



# FUTURE JOURNAL OF PHARMACEUTICALS AND HEALTH SCIENCES

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## Synthesis and Characterization of Silver Nano-Particles Prepared from *Pimenta dioica* Seed Extracts

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### Article History:

Received on: 20 Aug 2022

Revised on: 08 Sep 2022

Accepted on: 09 Sep 2022

### Keywords:

Silver,  
Nanoparticles,  
*Pimenta dioica*,  
Biogenetically

### ABSTRACT

Silver Nanoparticles were prepared biogenetically using aqueous extract of seeds of *Pimenta dioica*. Different volumes of extract were added to prepare formulations named AgNP-1, AgNP-2 and AgNP-3. The nanoparticles are confirmed to observe the color changes can observed at 420nm in UV Visible spectrophotometer. The so-formed silver nanoparticles have been evaluated for *in-vitro* antimicrobial activity against *S. aureus*, *P. vulgaris*, *B. subtilis*. The produced silver nanoparticles were found to be effective against all three bacteria. Significant activity was shown with the formulation AgNP-3 and AgNP-2 compared to standards. All the formulations exhibited good activity but comparatively higher against Bacillus and Putida. The toxicity of the silver particles against bacterial cells was hence proved. This infers that the increase in extract concentration results an increase in formation of silver nanoparticles.



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eISSN: 2583-116X

pISSN:

DOI: <https://doi.org/10.26452/fjphs.v2i4.320>



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### INTRODUCTION

The use of nanoparticles in medicine and agriculture has increased tremendously over recent years due to their unique size, shape, chemical composition, surface area, and reactivity [1]. NPs have been widely investigated as drug carriers and therapeutic agents, including anticancer drugs, antibiotics, anti-inflammatory agents, antimalarials, antivirals,

analgesics, antimicrobial agents, antioxidants, cardiovascular drugs and vaccines. NP synthesis methods can be classified into two categories based on their origin: natural and artificial [2]. Natural NP production includes biomineralization, biosilica formation, and peptide/protein self-assembly. These naturally occurring NPs are environmentally friendly and non-toxic, but lack control over particle size and shape. Artificial NP production includes bottom-up and top-down approaches. Bottom-up approaches take advantage of natural processes to synthesize NPs; these include physical vapor deposition, laser ablation, high-pressure liquid extraction, and electrochemical methods. Top-down approaches use mechanical milling or grinding to generate nanoscale materials [3]. A variety of NPs have already been produced using different types of mills, including ball milling, fluidized bed, vibratory ball milling, attritor milling, and planetary ball milling. However, none of these techniques can produce NPs precisely at the desired size and shape. Therefore, researchers need to combine multiple

steps to achieve the desired goal [4].

Antimicrobial agents have been used since ancient times to fight infection. However, overuse of antibiotics has seen bacteria develop resistance, making them less effective. Therefore, scientists have turned their attention to alternative methods of combating bacterial infections. Nano-sized materials such as gold and silver nanoparticles exhibit antimicrobial action against many pathogenic microorganisms. These nanoparticles interact with the surface of cells causing membrane damage leading to leakage of cytoplasmic contents [5]. Silver nanotechnology has become increasingly popular due to its broad spectrum antimicrobial activity. Silver nanoparticles (AgNPs) have shown promising results in treating various microbial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Candida albicans*, *Aspergillus niger*, and *Trichophyton mentagrophytes*. AgNPs are generally synthesized using green chemistry techniques, thus avoiding any toxic effects on human beings. In addition, they can easily be modified to target specific microbes. Due to their high biocompatibility and non-cytotoxic nature, these nanoparticles can be used in medical devices without any adverse effect on patients [6].

Silver nanoparticles (Ag NPs) have been widely investigated for their potential use in medicine, electronics, food packaging, and cosmetics. In addition, Ag NPs have shown excellent antibacterial activity against various pathogenic microbes including bacteria, viruses, fungi, and protozoa.

*Pimenta dioica* L., commonly known as Black pepper, is native to tropical regions of India and South-east Asia. *Pimenta dioica* is rich in essential oils and antioxidants that possess strong anti-inflammatory, anti-microbial, antinociceptive, antioxidant, analgesic, and wound healing activities [7]. Here we report the synthesis and characterization of Ag NPs using black pepper extract (BPE) as a reducing agent, followed by its assessment for biocidal efficacy against *S. aureus*, *P. vulgaris*, *B. subtilis*. BPE was characterized for total phenolic content, flavonoid content, and antioxidant activity. Characterization of synthesized Ag NPs was performed by UV-Visible spectroscopy [8].

## MATERIALS AND METHODS

### Collection of Plant Material

Seeds of *Pimenta dioica* were collected from S.V. University, Tirupathi and duly authenticated at

PARC, Chennai. The seeds were air dried; finely powdered and 50gm of the powder was macerated with water for 24hrs. The macerate was filtered under vacuum. The obtained filtrate was used for further experiments.

