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ECOFRIENDLY SYNTHESIS OF SILVER NANOPARTICLES FROM AQUEOUS LEAF EXTRACT OF PIPER NIGRUM L. AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY AGAINST INFECTIOUS PATHOGENS

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ABSTRACT

Piper nigrum commonly known as black pepper belonging to the family Piperaceae, is cultivated widely for its fruit which is usually dried and used as a spice. The current work analyzes the potential of the aqueous leaf extract of Piper nigrum to produce silver nanoparticle from AgNO3 solution and to assess its antibacterial activity. Formation of Ag nanoparticles was indicated by the change in the color of test solution from green brown to brown color. Formation of Ag nanoparticles was confirmed by UV visible spectral analysis, XRD and SEM studies. Ag particles obtained were found to be highly active against *Staphylococcus aureus* and *E. Coli* followed by *Pseudomonas aeruginosa* and *Bacillus subtilis.*

Key Words: Silver nanoparticle, aqueous extract, *Piper nigrum*, antibacterial activity

INTRODUCTION

Nanotechnology can be defined as the manipulation of matter through certain chemical and physical processes to create materials with specific properties which can be used in particular applications. A nanoparticle can be defined as a microscopic particle that has at least one dimension less than 100 nanometers in size with a surrounding interfacial layer. The interfacial layer is an integral part of nanoscale matter fundamentally affecting all of its properties. Different protocols have been designed for the production of metallic nanoparticles.

Piper nigrum commonly known as black pepper is a flowering vein belonging to the family Piperaceae, cultivated for its fruit known as pepper corn, which is usually dried and used as a spice. Black pepper is native either to Southeast Asia or South Asia. Pepper contains phytochemicals including amides, piperidines, Pyrolidines and trace amounts of safrole, which may be carcinogenic in laboratory rodents.

METHODOLOGY

Piper nigrum L. was collected from local areas of Thrissur. The plant material was dried in shade at ambient temperature and made it to powder by electric blender. The powdered material was stored in air tight containers for further use.

Preparation of plant extract

5g of fine powder was weighed and boiled with 100mL of deionized water at 80°c for 15 minutes using water bath. The plant extract was filtered and collected separately.

Synthesis of Silver Nanoparticles from plant extract

To 10ml of aqueous leaf extract of Piper nigrum L. 90mL of 1mM silver nitrate solution was added and observed the color change.

Characterization of Silver Nanoparticles

The synthesis of nanoparticles was confirmed and monitored with the help of the various

analytical methods like UV-Visible spectra, SEM, XRD and EDAX analysis

Phytochemical analysis of *Piper nigrum* leaves

Phytochemical screening was carried out in the deionized water extracts of *Piper nigrum* L. Primary metabolites analyzed were carbohydrates (Molisch's test), aldehyde (Fehling's test), sugar (Benedict test), ketose (Seliwanoff's test), starch, amino acids (ninhydrin test), fats (filter paper test).

Qualitative tests were conducted for various secondary metabolites like quinine (H_2SO_4 test), cardiac glycoside, steroids (Lieberman buchard test), alkaloids (Wagner's test), Terpenoides (saliskowski's test), phenol (folin's test), saponins (foam test), Tannins (iron salt test), coumarins, flavonoids, acid and resin. All the tests were carried out by using standard procedures (Harborne,1973; Sofowora,1982).

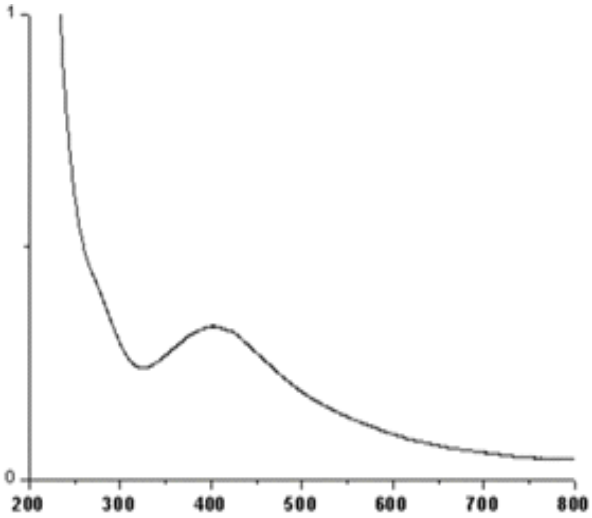
Antibacterial activity

Two human pathogenic gram negative (*Pseudomonas aeruginosa*, *E. Coli*) and two gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) bacterial strains were used for antimicrobial study of Silver Nanoparticles by well diffusion method.

RESULTS AND DISCUSSION

Plant extract contains several phytochemicals that react with silver nitrate and converts it into silver nanoparticles ie. silver ions which were primarily identified by the colour change from greenish brown to brown in the reaction mixture immediately on addition. Synthesis of Ag nanoparticles was confirmed by the UV visible spectrophotometric analysis that showed the absorption maxima at 400 nm characteristic peak of Ag nanoparticles (Fig.1).

