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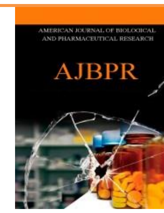

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PREPARATION AND INVESTIGATION OF AG-NANOPARTICLES USING LEUCAS EXTRACT

N. Deepa*, D.Tamilanban, P.Guna, S.Rohan, I.Daniel Prasanna

Department of Pharmacy, Saveetha College of Pharmacy, Saveetha Nagar, Thandalam, Chennai – 602105, Tamilnadu, India.

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ABSTRACT

Nanoparticles are in demand in diverse areas of science and technology, and their uses are also common in the medical and pharmaceutical fields. They have been used as preservatives, aids for diagnosis and effective antibacterial agents. But when it comes to cost, effectiveness and toxicity problems, and their development is a significant matter of concern. Green synthesis has taken advantage of its industrial and large-scale synthesis by addressing these limits. This study focuses on the synthesis of silver nanoparticles using Leucas aspera filtered leaf extract and examination using UV-Vis Spectrophotometry. In UV spectroscopy, the nanoparticles demonstrated surface plasmon resonance at 420nm. In addition, nanoparticles have been tested on Putida vulgaris, Staphylococcus aureus, and Bacillus subtillis for their antibacterial efficacy. The findings revealed that the eco-friendly synthesized silver nanoparticles have a strong antibacterial property and can be used successfully in treatments for diseases and contagious wounds.

INTRODUCTION

Nowadays, nanoparticles have acquired interest in diverse areas of medicine and science. They are commonly and exclusively used as diagnostic and surgical aids in terms of their benefits over other types[1]. With their own benefits and pitfalls, they can be synthesized using different approaches. Most notably, however, green synthesis of metallic nanoparticles has been found to be an emerging theme in nano biotechnology when it comes to health protection concerns. These methods are eco-friendly, less toxic, and the most powerful approach to nanoparticle synthesis[2].

Corresponding Author

Dr.N. Deepa
Email:- deepanatarajan@yahoo.com

Silver has been used as an antimicrobial agent since 500 AD, as proven by tradition. Silver has been used to store water, wine, vinegar and milk since ages on the basis of an argument that silver stops them from spoiling1 without empirical evidence. Although the process and toxicity have not been thoroughly studied, silver nano particles have been commonly used for health and household purposes[3]. The toxicity of silver nanoparticles, however, is still a matter of concern and is a topic on which research work needs to be performed. Silver's high efficacy and efficiency against any form of bacteria renders it the strongest antimicrobial agent known and recommended by doctors. While several derivatives that resolve bacterial resistance to synthetic antibiotics have been developed by scientists, these medications cause unnecessary or expensive side effects. So, in spite of the above-mentioned drawbacks of the antibacterial medicines, nano silver has developed itself as a potent and safer historical antibacterial agent. Not only as a prescription product, but also in electronics and related technologies, silver nanoparticles have a wide variety of applications[4].

The synthesis of silver nanoparticles by plants such as Neem leaf broth (Azadirachta indica), geranium leaves (P. graveolens), soap nuts (Sapindus trifoliandus), cinnamon (Cinnamomum zeylanicum), citrus (Citrus limon), tea, coffee, tannic acid and several micro-organisms has been discovered by many tests. So, this thesis focuses on the biogenesis of silver nanoparticles using the leaves of Leucas aspera. By the reduction of silver ions to metallic silver nanoparticles, silver nanoparticles are created. There are rich polyphenols3 in Leucas extract that can be used to decrease silver ions to form silver nanoparticles[5].

MATERIALS AND METHODS

Extraction of raw material

Leaves of Leucas aspera from S.V. University, Tirupathi, have been collected. The leaves were air-dried; the powder was finely powdered and 50gm of the powder was macerated for 24 hours with 250 ml of double distilled water with continuous stirring. The macerate was vacuum-filtered. Using a whattman filter paper, the filtrate obtained was filtered twice to get a transparent solution and was used directly for further experiments[6].

Cultures

Micro-organisms used in the experiment were procured from pure mother cultures. All these cultures were one day cultures freshly prepared from pure mother cultures obtained from the lab.

