Antibacterial activity of nanoparticles from *Allium* sp

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ABSTRACT

The plant material such as garlic and onion used in this study was collected from the nearby grocery, Thingal Nager, Kanyakumari District. The pathogens used in this study such as E.coli, Proteus sp., Klebsiella sp., Staphylococcus sp., Enterobacter sp., Bacillus sp. and Pseudomonas sp. were collected from Vivek Laboratory, Nagercoil. The plant material was ground in blender and extracted with distilled water. The plant extract were added with 3-10mm AgNO₃ and incubated for the synthesis of plant silver nano particles and it was confirmed by UV-visible spectroscopy. After that the antimicrobial activity was determined by disc diffusion method. Antibacterial disc were prepared in 5 different concentrations such as 100µl, 200µl, 300µl, 400µl, 500µl respectively. The antimicrobial activity of particles from garlic (16.65±0.10) showed greater activity than particles from onion (12.93±0.15). This study showed that the extract reveals the concentration dependent activity. The study revealed that the silver nano particles from plant extracts could be as a therapeutic agent for human microbial infections.

Key words: silver nano particles, pathogens, garlic, onion, antimicrobial activity.

INTRODUCTION

A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100 nm. Nanoparticle investigation is currently an area of passionate scientific research due to a wide variety of potential applications in biomedical, optical and electronic fields. Nanoparticles are of immense scientific interest as they are effectively bridge between bulk materials and atomic or molecular structures. To date metallic nanoparticles are mostly prepared from Nobel metals (ie., Ag, Pt, Au and Pd) [1]. The use of metallic nanoparticles in the field of catalysis, optoelectronics, pinpointing biological troubles and exhibit devices uncovered many significant findings.
Among the Nobel metals, silver (Ag) is the metal of preference in the field of biological systems, living organisms and medicine [2]. There are diverse methods for nanoparticles formation. In which biological methods are considered as safe and economically sound for the nano material fabrication as an alternative to conventional physical and chemical methods.

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process [3 & 4]. The most important application of silver and silver nanoparticles in medical industry is topical ointments to prevent infection against burn and open wounds [5]. The reduction of Ag+ ions by combinations of bio molecules found in these extracts such as vitamins, enzymes/proteins, organic acids such as citrates, amino acids, and polysaccharides [6]. The capability of some microorganisms such as bacteria [7] and fungi [8] to direct the synthesis of metallic nanoparticles should be employed in the hunt for new materials. Currently, the investigation of this fact has regained importance due to increase the bacterial resistance to antibiotics, caused by their overuse. Recently, silver nanoparticles exhibiting antimicrobial activity have been synthesized.

The metabolic activity of microorganisms can lead to precipitation of nanoparticles in external environment of a cell. The extracellular synthesis of silver and gold nanoparticles by the fungus Colletotrichum sp., Aspergillus sp. and Penicillium sp [9] has been reported. A two step mechanism for synthesis of silver nanoparticles using Verticellum was suggested by some researchers. The first step involves trapping of Ag+ ions at the surface of the fungal cells. In the second step, enzymes present in the cell reduce silver ions [8].

There are several reports on the synthesis and use of Helianthus annus, Basella alba, Oryza sativa, Saccharum officinarum, Sorghum bicolour, Zea mays, Azadirachta indica (neem) [10], Medicago sativa (alfa alfa) [11], Aloe vera, Diopyros kaki [12], Mangnolia kobus, Coriandrum sp [13] Carica papaya [14]; leaf extract of weed (Parthenium sp) and Ipomoea aquatic, Enhydra fluctuans Ludwigia adscendens (aquatic weed) [15] nanoparticles in pharmaceutical and biological applications were made.

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include: Diffusion (Kirby-Bauer and Stokes) and Dilution (E-Test method). The Kirby-Bauer and Stokes methods are usually used for antimicrobial susceptibility testing. Kirby-Bauer method was originally standardized by Bauer (the so called Kirby-Bauer method). This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values.

The Antibacterial testing of Allium cepa dried Scale leaves extract was evaluated by Agar well diffusion method using gram positive bacteria like Staphylococcus aureus, Bacillus subtilis, gram negative bacteria like Escherichia coli, Klebsiella pneumonia [16]. Jeya seelan et al., [17] study revealed the antibacterial activity of different solvent extracts of leaf, flower and fruit of Allium sativum against Pseudomonas solanacearum and Xanthomonas axonopodis pv. citri in vitro. By using this Allium sp we prepared the silver nanoparticles to enhance its antibacterial property.

The antibacterial characteristics of silver nanoparticles produced have been demonstrating by directly exposing bacteria to colloid silver particles solution. The present research highlights the
current knowledge regarding the potential organisms for biosynthesis of silver nanoparticles from Allium sp and its antibacterial activity.

MATERIALS AND METHODS

Collection of pathogens
The pathogens such as Proteus sp., Klebsiella sp., Staphylococcus sp., Bacillus sp., Pseudomonas sp. and Enterobacter sp. used for the antimicrobial activity were collected from Vivek Laboratory, Nagercoil.

Preparation of plant extract
Distilled water was used to prepare the extracts. 100 grams of onion and garlic were ground separately and filtered by using Whatman No.1 filter paper and the tiny solid particles were removed by centrifugation at 5000 rpm for 20 minutes.

