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Original Article

# EVALUATION OF ANTIBACTERIAL POTENTIAL OF SILVER NANOPARTICLES (SNPs) PRODUCED USING RHIZOME EXTRACT OF *HEDYCHIUM CORONARIUM* J. KOENIG

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**ABSTRACT**

**Objective:** The bio-reduction of silver ions in solution into silver nanoparticles by rhizome extract of *Hedychium coronarium* as capping, reducing and stabilizing agent. Further, to evaluate the antibacterial activity of the phytosynthesized silver nanoparticles.

**Methods:** Aqueous extract of dried rhizome powder of *Hedychium coronarium* were used for the green synthesis of silver nanoparticles. The synthesized nanoparticle was characterized by UV-vis spectroscopy, Scanning Electron Microscopy (SEM), X-ray diffraction (XRD) and Fourier Transform Infrared spectroscopy (FTIR) studies. Antibacterial activity of silver nanoparticles was carried out by disc diffusion method.

**Results:** The observation of the peak at 428 nm in the UV-vis spectrum for phytosynthesized silver nanoparticles reveals the reduction of silver metal ions into silver nanoparticles. The FTIR analysis was performed to identify the possible functional groups involved in the synthesis of silver nanoparticles. The SEM image shows that most of the phytosynthesized silver nanoparticles have spherical morphology. The average diameter of the particles was calculated from the XRD pattern and it was found to be 24 nm. Further, it was observed that silver nanoparticles have high antibacterial activity especially against gram negative organisms.

**Conclusion:** The rhizome extract of *Hedychium coronarium* could be a good candidate for the green synthesis of silver nanoparticles. The antibacterial property of silver nanoparticles is a beneficial application in the field of medical nanotechnology.

**Keywords:** *Hedychium coronarium*, Silver nanoparticles, Rhizome, SEM, FTIR.

## INTRODUCTION

Nanotechnology is expected to usher in many technological innovations in the 21st century. Research and development in this field is growing rapidly throughout the world. A major output of this is the development of new materials in the nanometre scale, including nanoparticles. Nanoparticles are materials at sub-micrometer scales, usually 1-100 nm, so they possess a large surface to volume ratio [1]. Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology [2]. Recently there has been considerable interest in the development of techniques for the biosynthesis of metal-nanoparticles of well-defined size, shape and composition, as they find applications in areas such as optics, electronics and medicine [3]. Production of nanoparticles can be achieved mainly through chemical, physical, and biological methods. Most of these methods are extremely expensive and also involve use of toxic, hazardous chemicals that pose potential environmental and biological risks. There is a growing need to develop environment friendly processes through bio mimetic approaches. Biological methods for nanoparticle synthesis using plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods [4].

For the last two decades, extensive work has been done to develop new drugs from natural products because of the resistance of microorganisms to the existing drugs. Nanotechnology advanced rapidly in recent years with its broad application in the health care sector, in imaging, diagnostics, drug delivery and therapeutics [5]. Silver nanoparticles (SNPs) have tremendous applications especially in disinfecting effect and have found useful applications in traditional medicines, culinary items, antimicrobial agents and sensors. Several salts of silver and their derivatives are commercially employed as antimicrobial agents [6]. Biological synthesis of nanoparticles by plant extracts is at present under exploitation and is testing for antimicrobial activities [7]. *Hedychium coronarium* J. Koenig, a medicinal herb belongs to the family Zingiberaceae, has been extensively used in traditional Indian medicine [8]. Its rhizomes have been used for the treatment of headache, contusion, inflammation and sharp pain due to rheumatism [9]. Various cytotoxic diterpenes, farnesane type sesquiterpenes and labdane-type diterpenes were isolated and characterized from the rhizomes of this plant [10, 11]. Anti-inflammatory, analgesic, antihypertensive, diuretic, leishmanicidal and antimalarial activities of rhizomes of this plant, have also been reported by several researchers [12]. In the present study, we describe an eco friendly approach toward silver nanoparticle biosynthesis using rhizome extract of *H. coronarium* and the evaluation of its antibacterial properties.

## MATERIALS AND METHODS

### Plant materials and chemicals

Rhizomes of *H. coronarium* were collected from Idukki, the southern part of Western Ghats. The voucher specimen (RHT 65120) was certified and documented in The Rapinat Herbarium, Tiruchirappalli Tamilnadu, India. Silver nitrate used for the production of SNPs was purchased from Sigma Aldrich.