Synthesis of silver nano particles

50ml of 1mM Silver nitrate was applied separately to various plant extract volumes such as 1ml, 5ml, 10ml and constituted a 200ml final solution and centrifuged for 25 minutes at 18000 rpm to separate any precipitates. The supernatant was heated to a temperature of 50oC to 60oC.4 During the heating process, a difference in the solution colour was observed. The resulting solutions were called, respectively, as SNP 1, SNP 5, SNP 10[7].

Evaluation

The reduction of pure silver ions was tracked by the analysis of the reaction medium UV-Vis spectrum at 30 min, 1, 2 and 3 hours after dilution of a small sample aliquot into distilled water. The spectral study of UV-Vis was carried out using the UV-Vis Spectrophotometer UV 2450 (SHIMADZU).

2.5. Antibacterial activity

The anti-microbial efficacy of various Silver Nano particle formulations was conducted using the Dip well process as per normal protocol on different microorganisms5. To assess the antimicrobial activity against three separate micro-organisms, i.e., Putida vulgaris, Staphylococcus aureus and Bacillus subtillis, three sterile Petri plates were taken.

The nutrient broth medium was prepared and poured into test tubes, and micro-organisms were inoculated and held for crop growth in the incubator at 370 for 24 hours. Cultured micro organisms were inoculated from the nutrient broth to the Solidified Nutrient Agar in the petri dishes. In the inoculated nutrient agar medium, four cavitites were rendered and filled with 25 µl of different Silver Nanoparticles concentrations in three cavities together with normal fourth cavity clindamycin gel. Take note that the samples are placed at the level of the cavity and incubated for 24 hours at 370 C. Petri plates were observed after 24 hours for anti-microbial activity. The size of the inhibition zone was measured and the results, showing the antimicrobial activity of silver nanoparticles, were compared to each other.

Statistical Analysis

The observations are presented with n=3 as mean SEM and subjected to One-way ANOVA followed by the examination of Dunnett. The $p<0.01$ values is found to be of importance[8].

RESULTS & DISCUSSION

The development of silver nano particles was shown by the colour shift of the silver nitrate-containing Leucas aspera extract after heating. Until heating, the solution was pale yellow and shifted to dark brown in Figure 1, which shows the development of silver nanoparticles.

The surface plasmon resonance was revealed by metallic nanoparticles at 420 nm by UV-Visible spectral analysis. This is attributable to the excitation of the shaped silver nanoparticles' surface plasmon vibrations6. At time intervals of 30 min, 1hr, 2hrs, 3hrs, respectively, peaks were observed and are shown in Figure 2. The absorption limit at 420nm was demonstrated by the spectrum obtained at 3hr. The height of the peaks rose, suggesting that the time was relative to the formation of nanoparticles. At the 3rd hour, the peak appears to become sharp and more intense, meaning that the formed nanoparticles are much larger than those formed at the 1st and 2nd hours. The absorption at shorter wavelengths means that certain organic molecules such as secondary metabolites from plant extract have shaped the silver nanoparticles due to the interference. Thus, it can be stated that the reduction of silver ions into metallic silver nanoparticles may be responsible for reducing agents and antioxidants such as polyphenols and flavonols present in the plant. Silver prevents a very low concentration of a number of pathogenic microorganisms and is comparatively less toxic to animal cells. Various species such as Putida vulgaris, Bacillus subtilis, Staphylococcus aureus have been examined for the antibacterial function of silver nanoparticles. Compared to SNP-5, SNP-1, and power, silver nanoparticles (SNP 10) had the highest inhibition region. The SNP-10 inhibition region for Putida vulgaris, Bacillus subtilis and Staphylococcus aureus is substantially higher ($p<0.01$) than for the remaining classes, as seen in Table 1. The low particle size of the prepared silver nanoparticles produced with 10 ml of extract may be attributed to this high efficacy. Smaller particles of nanoparticles have a comparatively greater effective surface region for contact with cells than larger particles and have a more bactericidal impact. Consisting of negatively charged phospholipids and lipoproteins, the bacterial cell walls attract positive charged silver nanoparticles, thereby disrupting permeability and cellular respiration7. They also reach into the bacterial cell and communicate with cell components such as DNA and RNA containing phosphorus and sulfur. The natural cell metabolism and division of cells is thereby disrupted, leading to bactericidal and bacteriostatic action.

