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Antimicrobial activity of Silver nanoparticles from *Capsicum* sp. against *Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp. and *Escherichia coli*

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Abstract
The Pepper extract acts as a reducing and capping agent in the formation of silver nanoparticles. A UV–Vis spectrum of the aqueous medium containing silver nanoparticles demonstrated a peak at 480 nm corresponding to the Plasmon absorbance of rapidly synthesized silver nanoparticles that was characterize by UV–Visible spectrophotometer.The Antibacterial testing of Pepper extract was evaluate by disk diffusion method using bacteria like *Staphylococcus aureus, Bacillus* sp., *Escherichia coli,* and *Pseudomonas* sp. Study revealed the antibacterial activity of solvent extracts by using pepper; we prepared the silver nanoparticles to enhance its antibacterial property.Antibacterial disc were prepared in five different concentrations such as 100µl, 200µl, 300µl, 400µl and 500µl respectively. The antimicrobial activity of particles showed higher activity in concentration 500µl against the pathogenic *Pseudomonas* sp. (15) mm of inhibition zone. The higher activity was followed by *Bacillus* sp. (13) mm of inhibition zone, *Staphylococcus* sp. (11) mm of inhibition zone and *E. coli* (10) mm of inhibition zone. This study showed that the extract reveals the concentration dependent activity. The study revealed that the silver nanoparticles from plant extracts could be as a therapeutic agent for human microbial infections. The zone of inhibition seems in both gram positive and gram negative bacterial strains. This study reports that synthesis is useful to avoid toxic chemicals with adverse effects in medical applications rather than physical and chemical methods.Thus, the aim of the study to synthesize silver based bionanoparticles. The present investigations revealed with the isolation and monitoring of silver nanoparticles to produce novel drugs to overcome drug resistance and advance reaction.
Keywords: Nanoparticles, Silver, Antimicrobial activity, Nano biosynthesis, standard disc diffusion method.

1. Introduction

A nanoparticle 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 [2].

The use of metallic nanoparticles in the field of catalysis, optoelectronics, pinpointing biological troubles and exhibit devices uncovered many significant findings [14].

Among the Nobel metals, silver (Ag) is the metal of preference in the field of biological systems, living organisms and medicine [12].

There are diverse methods for nanoparticles formation. In which biological methods are considered as safe and economically sound for the nanomaterial 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 [8, 9], the most important application of silver and silver nanoparticles in medical industry is topical ointments to prevent infection against burn and open wounds [7]. 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[16]. The capability of some microorganisms such as bacteria [15] and fungi [3] 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 had synthesized [17].

Pepper is a medicinal plant, it is described as a drug which increases digestive power, improves appetite, cures cold, cough, dyspnea, diseases of the throat, intermittent fever, colic, dysentery, worms and piles [10]

Numerous organisms have been found to synthesize nanoparticles. Biological production systems are of special interest due to their effectiveness and flexibility. Microbial cells are highly organized units, regarding morphology and metabolic pathways, capable of synthesizing reproducible particles with well-defined size and structure. Furthermore, biogenic nanoparticles often exhibit water soluble and biocompatible properties, which are essential for many applications. Presently metal accumulating bacteria have shown potential for material

science. Biomimetic is the area of research dealing with material science and engineering through biology. Bacteria are involved as workers in the living factory and plethora of novel nanostructured particles with unexpected properties are produced in the living factory which have applications in biomedical sciences, optics, magnetics, mechanics, catalysis and energy science. These biological materials can be used in their native form directly extracted from the living systems, or they can be processed after extraction and modified to their desired form [6]. Silver nanoparticle act as an antimicrobial and antibiotic agent when incorporated in proteins, nanofibre, first aid bandages plastics, soap and textiles, in cell cleaning fabrics and as a conductive filler. There is also an effort to incorporate silver nanoparticles into a wide range of medical devices, including but not limited to bone cement, surgical instruments, surgical masks, Wound dressings [11].

Currently most of the applications of silver nanoparticles are in antibacterial/antifungal agents in biotechnology and bioengineering, textile engineering, water treatment, and silver-based consumer products.

There is also an effort to incorporate silver nanoparticles into a wide range of medical devices, including but not limited to bone cement, surgical instruments, surgical masks, Wound dressings.

2. Materials and methods

2. 1. Collection of pathogens

The pathogens such as *Staphylococcus* sp., *Bacillus* sp., *Escherichia coli* and *Pseudomonas* sp. used for the antimicrobial activity were collected from Almustansiriya University Biology Department, Microbiology laboratories. Baghdad, Iraq.

2. .2. Preparation of plant extract

Distilled water was used to prepare the extracts. 25 grams of pepper powder with 100 ml of distilled water then boiled the mixture for 2 min. And filtered by Whatman cellulose filters with 2.5 µm pores, the tiny solid particles were removed by centrifugation at 5000 rpm for 20 minutes. And stored at 4 ^{o}C and used for further experiments

2. 3. Synthesis of silver nanoparticles

Silver nitrate was used as a precursor for the synthesis of silver nanoparticles. 10 ml of 1 mM $AgNO_3$ aqueous solution was added with 100 ml of clear plant extract in 250 ml conical flask at room temperature. The bioreduced aqueous component (1 ml) was used to measure UV–Vis spectra of the solution. The particle suspension was diluted to 1:10, with distilled water, to avoid the errors due to the high optical density of the solution.

2. 4. UV-visible spectroscopy analysis

The bioreduction of pure Ag+ ions was monitored by a periodic sampling of the aliquots (1 ml) of the suspension and subsequently measuring UV–Vis spectra of the resulting diluents. UV–Vis spectroscopic analyses of the silver nanoparticles produced were carried out as a function of bio reduction at room temperature on UV–Vis spectrometer.