Synthesis of silver nanoparticles
Silver nitrate was used as a precursor for the synthesis of silver nanoparticles. Five ml of 1mm AgNO₃ (99.99%) aqueous solution was added with 100ml of clear plant extract in 250 ml conical flask at room temperature. The flask was put into shaker (150 rpm) at 30°C and reaction was carried out for a period of 72 hours.

UV-visible spectroscopy analysis
The colour change in reaction mixture (metal ion solution + Allium sp extract) was recorded through visual observation. The bioreduction of silver ions in aqueous solution was monitored by periodic sampling of aliquots (1 ml) and subsequently measuring UV-Vis spectra of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on UV-Vis spectrophotometer operated at a resolution of 1 nm.

Preparation of disc
The sterile discs approximately 5mm in diameter was placed on Mueller Hinton agar (MHA) plates treated with garlic and onion nanoparticles. The disc was then placed over the swabbed MHA plates and incubated at 37°C for over night to study the antimicrobial activity.

Antibacterial activity of plant based silver nanoparticles against pathogen
The antibacterial assays were done on human pathogenic Escherichia coli, Proteus sp., Klebsiella sp., Staphylococcus sp., Enterobacter sp., Bacillus sp. and Pseudomonas sp., by standard disc diffusion method. Fresh overnight cultures of inoculums (100 µl) of each culture were spread on to MHA plates. Sterile paper discs of 5mm diameter containing silver nanoparticles were placed in each plate.

RESULTS
The Erlenmeyer flasks with the onion and garlic supernatants were a pale yellow color before the addition of Ag+ ions and this changed to a brownish color on completion of the reaction with Ag+. This event clearly indicating that the reduction of the ions occurs extracellularly through reducing agents released in to the solution by garlic and onion was analyzed by the UV-Vis
spectra. A strong, but broad, surface Plasmon peak located at 399nm (2.0786) and 397nm (2.2080) respectively was observed for the silver nanoparticles, prepared using garlic and onion respectively.

The antimicrobial activity of nanoparticles from garlic was given in the table1. The particles showed higher activity against the pathogenic Pseudomonas sp. (16.65±0.10). The activity was limited against Klebsiella sp. and Enterobacter sp. (10.63±0.15). The higher activity was followed by Bacillus sp. (14.20±0.30), Staphylococcus sp. (12.93±0.15), E.coli (12.45±0.15) and Proteus sp. (11.68 ±0.15). From this study, we revealed that the nanoparticles from garlic showed good activity against both the gram positive and gram negative organisms. Also it showed activity against cocci cells and rod cells.

The antimicrobial activity of nanoparticles from onion was given in the table2. The particles showed higher activity against the pathogenic klebsiella sp. (12.93 ±0.15). The activity was limited against Proteus sp. (10.13 ±0.15). After that the Klebsiella sp., the garlic particles were active against Bacillus sp. (12.80±0.10), Enterobacter sp. (11.41±0.15), Pseudomonas sp. (11.13±0.12), E.coli (10.63±0.20) and Staphylococcus sp. (10.17±0.13). These onion particles also showed the activity against gram positive and gram negative organism. Both the Allium sp. was good antimicrobial agent.

### Table 1. Antimicrobial activity of silver nanoparticles from garlic against pathogen

<table>
<thead>
<tr>
<th>Conc. of Ag nano from garlic(µl/disc)</th>
<th>Zone of Inhibition</th>
<th>E.coli</th>
<th>Proteus sp</th>
<th>Klebsiella sp</th>
<th>Staphylococcus sp</th>
<th>Enterobacter sp</th>
<th>Bacillus sp</th>
<th>Pseudomonas sp</th>
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<tr>
<td>100</td>
<td></td>
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<td>8.41±0.15</td>
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<td>8.63±0.25</td>
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<td>10.31±0.10</td>
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<td>400</td>
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<td>11.23±0.20</td>
<td>10.28±0.20</td>
<td>10.50±0.10</td>
<td>12.80±0.10</td>
<td>10.27±0.21</td>
<td>10.26±0.11</td>
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<td>500</td>
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<td>12.45±0.15</td>
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<td>10.63±0.20</td>
<td>12.93±0.15</td>
<td>10.63±0.15</td>
<td>14.20±0.30</td>
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### Table 2. Antimicrobial activity of silver nanoparticles from onion against pathogen

<table>
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<th>Conc. of Ag nano from onion(µl/disc)</th>
<th>Zone of Inhibition</th>
<th>E.coli</th>
<th>Proteus sp</th>
<th>Klebsiella sp</th>
<th>Staphylococcus sp</th>
<th>Enterobacter sp</th>
<th>Bacillus sp</th>
<th>Pseudomonas sp</th>
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<td>8.20±0.10</td>
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<td>8.41±0.19</td>
<td>10.13±0.26</td>
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<tr>
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### CONCLUSION

Unfortunately, there is a lack of interest of pharmaceutical companies in investing and developing Allium sp into a drug and performing clinical trials. This work determines the new way of preparing clove active extract with chemical approach ie., by applying nanotechnology. The antimicrobial activity of silver nanoparticles showed the concentration dependent activity. It gives activity against all the test organisms. Since this is an easily available throughout the nation and also is used in every house for cooking as a flavouring agent, the active nanocompound from this can be prepared and used effectively for preventing the growth of the microbial pathogens.
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