Plant based nanoparticle has gained more attention recent times due to their accessibility, rich source of phytocomponents, ease of preparation, cost effectiveness, efficiency and nontoxicity.[6] Secondary metabolites such as phenols, tannins, flavonoid, alkaloid, terpenoids and presence of functional groups in these phytocompounds serve as stabilizing and reducing agents enabling nanoparticle synthesis. Also, Phyto based nanoparticle are utilized in healing wounds, as delivery agents, as dental implants, as biosensors and so on.[7] Among the metal nanoparticle, silver nanoparticle is much desired due to their distinctive usage in different sectors and exert pathogen inhibitory activity, in treatment of cancer, as drug delivery agent, in anti-inflammatory and antiviral drugs.[8] Catalytic and optical properties of silver nanoparticle is mainly dependent upon its size, shape and surface characteristics and this can be attributed to the phytocomponents which plays a functional role as reducing agents thereby controlling the efficacy and properties of nanoparticle for use in different applications.[9] Silver nanoparticle is also synthesized in different shapes such as spherical, rod, triangular, cubical shaped and it mainly depends on

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the interaction of nanoparticle with stabilizers and the process of preparation.[10,11] Characterization is carried out to determine the size, morphology, stability, to understand the functional groups, crystalline nature, percentage distribution, surface roughness, topography of nanoparticle. Techniques used for characterizing nanoparticles involve UV-visible analysis, Dynamic light scattering, Transmission and Scanning electron microscopy with EDAX, Fourier transform infrared spectroscopy, X-ray diffraction studies and Zeta potential analysis.[12] In the present study, silver nanoparticle synthesis was carried out using the aqueous extracts of *Piper betle* L. leaf sample. Silver nanoparticles were synthesized using 1mM concentration of silver nitrate. The synthesized *Piper betle* L. silver nanoparticle was purified and characterized by means of UV-vis analysis, FTIR analysis, Particle size and Zeta potential analysis, SEM analysis with EDAX. The synthesized nanoparticle was further determined for its pathogen inhibitory activity against different bacterial organisms such as *Streptococcus mutans, Klebsiella pneumoniae, Staphylococcus aureus, Enterobacter* spp. and *Salmonella typhi*.

MATERIALS AND METHODS

Sample collection and authentication

Piper betle L. leaf samples were collected from different localities. Authentication was done at Plant Anatomy Research Centre, Chennai by Prof. Jayaraman. Samples were cleaned, washed, shade dried for about a month, powdered and kept for storage at 4ºC for further studies.

Preparation of aqueous extract

Powdered *Piper betle* L. leaf samples weighing about 5 g were mixed with 50 mL distilled water and heated at 65ºC for 20-25 min on water bath. It was allowed to cool down, filtered and the aqueous extract was stored in bottle at 4ºC and used for silver nanoparticle synthesis.[13]

Synthesis and Purification of Silver nanoparticle

Piper betle L. leaf aqueous extract of about 10 mL was added to 100 mL of silver nitrate solution taken at a concentration of 1 mM and stirred continuously on magnetic stirrer at 37ºC. Colour change of solution from colourless to dark brown indicates silver nanoparticle formation. The obtained nanoparticle suspension was incubated in dark for about 24 hr to enhance complete settling of the particles. It was then centrifuged thrice by washing with double distilled water at 5,500 rpm for about 15 min. The nanoparticle pellet obtained was mixed and diluted with double distilled water and kept for drying at 60-70ºC in hot air oven for 1 hr. The dried silver nano pellet was scrapped and used for characterization studies.[14,15]

Characterization of Silver nanoparticle

Characterization of *Piper betle* L. Silver nanoparticle was carried out by UV-vis Spectrophotometer, Perkin Elmer in the range of 200 to1100 nm, Fourier Transform Infrared Spectroscopic analysis using FTIR Instrument, Perkin Elmer by KBR pellet method at a resolution of 1.0 cm^{-1}, Zeta Potential analysis was carried out using Zetasizer, Malvern, Particle size analysis was carried out by means of Nano plus, Particulate system and SEM with EDAX analysis was carried out by using FEI Quanta Field Emission Gun 200F Scanning electron microscope.