### Preparation of the plant extract and synthesis of SNPs

Fresh rhizomes of *H. coronarium* were air dried for 10 days and kept in the hot air oven at 60ºC for 24 hours. The dried rhizomes were ground to a fine powder and 50 gms were taken in a conical flask and added 500 ml of deionised water. The mixture was kept in the hot water bath for 30 min at 70°C.

After the desired reaction period the samples were filtered through Whattman filter paper No. 1 to get the aqueous extracts. In the phytosynthesis protocol, for the reduction of silver ions, 20 ml of aqueous leaf extract was added dropwise into 100 ml of silver nitrate solution of different concentrations (0.5–1 mM) with constant stirring. As soon as, the extract was mixed in an aqueous solution of silver ion, the preliminary detection of SNPs formation was carried out by visual observation of colour change. The samples were centrifuged at 6000rpm for 20 min, the pellet was washed in

ethanol, dried and powdered. The nanoparticles were stored at 4ºC for further analysis [13].

**Characterization of SNPs**

The formation of silver nanoparticles in the reaction mixture was confirmed by UV-vis spectroscopic measurements. Morphology and average size of the purified and dried powder of silver nanoparticles were determined by Scanning Electron Microscopy (SEM). The structural properties and average size of the particles were investigated and confirmed by X-ray diffraction analysis. FTIR analysis was carried out to determine the biomolecules present in the leaf extract responsible for the reduction of Ag ions with 400 - 4000 $cm^{-1}$ of spectral range.

**Microorganisms**

The microbial strains of *Enterobacter aerogenes, Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis, Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella paratyphi, Vibrio cholerae, Bacillus cereus, Bacillus subtilis*, *Serratia marcescens*, *Staphylococcus aureus* and *Streptococcus faecalis* were used in the present study.

**Antibacterial test**

The antibacterial activities of SNPs were carried out by disc diffusion method [14]. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were loaded with 30 µl of silver nanoparticles solution (1mg/ml) and placed in the nutrient agar plate and kept for incubation at 37°C for 24 hours. Discs loaded with 30 µl (1mg/ml) of streptomycin were used as standard. The plates were examined for evidence of zones of inhibition, which appeared as a clear area around the discs. The diameters of zones of inhibition were measured using a meter ruler and was recorded and expressed in millimetre. The experiments were repeated thrice.

**RESULTS**

Synthesis of silver nanoparticles from silver nitrate is one of the most widely used methods for the synthesis of silver colloids. During the biosynthesis of SNPs, using the rhizome extract of *Hedychium coronarium,* the colour of the reaction medium changed from light yellow to dark brown (Figure 1) in 12 minutes. The change in colour of the reaction medium was varied with concentration of silver nitrate. From the different concentrations used, reaction mixture with a concentration of 0.5 mM silver nitrate gave better and rapid result. Thus the optimum concentration was found to be 0.5 mM silver nitrate.

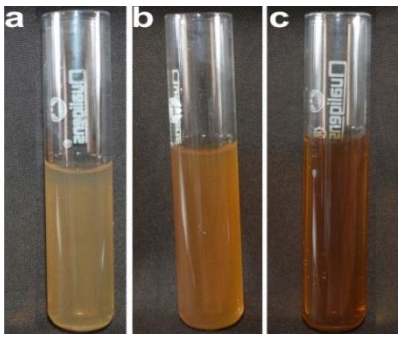


**Fig. 1: Various stages in the synthesis of silver nanoparticles using rhizome extract of *Hedychium coronarium***

The reduction of aqueous silver ions to silver nanoparticles was monitored by UV-Visible spectroscopy analysis and shown in figure 2. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectrum of the reaction medium at 12 min has an absorbance peak at 428 nm (Figure 2) indicates the presence of silver nanoparticles in the reaction mixture.

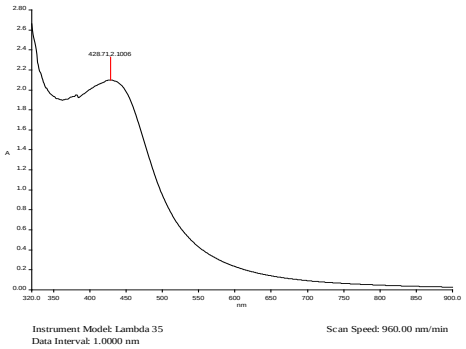


**Fig. 2: UV-VIS absorbtion spectra of silver nanoparticles synthesized using rhizome extract of *Hedychium coronarium* at a concentration of 0.5 mM silver nitrate.**

SEM is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. SEM provided insight into the morphology and size details of the silver nanoparticles produced. The size of the particles was from nano to micron range with an average size of 100 nm and morphology of particles was nearly spherical (Figure 3).

