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**Antimicrobial activity and characterization of  
biosynthesized silver nanoparticles from  
*Anisochilus carnosus***

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**ABSTRACT**

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Silver nanoparticles with size of 6- 57nm were synthesized by bioreduction method from *Anisochilus carnosus* aqueous and ethanolic extract. Biosynthesized nanoparticles showed maximum antimicrobial activity against *K. pneumonia* (13±1.67mm), *E.coli* (13±1.97mm) and *B. subtilis* (13±1.07mm) followed by *P. aeruginosa* and minimum against *S. aureus*. The quantitative formation, characteristics and impurities of silver nanoparticles were studied using UV-Vis absorption spectroscopy, X-ray diffraction analysis, EDX spectrum, scanning electron microscopy (SEM) analysis. Based on this investigation these silver nanoparticles may be used in effluent treatment process for reducing the microbial load.

**Keywords:** *Anisochilus carnosus*; Silver nanoparticles; Antimicrobial activity; SEM; EDX; XRD.

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**INTRODUCTION**

Two major applications of nanomedicine are targeted drug delivery and molecular imaging. Today, nano structures are being intensively investigated and some products are undergoing clinical trials [1-4]. For drug delivery, polymer capsules in nanoscale, or nanoshells, are designed to break down and release drugs at controlled rate to release drugs in specific environments, thereby extending the duration of action of the drug [5]. Another advantage of being nano-size particles is that the drug or the carrier can bypass physiological barriers that normally do not allow drugs to pass through, such as the blood-brain barrier, a unique membrane that tightly segregates the brain from the circulating blood, the branching pathways of the pulmonary system, and the tight epithelial junctions of skin [6]. Furthermore, the clearing mechanism of nano sized drugs can be modulated by altering the particle surface structures to either suppress macrophage detection or control particle self-aggregation [6].

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Recently many studies has been conducted to explore the synthesis of nanoparticles uses of microorganisms as a potential, bio sources; such as Au and Ag, Basavaraja et al. [7] use *Fusarium semitectum* for biosynthesis of silver nanoparticles. Sastry et al. [8] have reported that fungus *Fusarium oxysporum* and *Verticillium sp*, when exposed to Au and Ag<sup>+</sup> ions formed respective metallic bionanoparticles and Holmes et al. [9] have shown that the bacteria *Klebsiella aerogenes* can be used for intracellular synthesis of Cds nanoparticles. Recently few studies have been conducted for characterization and antimicrobial effect of silver nanoparticles [10-12] showed, the silver nanoparticles like its bulk counterpart are an effective antimicrobial agent against various pathogenic microorganisms, Shrivastava et al [13] has reported the silver nanoparticles in the range 10-15 nm with increased stability and enhanced antimicrobial potency.

Silver with a positive charge, abbreviated as Ag<sup>+</sup>, is known as ionic silver or silver cation. The ionic charge allows silver cation to bind to and damage bacterial cells at multiple sites. It has been found that the antibacterial activity of silver can act against a broad range of aerobic, anaerobic, gram-negative, gram-positive bacteria, yeast, filamentous fungi, and viruses. The plant *Anisochilus carnosus* belonging to the family Lamiaceae have been used in malarial fever, hepatopathy, renal and vesicle calculi, cough, chronic asthma, hiccough, bronchitis, anthelmintic, colic and convulsions. Stimulant has expectorant and diaphoretic. Juice of fresh leaves is used in urinary and other allergic conditions; antibacterial, antitubercular. The oil exhibits antihistaminic property in vitro on smooth muscles of the uterus and the intestines. It also possesses muscle-relaxant action; bactericidal and fungicidal properties. The leaves contain glycosides of luteolin and apigenin. In the present investigation we report the extracellular synthesis, of highly stable bionanoparticles and the evaluation of antimicrobial activity against multi and the study also includes the characterizations of bionanoparticles by UV-Visible spectrophotometer, Scanning electron microscope and XRD spectral analysis.

## EXPERIMENTAL

### *Collection of plant material*

Fresh and healthy *Anisochilus carnosus* leaves were collected, washed thoroughly with distilled water, incised into small pieces and air-dried. The medicinal plants selected for the study were collected from Navamalai, Pollachi, India.