## Methodology

### Formulation of Silver Nano Particles

50ml of 1mm Silver nitrate was added to different concentrations of plant extract like 1ml, 5ml, 10ml separately and make up a final solution of 200ml and centrifuged at 18000 rpm for 25 min. The supernatant was heated at 50°C to 95°C. A change in the colour of the solution was observed during the heating process [9]. The resultant solutions were named as AgNP 1, AgNP 2, AgNP 3 respectively.

## Evaluation

### UV-Visible Spectral Analysis

The reduction of pure silver ions was monitored by measuring the UV-Visible spectroscopy of the reaction medium at 5hrs after diluting a small aliquot of the sample into distilled water [10]. UV-Visible spectroscopy was done by using UV-Vis Spectrophotometer UV 2450 (Shimadzu).

### Antibacterial Activity

The Anti-microbial efficacy of different formulations of *Pimenta dioica* Silver Nanoparticles and were performed on various microorganisms by using Dip well method as per standard procedure. Three sterile Petri plates were taken for testing the antimicrobial activity against three different microorganisms' i.e *Putida vulgaris*, *Staphylococcus aureus*, and *Bacillus subtilis* [11]. Nutrient broth solution was prepared, microorganisms were inoculated and kept in incubator at 37°C for 24 hours for the growth of microorganisms. To the Solidified Nutrient Agar in the petriplates, microorganisms were inoculated from nutrient broth. Four cavities were made in the nutrient agar media and filled with different concentrations of *Pimenta dioica* Silver Nanoparticles in three different cavities along with standard clindamycin in fourth cavity. It was taken care that the samples should be placed at the level of cavity and incubated at 37°C to test the activity. Observe the petriplates for anti-microbial activity of microorganisms after 24 hrs. The extent of zone of inhibition shows the antimicrobial activity [12].

## RESULTS AND DISCUSSION

### Formation of Silver Nano Particles

The formation of silver nanoparticles was evidenced with the change in colour of the *Pimenta dioica*

extracts containing Silver nitrate after heating from the pale yellow to brown colour [Figure 1].



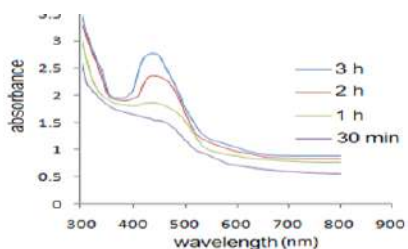
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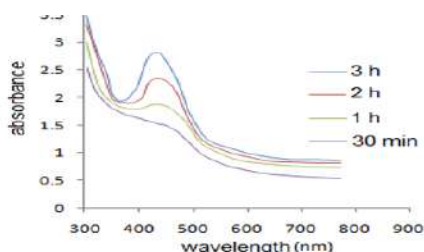
**Figure 1: Formation of Silver Nanoparticles**

### UV-Visible Spectral Analysis

The formation of silver nanoparticles was monitored through UV-Vis spectrophotometer at time intervals of 30 min, 1hr, 2hrs, 3hrs respectively. The spectrum obtained at 3hr shows the absorption maximum at 420nm. The intensity of the peak with respect to the height increases gradually either increase of time. The UV-Visible spectral analysis shows the formation of silver nanoparticles. Hence the peak maxima at 420nm are characteristic of silver nanoparticles [Figure 2, Figure 3 & Figure 4].



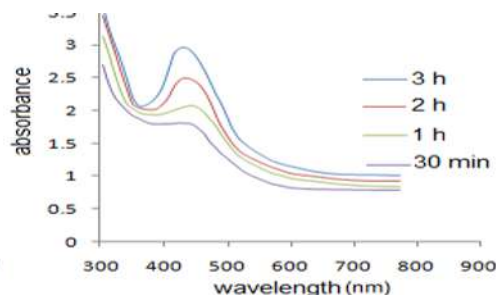
**Figure 2: UV Spectrum of Silver Nanoparticles AgNP 1**



**Figure 3: UV Spectrum of Silver Nanoparticles AgNP 2**

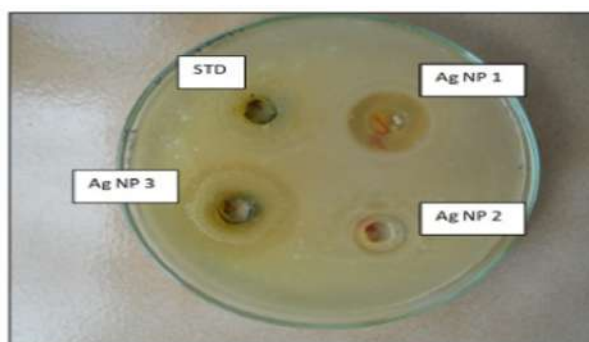
### Anti-Microbial Activity

Silver inhibits a variety of pathogenic microorganisms in very low concentration and it is less toxic to animal cells. The antibacterial activity for silver nanoparticles was done with various organisms