Antibacterial activity of Silver nanoparticle

Piper betle L. silver nanoparticle was determined for its antibacterial activity against *Streptococcus mutans, Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter* spp. and *Salmonella typhi* by well diffusion and diameter of inhibition zone was observed and measured in mm.[16]

RESULTS

The powdered betel leaf samples were extracted using double distilled water as the extraction medium. *Piper betle* L. extract thus obtained was used for silver nanoparticle synthesis. Nanoparticle synthesis was observed through immediate colour change of the reaction medium from colourless to reddish brown or colloidal brown.[17] Silver nanoparticle formation was confirmed through UV-visible analysis. *Piper betle* L. silver nanoparticle showed an absorption peak at 392.6 nm with absorbance O.D value of 0.070 (Figure 1). FTIR [Fourier Transform Infrared Spectroscopy] analysis for *Piper betle* L. silver nanoparticle was performed for determining the functional groups present. FTIR spectrum for nanoparticles has been given (Figure 2). FTIR spectrum of *Piper betle* L. silver nanoparticle showed the presence of peaks and their corresponding functional groups such as 3298.74 cm^{-1} (N-H Stretching, Aliphatic primary amine; O-H Stretching, Alcohol; O-H Stretching, Carboxylic acid; C-H Stretching, Alkyne); 2915.42 cm^{-1} and 2846.45 cm^{-1} (O-H Stretching, Alcohol; N-H Stretching, amine; O-H Stretching, Carboxylic acid; C-H Stretching, Aromatic compound); 2115.07 cm^{-1} (N=C=S Stretching, Isothiocyanate; C≡C Stretching, Alkyne); 1991.28 cm^{-1} (N=C=S Stretching, Isothiocyanate; C=C=C Stretching, Allene; C-H Bending, Aromatic compound); 1885.43 cm^{-1} (C-H Bending, Aromatic compound); 1625.77 cm^{-1} (C=C Stretching, Cyclic alkene and Conjugated alkene; N-H Bending, Amine); 1540.93 cm^{-1} (N-O Stretching, Nitro compound); 1404.88 cm^{-1} (O-H Bending, Alcohol and Carboxylic acid); 1035.52 cm^{-1} (C-N Stretching, Amine; C-O Stretching, Alkyl aryl ether); 777.86 cm^{-1} (C-H Bending; C-Cl Stretching, Halo compound); 530.69 cm^{-1} (C-I Stretching, Halo compound and C-Br Stretching).[18-21] Zeta potential analysis was carried out for *Piper betle* L. silver nanoparticle and it was determined using Zetasizer, Malvern. *Piper betle* L. silver nanoparticle showed zeta potential of -35.7 mV