### *Extract of plant materials*

Shade dried sample were percolated by soxhlet apparatus using different solvents. The phytochemicals present in the plant material was extracted by the distillation method using soxhlet apparatus. Two different solvent were used for the separation (Ethanol and aqueous). About 100g of plant were weighed and shade dried for 14 days. The dried materials were powdered and 50g of powdered sample was packed in a thimble and extracted with 100 ml water and 70% ethanol successively up to 48 h. Each of the solvent extracts was concentrated separately under reduced pressure. The whole apparatus was kept over a heating mantle and was heated continuously for 8 hours at boiling point of each solvent. The extract was concentrated to dryness and the solid residues were transferred to a preweighed sample bottle after complete solvent evaporation.

### *Preliminary screening for Phytochemical*

Natural chemical groups such as amino acids, alkaloids, carbohydrates, flavonoids, saponins, sterols, tannins, terpenoids, proteins and phenolic compounds were probed. The aqueous and ethanolic extracts were screening for phytochemical analysis to reveal the characteristic of the extract.

### *Bio Synthesis of silver nanoparticles*

Aqueous solution (5mM) of silver nitrate (AgNO<sub>3</sub>) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Anisochilus carnosus* leaf extract was added into 95ml of aqueous solution of 5mM silver nitrate for reduction into Ag<sup>+</sup> ions. Reduction of silver nitrate to silver ions was confirmed by the color change from colorless to brown black. The formation of silver nanoparticles was also confirmed by spectrophotometric determination. The fully reduced solution was centrifuged at 10,000 rpm for 15 min. The supernatant liquid was discarded and

the pellet obtained was redispersed in deionized water. The centrifuge process was repeated two to three times to wash off and air dried the sample and makes up to in a powder form any absorbed substances on the surface of the silver nanoparticles. Same protocol was followed for synthesis of nanoparticles in ethanol extract.

#### **Anti-bacterial efficacy by well diffusion method**

Bacterial cultures such as *Escherichia coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 530), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (ATCC 6633) to evaluate the anti-bacterial efficacy of aqueous, ethanolic extracts and biosynthesized silver nanoparticles from *Anisochilus carnosus* along with 5 mM of silver nitrate as positive control and DMSO as negative control. Freshly prepared Muller Hinton agar plates were inoculated with exponential bacterial cultures by spread plate technique. The agar well diffusion method was used to determine the growth inhibition.

#### **Characterization of bio synthesised silver nanoparticles**

- **UV-VIS studies**

The reduction of silver ions was monitored by measuring the UV-VIS spectrum of the Colloidal solution obtained after 10 min of 300µl sample and 3 ml deionized water. Absorbance was recorded at a resolution of 0.5nm at 200-800nm using UV-VIS spectrophotometer.

- **SEM Analysis**

For electron microscopic studies, Scanning Electron Microscopic (SEM) analysis was done using AMETEK SEM machine. 1µg thin films of the sample were prepared and placed in the sputter coated on copper stub then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min and the images of nanoparticles were studied using SEM(JPEG, Model JFC-1600).

- **EDAX analysis**

Energy dispersive spectroscopy (EDS) was carried out on AMETEK micro analysis report and addition presence of metals in the sample was analyzed.

- **XRD analysis**

The purified pellet was dried, mixture of silver nanoparticles was further analyzed with X-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV, and a current of 30 mA, with Cu Ka radiation in a h-2θ configuration. In addition, a thin film of sample was also prepared in the cover slip with 100ll synthesised silver nanoparticles solution, and allowed to dry for 5 min, and the slides were analyzed with atomic force microscopy.

## **RESULTS AND DISCUSSION**

#### **Phytochemical analysis**

The preliminary phytochemical studies reveal that the extracts of leaves from *A. carnosus* have variety of phytochemical constituents, namely alkaloids, coumarins, flavonoids, saponins, xanthoprotein, protein, Glucoside, cardiac glycosides, terpenoids, tannins and sugar (Table 1).