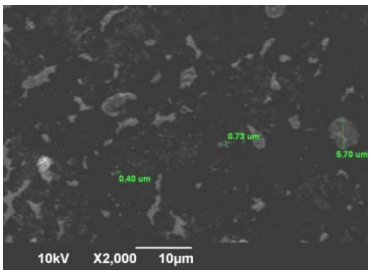


**Fig. 3: SEM image of silver nanoparticles synthesized using rhizome extract of *Hedychium coronarium* at a concentration of 0.5 mM silver nitrate**

The exact structure and an average grain size of the phytosynthesized silver nanostructures were demonstrated and confirmed by characteristic peaks observed in the XRD image (Figure 4). The XRD spectrum showed four distinct diffraction peaks at 38.28°, 44.51°, 64.69° and 77.57° corresponds to (111), (200), (220) and (311) planes of silver, respectively. The average grain size of the SNPs formed in the process was done at (111) plane using Scherrer equation [(d=K λ / β Cos θ), by using the reference peak width at angle θ, where λ is the wavelength of the X-rays (1.54060 A°), K is Scherrer constant (0.9), and β is the width of the XRD peak at half height]. The average grain size of the SNPs estimated from the Scherrer formula was 24 nm.

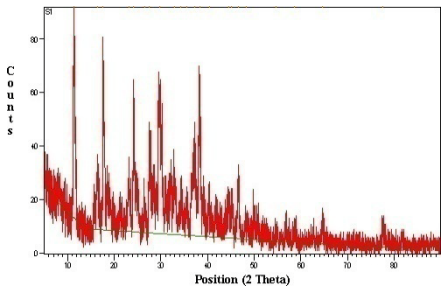


**Fig. 4: X-ray diffraction pattern of silver nanoparticles synthesized using rhizome extract of *Hedychium coronarium* at a concentration of 0.5 mM silver nitrate.**

FTIR measurements were performed to identify the biomolecules, responsible for capping, reducing and stabilizing the silver nanoparticles, present in the rhizome extract of *Hedychium coronarium*. Figure 4 shows the FTIR spectrum of the SNPs, which clearly shows peaks at 3905, 3856, 3466, 3434, 3412, 2361, 2079, 1635 and 668 $cm^{-1}$.

The absorption band at 3905 and 3856 $cm^{-1}$ was assigned as –OH stretching of the hydroxyl group of alcohol and phenolic compounds. The strong band observed at 3466, 3434 and 3412 $cm^{-1}$ represents the H-bonded –OH stretching of alcohol and phenolic compounds. Whereas the band observed at 2361 $cm^{-1}$ denotes the presence of hydrogen bonded –OH stretching of carboxylic acids in the rhizome extract. Peak at 1635 $cm^{-1}$ was a strong absorption peak which corresponds to C=O stretching (amides). The absorption band at 2079 $cm^{-1}$ and 668 $cm^{-1}$ corresponds to C≡C and C-H stretching respectively of alkynes.

The biologically synthesized silver nanoparticles from *H. coronarium* were found to be highly toxic against different gram positive and gram negative pathogenic bacteria. Among the tested organisms, *Escherichia coli* was most susceptible (21.66±1.52 mm) followed by *Streptococcus pneumoniae* (19.67±0.57 mm), *Proteus mirabilis* (19.00±1.00 mm), *Proteus vulgaris* (18.33±0.60 mm), *Enterobacter aerogenes* (18.00±1.00 mm), and *Bacillus cereus* (18.00±1.00 mm).

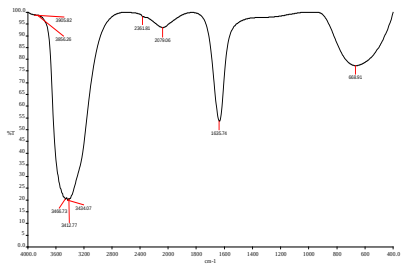

**Fig. 5: FTIR spectrum of silver nanoparticles synthesized using rhizome extract of *Hedychium coronarium* at a concentration of 0.5 mM silver nitrate.**

The result of antibacterial activity of silver nanoparticles is depicted in table 1.