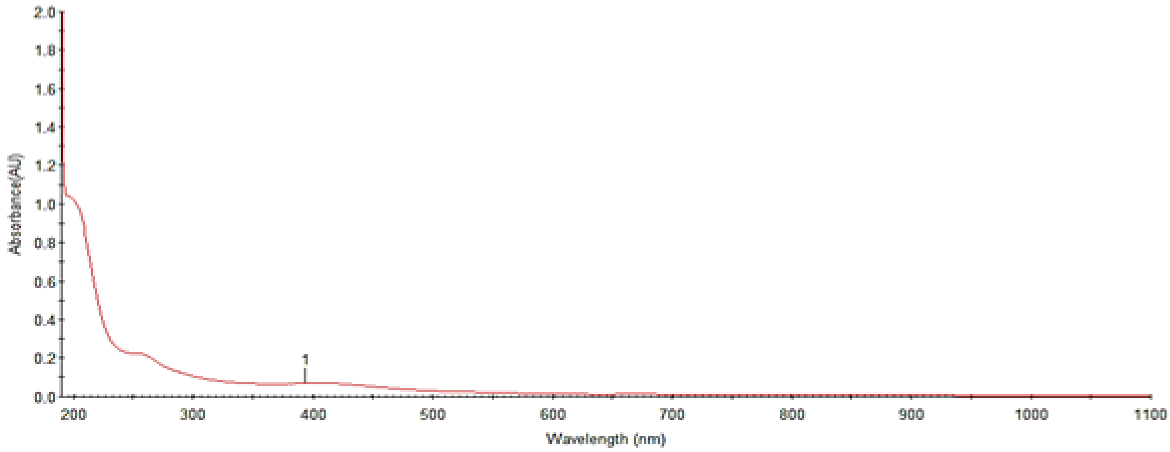

Figure 1: UV-visible Spectroscopic analysis of *Piper betle* L. Silver nanoparticle.

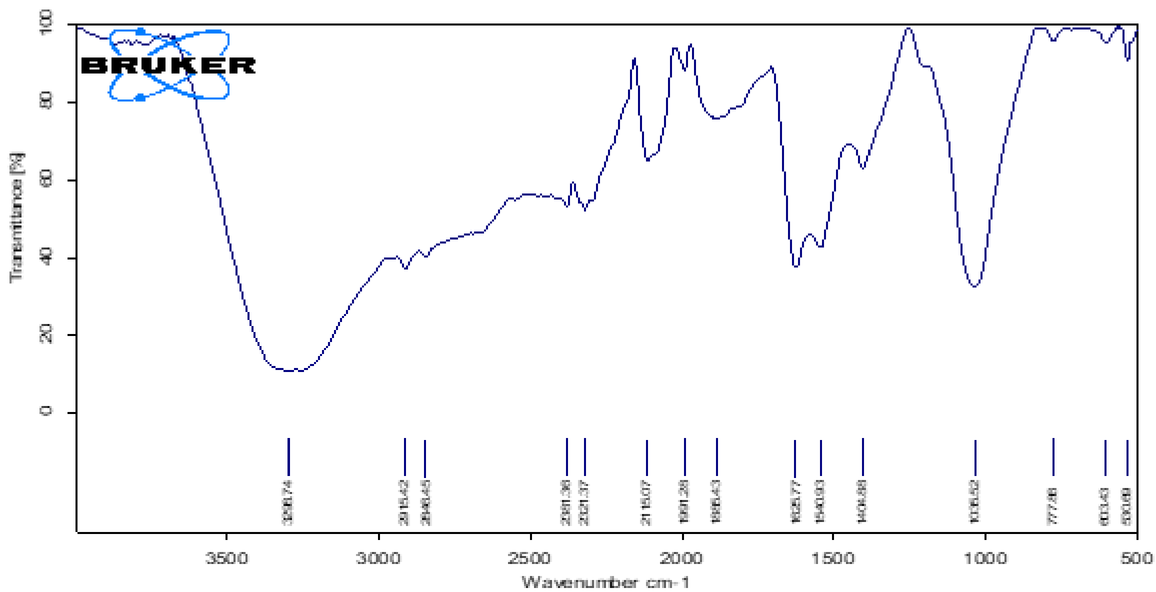

Figure 2: FTIR Spectrum for *Piper betle* L. Silver nanoparticle.