#### **Anti-bacterial efficacy**

Ethanolic extract of *A. carnosus* showed the maximum zone of inhibition with the *K. pneumonia* (12±0.97mm), *E. coli* (12±0.97mm) and *B. subtilis* (12±0.97mm) followed by *P. aeruginosa* (10 ± 0.91 mm) but the minimum zone (10 ± 1.33 mm) of inhibition was identified with the *S. aureus* species. Moreover, aqueous extract of *A. carnosus* showed the maximum zone of inhibition with the *E. coli* (10±0.91mm) followed by *K. pneumonia* (9±0.87mm) (Table 1). Previously reported that, *Anisochilus carnosus* (L) Wall and *Melaleuca alternifolia* (Maiden & Betche), against opportunistic pathogen *Candida albicans*. The plant extracts showed a wide range antifungal activity [14]

Similarly, biosynthesized of nanoparticles from *A. carnosus* showed maximum zone of inhibition with the *K. pneumonia* (13±1.67mm), *E. coli* (13±1.97mm) and *B. subtilis* (13±1.07mm) followed by *P. aeruginosa* (12 ± 0.91 mm) but minimum zone (10± 1.33) of inhibition was identified with *S. aureus* specie in ethanolic extracts. Moreover, the synthesis of nanoparticles from aqueous extract of *A. carnosus* showed maximum zone of inhibition with the *E. coli* and *B. subtilis* (12±0.98mm) followed by *K. pneumonia* (11±0.97mm) respectively Table 2.

**Table 1.** Phytochemicals analysis of *Anisochilus carnosus*

Different solvent extracts	Carbohydrate	Flavonoids	Glucoside	Cardiac glycosides	Alkaloids	Coumarins	Tannins	Terpenoids	Anthraquinone glycosides	Saponin
Ethanol	+	+	+	+	+	+	+	+	-	+
Aqueous	+	+	+	+	+	+	+	+	-	+

**Table 2.** *In vitro* antibacterial potential of biosynthesized silver nanoparticles using *A. carnosus* leaf extract (Disk diffusion assay mm in dm)

Bacterial Pathogens	Leaf Extracts of <i>A. carnosus</i>		Biosynthesized silver nanoparticles of <i>A. carnosus</i>	
	Aqueous extract	Ethanollic extract	Aqueous extract	Ethanollic extract
<i>Escherichia coli</i>	10±0.96	12±0.91	12±0.981	13±1.67
<i>Klebsiella pneumonia,</i>	7±0.98	10±0.91	10±1.2	12±0.91
<i>Pseudomonas aeruginosa,</i>	6±0.26	10±1.33	8±0.48	10±1.33
<i>Staphylococcus aureus,</i>	9±0.36	12±0.97	11±0.91	13±1.67
<i>Bacillus subtilis</i>	7±0.45	12±0.97	12±0.981	12±1.67

Biosynthesized silver nanoparticles using different plant parts (leaves, bark and root) of *Avicenna marina* mangrove plant. Of the selected three different parts, the leaf extract showed the maximum synthesis of silver nanoparticles. The *in vitro* antibacterial assay (100 µg/disk concentration) showed the results of maximum zone of inhibition with the *E. coli* (18.40 ± 0.97 mm), and minimum (10.87 ± 1.33 mm) zone of inhibition with *S. aureus* but the concentrations of MIC and MBC values ranged between 6.25 and 50.0 µg. ml<sup>-1</sup> between the selected bacterial strains [12]. Lee et al. [15] reported on possibility of using cotton cloth immobilized with silver nanoparticles synthesized chemically for wound dressing. They reported the bactericidal effect of the silver

nanoparticle-immobilized cloth material against gram-positive and -negative and antibiotic-resistant bacteria. Skin-irritation tests of the silver nanoparticle-immobilized cloth material on guinea pigs revealed no side effects [15]. Interaction of silver nanoparticles with human fibroblasts affected their fission but was not toxic [16].

Moreover, the present study also proved to have potential antibacterial activities with the *Anisochilus carnosus* leaf extract synthesised silver nanoparticles and this might be due to denaturation of bacterial cell wall, blocking bacterial respiration, destabilization of outer membrane and depletion of intracellular ATP [17]. Further, the variation of the sensitivity between Gram positive and negative bacterial isolates might be attributed by the

membrane permeability [18]. In spite of this permeability barrier, the synthesized silver nanoparticles exhibited the strong inhibition with gram-negative bacterial strains. The size and shape of nanoparticles play an important role in many of the pharmaceutical applications. In this regard, the size of the synthesised nanoparticles was identified between 71 and 110 nm with various spherical shapes, which falls closer to many of the silver nanoparticles produced by other plant materials [12, 17, and 19]. It is concluded from the present findings that, the biosynthesized silver nanoparticles using leaf aqueous extract of *Anisochilus carnosus* showed potential antibacterial activity with various bacterial pathogens which could be further used as a potential antibacterial agents.

