SYNTHESIS OF SILVER NANO PARTICLES USING PIPER BETLE AND ITS ANTIBACTERIAL ACTIVITY

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Most of the researchers have been reported about the nontoxic biosynthesis of silver nanoparticles using several microorganism and plant extracts. This ecofriendly silver nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution, morphology and many applications. In this research the synthesis of silver nanoparticles using *Piper betle* has been investigated. We have synthesized silver nano particles using 1 mM silver nitrate solution into the plant extract and characterized by UV-vis absorption spectroscopy. The antibacterial efficacy also determined by disc diffusion method with *Bacillus cereus*, *Escherchia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and it showed that high level of inhibition. The most outcome of this research will be suggested that biologically synthesized silver nanoparticle has more effective against various disease causing pathogens.

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Introduction

Biological methods of synthesis have paved way for the "green synthesis" of nanoparticles and these have proven to be better methods due to slower kinetics, they often better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nanotechnological applications. The use of environmentally benign materials like plant extract,[1] bacteria,[2] fungi[3] and enzymes[4] for the synthesis of silver nanoparticles after numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily sealed up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Various approaches are available for the synthesis of silver. NPs include chemical,[5] electrochemical,[6] radiation,[7] photochemical methods,[8] Langmuir-Blodgett,[9,10] and biological techniques.[11]

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrials process.[11,12] The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds.[13]

Existing literature also reports successful synthesis of silver nanoparticles through green route where the reducing and capping agent selected was the latex obtained from *Jatropha curcas*.[14] AgNPs were also obtained using *Aloe vera*,[15] *Acalyphaindica*,[16] *Garcinia mangostana*[17] as reducing agent.

Figure 1. Picture of *Piper betle* plant

Here we report green synthesis of silver nanoparticles using *Piper betle* extract which was confirmed by using various characterization techniques and application of antibacterial activity.

Materials and Methods

Reagents and chemicals: Silver nitrate was obtained from sigma Aldrich. Freshly prepared triple distilled water was used throughout the experiment.

Preparation of leaf extract by boiling method

Piper betle leaves were collected and washed several times with deionized water. 20 g of finely cut *Piper betle* leaves were taken and boiled in 200 ml of double distilled water for 3 min and filtered through Whatman No 1 filter paper. The filtrate was collected and stored at 4 °C for further use.

UV-Vis spectra Analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 3 hours after diluting a 1 ml of the sample into 4 ml of distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer(Lambda 35).

FTIR analysis

For FTIR measurements, Silver nanoparticle solution was centrifuged at 12,000 rpm for 12 minutes. The pellet was washed three times with 20 ml of deionized water. The samples were dried and analyzed on IR-Prestige-21[SHIMADZU].

Antibacterial activity

The anti bacterial activity was done on human pathogenic *Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* by the standard disc diffusion method. Nutrient agar (NA) plates were seeded with 8 h broth culture of different bacteria. In each of these plates, well were cut out using sterile cor borer. Using sterilized dropping pipettes, different concentrations (10, 20, 30, 40 µl/well) of sample was carefully added into the wells and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 37 °C for 18-24 h. Gentamicin (10 µg) was used as positive control. The antibacterial activity was evaluated by measuring the diameter of inhibition zone.

Results and Discussion

Synthesis of silver nanoparticles

Piper betle extract is used to produce silver nanoparticles in this experiment Ag^+ ions were reduced to Ag nanoparticles when plant extract is mixed with $AgNO_3$ solution in 1:8 ratio reduction is followed by on immediate change in yellowish to brown color in the aqueous solution of the plant extract due to excitation of surface plasmon vibration in silver nanoparticle.[18] Further formation of AgNPs in aqueous extract can be monitored by color change. Fig. 1. Shows the color changes when the aqueous extract of *Piper betle* plant was mixed with a $AgNO_3$ solution. The mixture was kept at room temperature for 24 hours. The appearance of a yellowish-brown color in the reaction vessel indicated formation of AgNPs. AgNPs exhibit this yellowish-brown color in aqueous solution due to excitation of surface plasmon resonance in the AgNPs.

UV-Vis spectroscopy analysis

UV-Vis spectroscopy is most widely used technique for structural characterization of silver nanoparticles. This was confirmed by the UV-Vis spectroscopy of the colloidal solution of silver nanoparticles has been recorded. Fig. 3 shows that absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 444 nm. Broadening of peak indicated that the particles are polydispersed.[19]

Figure 2. Color changes after the addition of leaf extract with aqueous silver nitrate solution: (A) silver nitrate solution (control), (B) silver nitrate with boiled leaf extracts

As shown in Fig. 3, the surface plasmon resonance of the AgNPs was centered at approximately 444 nm, indicating the presence of AgNPs in the solution.