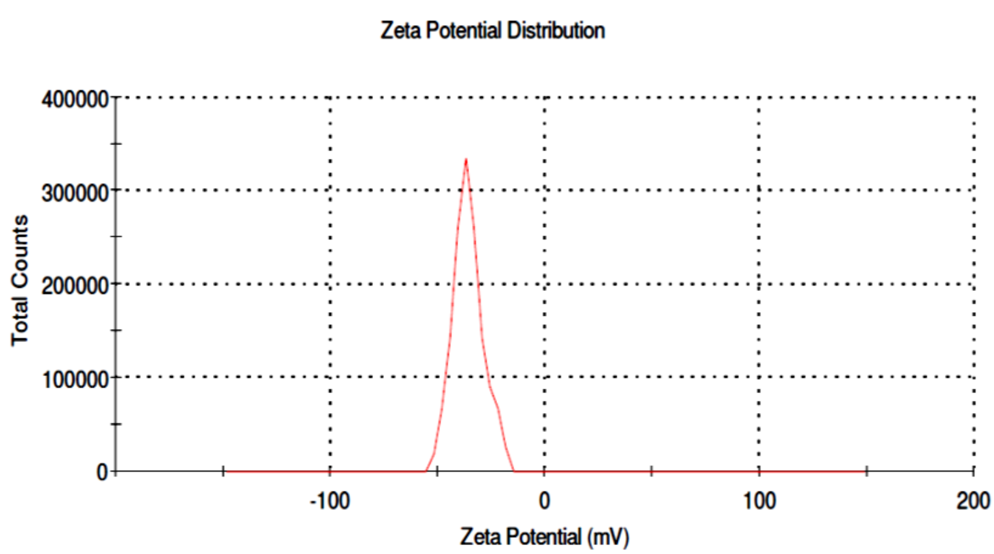

Figure 3: Zeta potential analysis of *Piper betle* L. Silver nanoparticle.

with 100% area (Figure 3). The average hydrodynamic diameter of nanoparticle measured by particle size analyzer was found to be about 115.9 nm with Polydispersity Index (PDI) value of 0.288 respectively. As a result of surface plasmon resonance, silver nanoparticles exhibit absorption peak at around 3KeV.[22,23] *Piper betle* L. silver nanoparticle was observed to be spherical shaped, and the percentage (%) weight of silver was found to be 70.60% in EDAX spectrum (Figure 4).

Piper betle L. silver nanoparticle showed highest inhibition against *Streptococcus mutans* with an inhibition zone of around 12 mm followed by *Salmonella typhi* with 10 mm, *Enterobacter* spp. with 8 mm and least inhibition against *Staphylococcus aureus* and *Klebsiella pneumoniae* with an inhibition zone of 6 mm (Table 1).

DISCUSSION

Silver nanoparticle synthesis was carried out at the ratio of 1:10 with 1mM concentration of silver nitrate. The time duration taken for synthesis was observed to be 30 min. The synthesized silver nanoparticle was purified by centrifugation, dried in hot air oven and the nano pellet obtained was further characterized by means of UV-visible analysis to understand silver nanoparticle formation, Fourier Transform Infrared Spectroscopic analysis for identifying the functional groups present, Zeta Potential analysis to check the stability of nanoparticle in solution, Particle Size analysis to measure the hydrodynamic diameter of nanoparticle and Scanning Electron Microscope with EDAX analysis to determine the morphology of silver nanoparticle and percentage of silver content (Figures 1-4). Bioactive molecules in *Piper betle* L. aqueous extract serves as reducing and capping agents as well as aids in the reduction of Ag^+ ions in silver nitrate into silver nanoparticles which can be observed through the formation of an absorption peak called as SPR (Surface Plasmon Resonance) peak. A Study has reported peak formation at 350 nm for silver nanoparticle from *Thymus vulgaris*[24] while, another study has reported absorption peak formation at 320 nm for silver nanoparticle from *Ulva fasciata*.[25] Absorbance peak or surface plasmon band in the wavelength range of between 300-450 nm confirms the formation of silver nanoparticle.[26,27] Negative zeta potential indicates the stability and presence of repulsive force thereby preventing aggregation of nanoparticle.[28] Polydispersity value of less than 0.5 indicates monodispersed nature of nanoparticle.[29] Thus, the silver nanoparticle synthesized from *Piper betle* L. leaf was found to be highly stable and also monodispersed. It has been reported that silver nanoparticle release silver ion which binds to the bacterial cell wall as a result of electrostatic attraction thereby disrupting the bacterial envelope and ultimately leading to inhibition and killing of microbes.[30]

Table 1: Antibacterial activity of *Piper betle* L. Silver Nanoparticle.