Fig.1. UV-VIS Spectrophotograph of AgNP



XRD analysis was performed to verify the presence of Ag particles at its domain planes. The result showed typical XRD pattern of Ag nanoparticle. The observed peaks were compared with the standard powder diffraction card (JCPDS file No. 87-0720) and the results were in agreement with the standard patterns of XRDs of AgNPs (fig.2). Energy dispersive X-Ray Analysis (EDAX) spectrum of the synthesized nanoparticles, suggests the presence of silver as the ingredient element. A typically strong signal peak at 3 keV in dispersive spectrum confirms the presence of Ag (fig.3). The SEM micrograph revealed that the synthesized silver nanoparticles were aggregated as irregular spherical shapes and ranges from 100-200 NM with inter particle distance (plate 1)

Fig.2. XRD of AgNPs

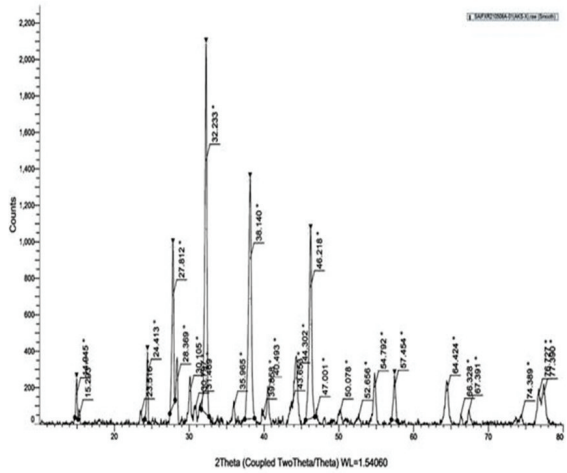


Fig.3. SEM –EDAX of AgNPs

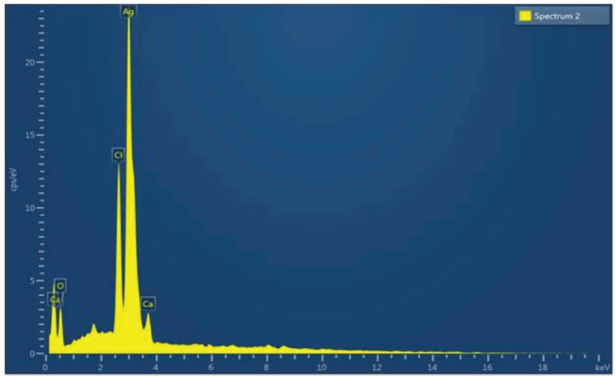
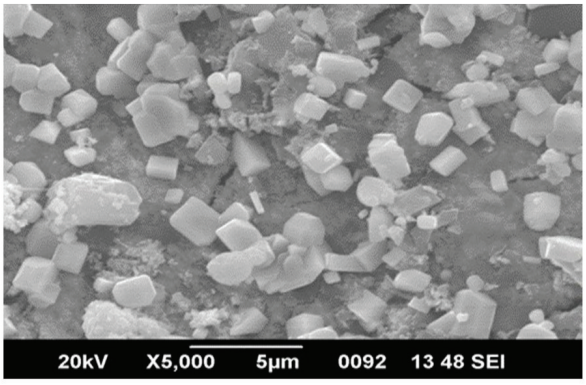


Plate 1. SEM analysis 500 X



Analysis of antibacterial activity revealed that the AgNPs were highly active against *staphylococcus aureus* and *E. Coli* followed by *Pseudomonas aeruginosa* and

then *Bacillus subtilis*. The results are depicted in table 1. Similar antibacterial activity of silver nano-particles was reported against *E.coli* and *Pseudomonas aeruginosa* (Jain et al., 2009); *Bacillus cereus* and *Pseudomonas aeruginosa* (Elumalai et al., 2010) *; Staphylococcus aureus*, *E.coli*, *Salmonella typhi* and *Candida albicans* (Dar et al., 2013).

CONCLUSION

The current reveals the potential of Piper nigrum leaves in the production of Ag nanoparticles from AgNO3 solution. Various techniques adopted for the analysis of nanoparticles confirmed the synthesis of Ag nanoparticles. Nanoparticles thus produced were found to be effective against various human pathogenic bacteria. The mechanism of synthesis of silver nanoparticles by plants can be used in the drug production for diseases which are caused by multidrug resistant bacteria.

Table 1. Antibacterial activity against various strains of bacteria

Organism	Zone of Inhibition in AgNPs (in mm)	Zone of Inhibition in Amoxycillin(in mm)
Staphylococcus aureus	**5**	**15**
Bacillus subtilis	**3**	**0**
Pseudomonas aeruginosa	**4**	**8**
E. coli	**5**	**10**

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