### Characterization of Biosynthesised Silver Nanoparticles

#### • UV- VIS spectrum analysis

After the extraction of silver nanoparticles, UV-Vis spectrophotometer measurements were performed for two samples. The reduction of silver ions in the aqueous and ethanolic solution of silver complex during the reaction with the ingredients of the leaf extracts revealed that silver nanoparticles in the solution could be correlated with the respective UV-Vis spectra (Figure 1). Using spectrophotometric analysis, the colloidal solution of each sample studied exhibited a strong absorption between 200 and 400 nm.

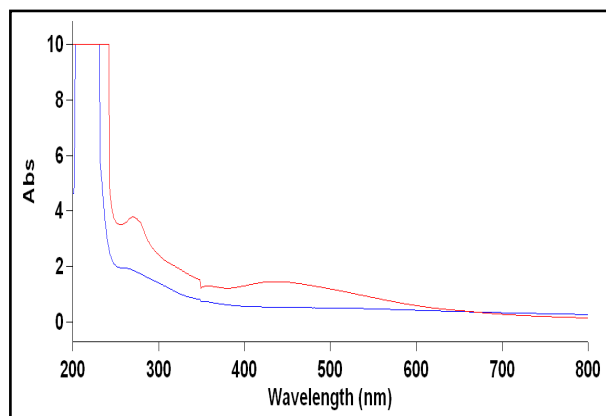


Fig. 1. UV-Vis spectral analysis of silver nanoparticles  
Red – aqueous, blue – ethanolic

#### • SEM Analysis

The presence of nanoparticles was confirmed by carrying out SEM. It is observed that most of Ag nanoparticles were spherical in shape and also cubic shape. It is evident that there is variation in particle sizes and the average size estimated was 35 nm and the particle size ranged from 6 to 57 nm (Figure 2).

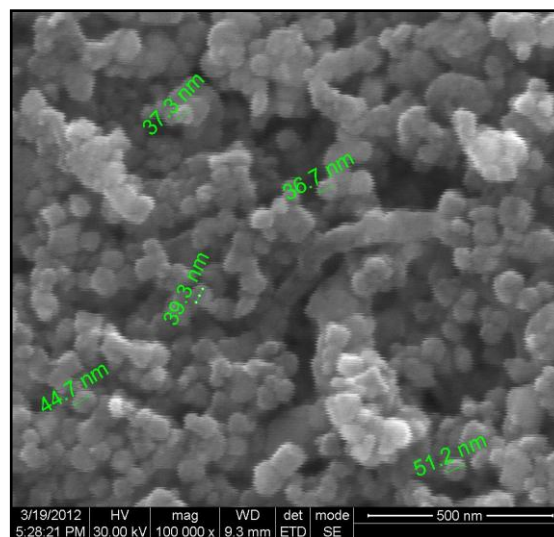


Fig. 2. Image of SEM observation of synthesized AgNO<sub>3</sub>

#### • EDX Analysis

Standard EDX spectrum recorded on the examined sample is shown. In the middle part of the presented spectrum one can clearly see five peaks located between 2 kV and 4 kV. Those maxima are directly related to the silver characteristic lines K and L. The maximum located on the left part of the spectrum at 0.2 kV clearly comes from carbon. The hardly visible maximum located at 0.5 keV is connected with the oxygen characteristic line. The carbon and oxygen spots in the examined samples confirm the presence of stabilizers composed of alkyl chains. The spectra obtained during EDX studies were used for carrying out the quantitative analysis. For that purpose, SEMQuant software and the ZAF procedure were applied. Quantitative analysis proved high silver contents (74%) in the examined samples. Except silver, we also show the presence of coal and oxygen, of the contents which amounted to 21% and 5%, respectively. In this study, elemental silver can be seen in the graph



presented by the EDX analysis in support of XRD results, which indicated the reduction of silver ions to elemental silver (Figure 3).