Sl. No.	Organisms	Diameter of Zone of Inhibition (in mm)
		Piper betle L. Silver nanoparticle
1	*Staphylococcus aureus*	6 mm
2	*Streptococcus mutans*	12 mm
3	*Klebsiella pneumoniae*	6 mm
4	*Salmonella typhi*	10 mm
5	*Enterobacter* spp.	8 mm

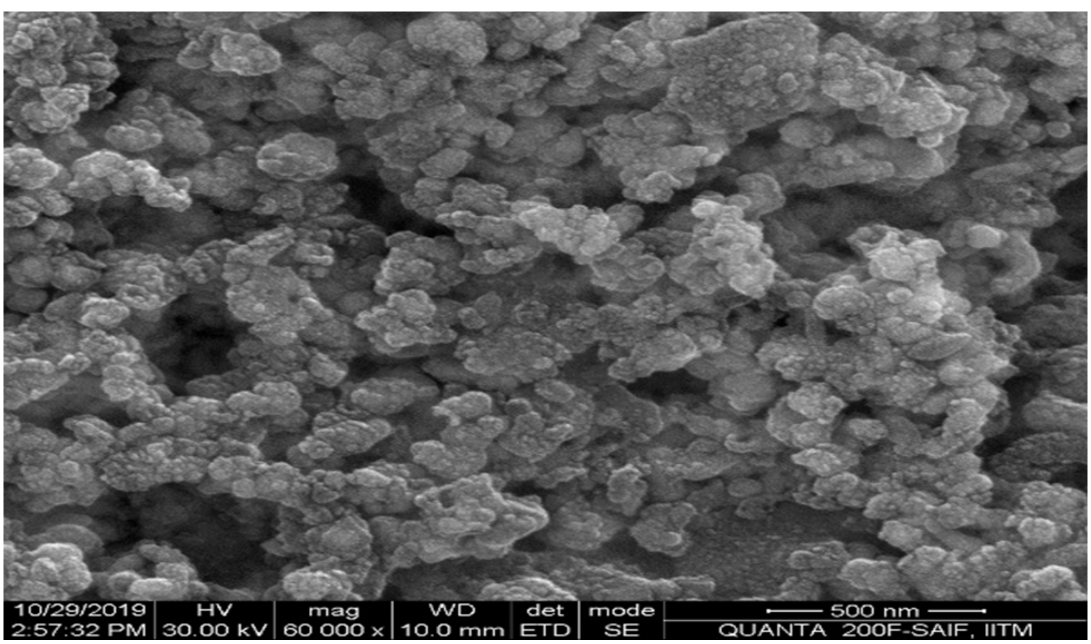

Figure 4: SEM analysis of *Piper betle* L. Silver nanoparticle

CONCLUSION

Nanotechnology is an advancing and highly demanding field of science that is being continuously exploited towards diverse applications. In this study, silver nanoparticle synthesis was carried out using the aqueous extract prepared from dried and powdered leaves of *Piper betle* L. The synthesized silver nanoparticle was characterized by several techniques and its antibacterial activity was also determined against the bacterial pathogens. Further studies can also be carried out on nano-based drugs thereby exploring the pharmacological importance of silver nanoparticle in the treatment forefront.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

UV-vis: Ultra violet-visible; **FTIR:** Fourier Transform Infrared; **SEM:** Scanning Electron Microscope**; EDAX:** Energy Dispersive X-ray Analysis; **SPR:** Surface Plasmon Resonance; **PDI:** Polydispersity index.

SUMMARY

In this study, aqueous extract prepared using *Piper betle* L. dried leaves was employed as the source for silver nanoparticle synthesis with silver nitrate at a concentration of 1 mM and characterized by various techniques which further confirmed the synthesis of nanoparticle with peak formation at 392.6 nm, functional groups present, negative zeta potential of -35.7 mV indicating high stability of nanosuspension, polydispersity index of 0.288 showing its monodispersed nature, formation of spherical shaped nanoparticles with 70.60% silver content and was also found to exhibit good inhibition zones against all organisms determining its efficacy as an antibacterial agent.

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