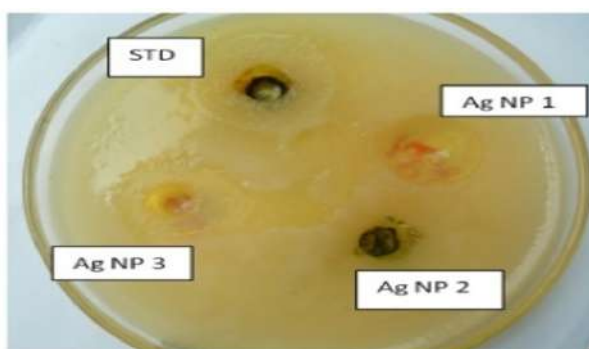


**Figure 4: UV Spectrum of Silver Nanoparticles AgNP 3**

like *Putida vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus* [Figure 7]. *Pimenta dioica* silver nanoparticles at 10ml concentration shown highest zone of inhibition compared to 5ml concentration and 1ml concentration [Figure 5, Figure 6 & Figure 7] [Table 1].



**Figure 5: Antimicrobial Activity of Silver Nanoparticles Against *Staphylococcus aureus***



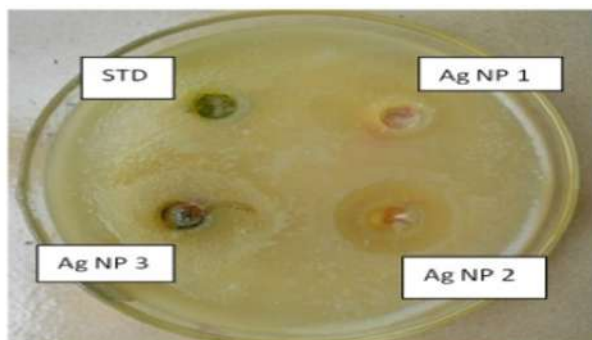
**Figure 6: Antimicrobial Activity of Silver Nanoparticles Against *Putida vulgaris***

The attempt to produce silver nanoparticles is successful from the above results. Nanoparticles produced were determined by observing the colour change as a preliminary test. Then the UV results support the same. Any metal, in nano size exhibit a principle called Surface Plasmon resonance when exposed to UV light. The silver nanoparticles were determined by observing the peak exhibited at 420 nm. The peak intensity grew through the time of

**Table 1: Antimicrobial Activity of Silver Nanoparticles**

<i>Putida vulgaris</i>		<i>Bacillus subtilis</i>		<i>Staphylococcus aureus</i>	
Group	Zone of Inhibition	Group	Zone of Inhibition	Group	Zone of Inhibition
STD	4.5±0.54mm	STD	4.9±0.34mm	STD	2.1±0.12mm
AgNP-1	2.6±0.22mm	AgNP-1	1.7±0.3mm	AgNP-1	1.9±0.8mm
AgNP-2	4.9±0.12mm*	AgNP-2	5.2±0.26mm*	AgNP-2	3.6±0.32mm**
AgNP-3	5.9±0.28mm**	AgNP-3	6.4±0.17mm**	AgNP-3	5.4±0.18mm**

Results were Represented as M±SEM (Mean and Standard Error of Mean); n=3; \*P<0.01 Significant Compared to std; \*\*More Significant



**Figure 7: Antimicrobial Activity of Silver Nanoparticles Against *Bacillus subtilis***

heating. The highest peak was observed after heating to 3hrs at a concentration of 10 ml of extract in the formulation AgNP 3. This implies that the extract concentration influences the formation of nanoparticles. Higher the extract higher the formation of silver nanoparticles.

The produced silver nanoparticles were found to be effective against all three bacteria. Significant activity was shown with the formulation AgNP-3 and AgNP-2 compared to standard. All the formulations exhibited good activity but comparatively higher against *Bacillus* and *Putida*. The toxicity of the silver particles against bacterial cells was hence proved.

**CONCLUSION**

Biogenetical production of silver nanoparticles is being considered to a greater extent due to their antibacterial potency besides their safety. They are relatively safe and effective compared to available antibacterial drugs and chemically produced silver nanoparticles either. Silver Nanoparticles were synthesized using aqueous extract of seeds of *Pimenta dioica*. The biogenetically produced silver nanoparticles are proved to be effective against all the three bacteria. Characterization of silver nanoparticles to its details like particle size, morphology, distribution, elemental analysis, quantitative estimation

would be studied further studied.

**ACKNOWLEDGEMENT**

We would like to thank our Principal sir Dr. P. Shanmugapandiyar for School of Pharmacy, Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai-600119, Tamil Nadu, India.

**Funding Support**

The authors declare that they have no funding support for this study.

**Conflict of Interest**

The authors declare that there is no conflict of interest for this study.

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**Cite this article:** Voleti Vijay Kumar, Govinda Rao Y, Niranjana Kumar R. **Synthesis and Characterization of Silver Nanoparticles Prepared from *Pimenta dioica* Seed Extracts.** *Future J. Pharm. Health. Sci.* 2022; 2(4): 288-292.