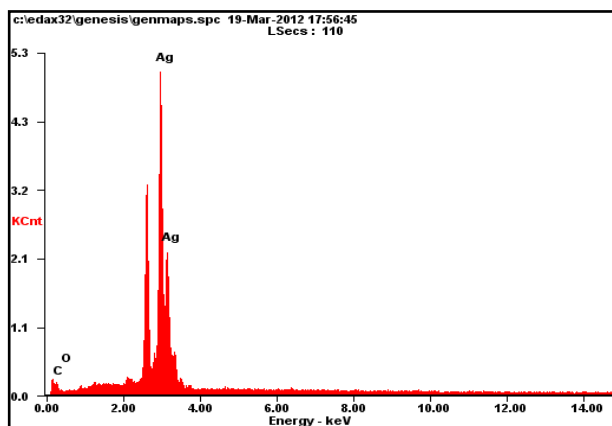


Fig. 3. EDX graph of biosynthesized silver nanoparticles

#### • XRD Analysis

The powder diffraction (XRD) patterns of the  $\text{AgNO}_3$ s were shown in Figure 4.  $\text{AgNO}_3$ s showed diffraction peaks which indicated characteristic of metallic face-centered cubic silver phase (PDF-2 4-0738) at  $38.1^\circ$ ,  $44.2^\circ$ ,  $64.5^\circ$ ,  $77.4^\circ$ ,  $81.5^\circ$ ,  $98.0^\circ$   $110.6^\circ$  and  $114.8^\circ$  in  $2\theta$ . It can be noted that diffraction peaks corresponding to potential silver nitrate (were calculated by applying Scherrer equation and found to be of size of 12 and 11 nm, respectively. In this results of XRD  $2\theta$  indicated intense values ranged with different degree (38.11 and 70.57), and these results correspond to the (111) and (311) Bragg's reflection, respectively. Further, the biosynthesis of silver nanoparticles can be confirmed by the formation of yellowish brown colour, and this might be due to the excitation of the surface plasmon vibration of the synthesised silver nanoparticles [20]. In addition, the results of XRD pattern further corroborate the synthesis of silver nanoparticles with sharp bands of Bragg peaks, and this might be due to the stabilization of the synthesised nanoparticles by the various reducing agents of the *A. marina* leaf extract, and thus provided the crystallization nature of the silver nanoparticles [21].

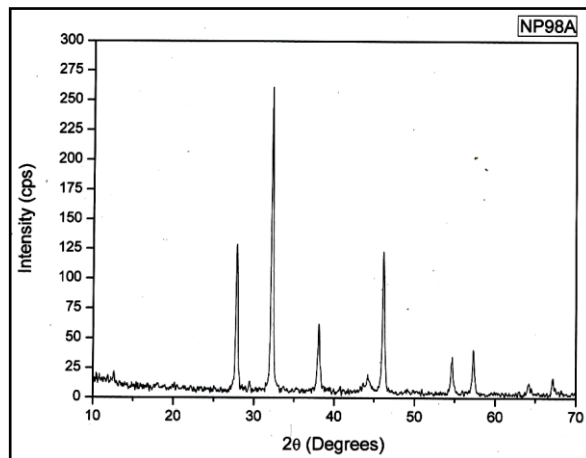


Fig. 4. XRD analysis of biosynthesized silver nanoparticles from *A. caronsus* leaf extract

## CONCLUSIONS

Nanoparticles play an important role in pharmaceutical, industrial and biotechnological applications. Synthesis of nanoparticles using chemical and physical methods requires high pressure, energy, temperature and toxic chemicals. In this regard, plants and plant part extracts based biosynthesis has been found to be cost effective and environmental friendly (Casida and Quistad, 2005). Development of reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. In this investigation the results confirmed the reduction of silver nitrate to silver nanoparticles with high stability and without any impurity. The biosynthesized silver nanoparticles using *A. caronsus* leaves extract proved excellent antimicrobial activity. The antimicrobial activity is well demonstrated change in membrane permeability and respiration activity of bacterial cells treated with silver nanoparticles. The characteristics of the obtained silver nanoparticles were studied using UV-Vis, XRD, EDX, and SEM techniques. Hence, the biological approach appears to be cost efficient alternative to conventional physical and chemical methods of silver nanoparticles synthesis and would be suitable for developing a biological process for large-scale production. These silver nanoparticles may be used in effluent treatment process for reducing the microbial load.

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