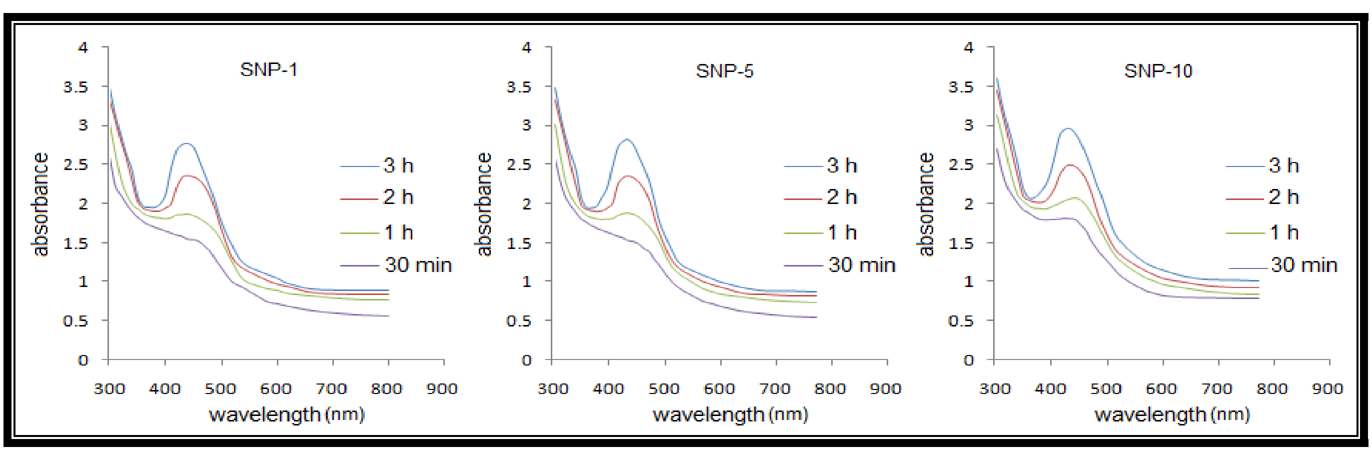
Table 1. Antibacterial activity of silver produced by Leucas extract

Putida vulgaris		*Bacillus subtilis*		*Staphylococcus aureus*	
Group	**zone of inhibition**	**Group**	**zone of inhibition**	**Group**	**zone of inhibition**
STD	5.2±0.57mm	**STD**	5.1±0.43mm	**STD**	3.4±0.25mm
SNP-1	3.8±0.26mm	**SNP-1**	2.4±0.7mm	**SNP-1**	2.9±0.11mm
SNP-5	6.3±0.2mm*	**SNP-5**	6.3±0.26mm*	**SNP-5**	4.5±0.38mm**
SNP-10	7.4±0.29mm**	**SNP-10**	7.8±0.31mm**	**SNP-10**	5.2±0.32mm**

Results were represented as mean±SEM, n=3; *P<0.01 significant compared to std; **more significant

Figure 1. Color change in extract solution; a. before heating b. after heating

Figure 2. UV-Vis spectrum of silver nanoparticles



CONCLUSION

As a therapeutic and diagnostic agent, silver has been used and it is apparent from the literature that nano particles are potent and effective relative to standard medicines. It was discovered that silver nanoparticles have possible advantages over many drugs available. But the cost and toxicity of their synthesis became a significant concern. Thus, using leaf extract of Leucas aspera, the bio-mimetic synthesis of silver nanoparticles was carried out and research showed that the prepared nanoparticles are potent antibacterial agents. The bio-mimetic synthesis of metal nanoparticles has been shown to be safer and more cost-effective. They are relatively safe and effective compared to available antibacterial drugs and chemically produced silver nanoparticles either. The potency and effect of silver nanoparticles was proven yet the toxicity was to be considered for establishing it as a therapeutic agent.

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