**Table 1: The antibacterial activity of silver nanoparticles synthesized using *H. coronarium* rhizome extract**

| S. No. | Organisms | Width of zone of inhibition (mm) | |
|---|---|---|---|
| | | Silver nanoparticles | Antibiotic |
| 1 | *Escherichia coli* (-) | 21.66±1.52 | 18.00±0.00 |
| 2 | *Streptococcus pneumoniae* (+) | 19.67±0.57 | 19.00±0.00 |
| 3 | *Proteus mirabilis* (-) | 19.00±1.00 | 21.33±0.60 |
| 4 | *Proteus vulgaris* (-) | 18.33±0.60 | 18.33±0.60 |
| 5 | *Enterobacter aerogens* (-) | 18.00±1.00 | 18.00±0.00 |
| 6 | *Bacillus cereus* (+) | 18.00±1.00 | 17.00±0.00 |
| 7 | *Bacillus subtilis* (+) | 17.67±0.57 | 18.00±0.00 |
| 8 | *Serratia marcescens* (-) | 17.33±0.60 | 15.00±0.00 |
| 9 | *Staphylococcus aureus* (+) | 15.33±0.60 | 14.66±0.57 |
| 10 | *Salmonella paratyphi* (-) | 14.00±1.00 | 14.00±0.00 |
| 11 | *Vibrio cholerae* (-) | 10.67±0.57 | 11.00±0.00 |

Values are mean± standard deviation of three experiments, (+) gram positive, (-) gram negative

## DISCUSSION

It is well known that silver nanoparticles exhibit yellowish–brown colour in aqueous solution. Reduction of silver ions into silver nanoparticles during exposure of the rhizome extract could be followed by color change is a clear indication of the formation of SNPs *in vitro*. The colour change of the reaction mixture is due to the presence of the surface plasmon vibrations of the SNPs well dispersed in the solution [15].

A single broad peak was observed in the UV-vis spectrum at 428 nm, which corresponds to plasmon excitation of the SNPs. The time duration of change in colour (silver ion reduction) has a direct correlation with plant extract. From the results it can be suggests that the rhizome extract of *H. coronarium* is a good candidate for the *in vitro* production of SNPs in short time. Similar results were also found in *Boswellia ovalifoliolata, Shorea tumbuggaia* and *Svensonia hyderobadensis* [16]. SEM and XRD results provide the morphology, structure and size of the SNPs formed in the phyto synthesis process. The XRD result indicates that the specific peaks in XRD pattern can be indexed to a face-centered cubic structure of silver as per standards (JCPDS, file No.04-0783). Thus, XRD spectrum confirmed the crystalline structure of SNPs. The unassigned peaks could be due to the crystallization of bioorganic plane that occurs on the surface of the SNPs. Further, SEM and XRD analysis proved the efficiency of rhizome extract of *H. coronarium* as a reducing agent for the production of silver nanoparticles.

The secondary metabolites present in plants may be responsible for the reduction of silver ions in solution and synthesis of nanoparticles [17]. The FTIR gives information on the vibration and rotational modes of motion of a molecule and hence an important technique for identification and characterisation of a substance. The FTIR results of the present study suggest that the presence of secondary metabolites with various functional groups in the rhizome extract and as well as in synthesized SNPs. From the analysis of FTIR studies we confirmed that the carbonyl groups (1635 $cm^{-1}$) from the amino acid residues and proteins in the extract has strong ability to bind metals indicating that the proteins could possibly from the silver nanoparticles (capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of SNPs in the aqueous medium. Toxicity studies on pathogens open a door for nanotechnology applications in medicine. The SNPs synthesised using rhizome extract of *H. coronarium* showed good antibacterial properties and it might be due to the various actions of silver nanoparticles. The ionic silver strongly interacts with thiol group of vital enzymes and inactivates the enzyme activity [18] and DNA loses its replication ability once the bacteria have been treated with silver ions [19]. Ahmad et al., [17] mentioned that the pathogenic effect of nanoparticles can be attributed to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the pathogen proteins and arrests its growth.

The growth of microorganisms was inhibited by the green synthesized SNPs and showed variation in the inhibition of growth of microorganisms which may be due to the presence of peptidoglycan. The later is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria [17].

## CONCLUSION

The present study aimed at the bio-reduction of silver ions through medicinal plants extracts and its effectiveness as antimicrobial agent. The secondary metabolites present in the rhizome extract of *H. coronarium* responsible for the reduction of silver ions into silver nanoparticles whereas amino acids and proteins play an important role in capping and stabilization of the nanoparticles. The results have indicated that silver nanoparticles synthesised in the experiments have definite antimicrobial activity against different microorganisms. It is confirmed that silver nanoparticles are capable of rendering high antibacterial efficacy and hence have a great potential in the preparation of drugs against bacterial diseases. Moreover the synthesized SNPs enhance the therapeutic efficacy and strengthen the medicinal values of this plant.

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## CONFLICT OF INTEREST

The authors declare that we have no conflict of interest.

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