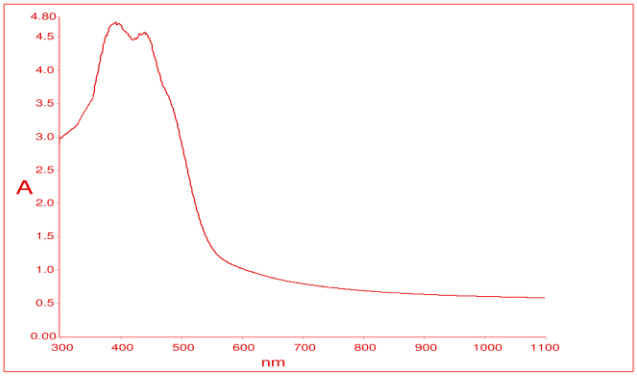

Figure 3. UV–Vis bioreduction kinetics in the range 300–700 nm for colloidal AgNPs solution with *Piper betle* leaf extract.

FTIR Spectroscopy Analysis

The FTIR spectrum of Ag nanoparticle is shown in Fig 4. The IR spectrum of Ag nanoparticles shown band at 3433 cm^{-1}, 1657 cm^{-1}, 2395 cm^{-1}, 1384 cm^{-1}, 1020 cm^{-1} corresponds to O-H stretching for alcohols and phenols, carbonyl stretching, N-H bond stretching of amines, C-N stretching of the aromatic amino group and C-O stretching of alcohols and ethers respectively. FTIR spectrum of Ag nanoparticles suggested that Ag nanoparticles were surrounded by different organic molecules such as terpenoids, alcohols, ketones, aldehydes and carboxylic acids. Fig. 4. Shows the plant *Piper betle* has been effectively involved in the antibacterial activity and also which is used in syntheses the silver nanoparticles.

Antibacterial activity

The anti bacterial activity was done on human pathogenic *Bacillus cereus, Escherchia coli, Klebsiella pneumonia, Staphylococcus aureus* by the standard disc diffusion method. (Fig. 5.)

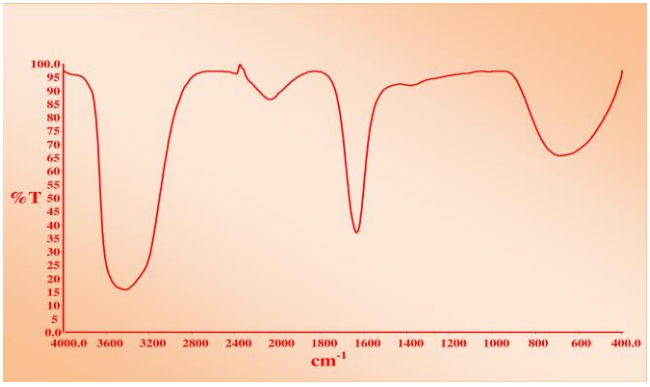

Figure 4. FTIR spectra of silver nanoparticles synthesized using *Piper betle* leaf extract

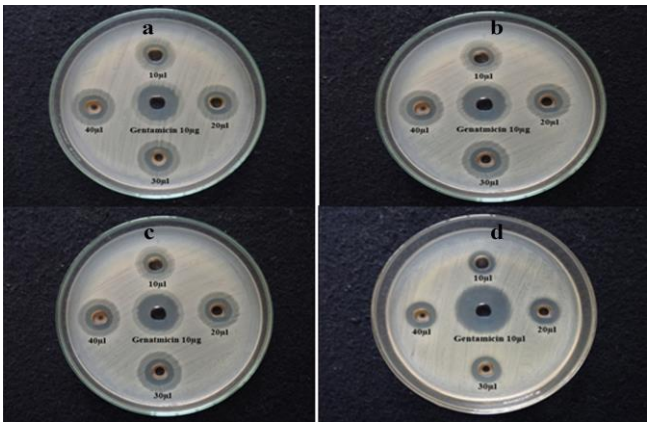

Figure 5. Antibacterial activity of synthesized silver nanoparticles (*Piper Betle* plant extract) on a) human pathogenic *Bacillus cereus*, b) *Escherchia coli*, c) *Klebsiella pneumonia*, d) *Staphylococcus aureus* by the disk diffusion method.

Table 1. Effect of silver nanoparticles on human pathogens

Pathogens	Zone of Inhibition	
	Plant silver	Gentamicin
Bacillus cereus	17mm	22mm
Escherchia coli	15mm	24mm
Klebsiella pneumonia	15mm	24mm
Staphylococcus aureus	16mm	26mm

Conclusion

We have demonstrated a good method for developing a simple, safe, cost-effective, and ecofriendly preparation of AgNPs in an aqueous extract of *Piper betle*. The present study concluded that the *Piper betle* plant extract can be used as an excellent source for synthesizing the silver nanoparticles. The primary confirmatory for the silver nanoparticles was color change of the solution, and UV-Vis absorption spectra of silver nanoparticles containing solution showed a peak at 444 nm, which confirms the presence of silver nanoparticles. FTIR spectrum of Ag nanoparticles suggested that Ag nanoparticles were surrounded by different organic molecules such as terpenoids, alcohols, ketones, aldehydes and carboxylic acids. The green synthesized nanoparticles have more effective antibacterial activity to the pathogens. So green synthesis of nanoparticles can be ecofriendly involved in the many applications of clinical, biomedical sectors and etc.

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