2. 5. Preparation of disc

The sterile discs 5mm in diameter was placed on Mueller Hinton agar (MHA) plates treated with pepper nanoparticles. The disc was then placed over the swabbed MHA plates and incubated at 37^{o}C for overnight to study the antimicrobial activity.

2. 6. Antibacterial activity of plant based silver nanoparticles against pathogen

The antibacterial assays were done on human pathogenic *Staphylococcus* sp., *Bacillus* sp., *Escherichia coli* and *Pseudomonas* sp. by standard disc diffusion method. Fresh overnight cultures of inoculums (100 µl) of each culture (100, 200, 300, 400 and 500) were spread on to MHA plates. Sterile paper discs of 5mm diameter containing silver nanoparticles were placed in each plate.

3. Result and Discussion

3. 1. Determine the silver nanoparticles

Reduction of silver ions was visually evident from the color change and was completed within 15 min with a stable brown color indicating the formation of the silver nanoparticles in aqueous solution. The progress of the reaction between metal ions and the pepper extracts were monitored by UV–Vis spectra of Ag nanoparticles in aqueous solution as shown in figure (1) The UV–Vis absorption spectra of silver nanoparticles by exposure of pepper with 0.001 M silver nitrate showed the appearance of single and strong absorption peaks centered at 480 nm respectively where the absorbance located 0.1222. This band called the surface Plasmon resonance. It is one of the useful way to analyzing metallic nanoparticles with their localized surface Plasmon resonance (LSPR) [4].

3. 2. Antimicrobial activity against pathogens

Silver nitrate has long been considered as a powerful and natural antibiotic and antibacterial agent. Silver nanoparticles exhibited antibacterial properties against bacterial pathogens with close attachment of the nanoparticles themselves with the microbial cells.

The antimicrobial activity of nanoparticles from pepper as shown in the table (1) with the concentration 500

µl/disc of Nano silver from pepper. The particles showed higher activity against the pathogenic *Pseudomonas* sp. (15 mm). The higher activity was followed by *Bacillus* sp. (13 mm), *Staphylococcus* sp. (11 mm) and *E. coli* (10 mm). From this study, we revealed that the nanoparticles from pepper showed good activity against both the gram positive and gram negative organisms. Also it showed activity against cocci cells and rod cells.

3. 3. Antimicrobial agent based on silver

Silver kills bacteria by strangling them in a warm and moist environment [1, 5]. Highly bioactive silver ions bind with proteins inside and outside bacterial cell membranes, thus inhibiting cell respiration and reproduction. Silver is 3- 4 times more active at pH 8 than at pH 6. Silver products are effective against bacteria but not as effective against other organisms like fungi, mold, and mildew; they can be used with polyester where many other products cannot [13].

3. 4. Nano silver

Nano silver is a powerful and natural antimicrobial agent that has been proven highly effective in fighting a whole range of microbes. Acting as a catalyst, it reportedly disables the enzyme that one-celled bacteria, viruses, and fungi need for their oxygen intake without causing corresponding harm to human enzymes or other parts of the human body chemistry. The result is the destruction of disease-causing organisms without any detrimental effects on the surrounding human tissue [18].

3. 5. Antimicrobial agents act in various ways. The main modes of action are: [18]

- Protein coagulation.
- Disruption of cell membranes resulting in exposure, damage, or loss of the contents.
- Removal of free sulfhydryl groups essential for the functioning of enzymes.
- Substrate competition. A compound resembling the essential substrate of the enzyme diverts or misleads the enzymes necessary for the metabolism of the cell and causes cell death.

3. 6. Antimicrobial mechanism of Nano silver

Silver nanoparticles making a break through the permeability of outer membrane firstly, resulting in the leakage of cellular materials. **Secondly**, silver nanoparticles enter the inner membrane and inactivate respiratory chain dehydrogenases, thus inhibiting respiration and growth of cells.

Simultaneously, silver nanoparticles could affect some proteins and phosphate lipids and induce collapse of membrane, resulting in cell decomposition and death eventually. Taking into account the mobility of silver nanoparticles into cells and their fate in a bioprocess or even in the environment, the risk aspects for the application in larger scales and in the environment should be strengthened in future study [19].

3. 7. Nano silver versus other antibiotics

3. 7. 1. Effective but harmless

- Silver attacks bacteria by either denaturation or oxidization. For these reasons, bacteria cannot build resistance against silver.
- As human cells are a tissue type, they are unaffected by these actions [18].

3. 7. 2. Permanent solution

Unlike most antibiotics, which consumed while destroying bacteria, silver remains unconsumed while constantly working as a catalyst [18].

4. Conclusion

The study included the synthesis of silver nanoparticles from plant extrats. Presences of nanoparticles were confirmed by color change of media from pale yellow to brown color.

Unfortunately, there is a lack of interest of pharmaceutical companies in investing and developing Capsicum sp. into a drug and performing clinical trials. This work determines the new way of preparing clove active extract with chemical approach i.e., 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 flavoring agent, the active Nano compound from this can be prepared and used effectively for preventing the growth of the microbial pathogens. Hence, it has wide application in medical field.

References

[1] Achwal, W., B. (2003), “Antimicrobial finishes and their modifications”, *Colourage* 50, 58-59.

[2] Arangasamy, L., Munusamy, V. (2008), “Tapping the unexploited plant resources for the synthesis of silver nanoparticles”, *African.J.of Biotechnol* 7, 3162-3105.

[3] Bhainsa, K., C., D’Souza, S., F. (2006) “Extracellular Biosynthesis of Silver Nanoparticles Using the Fungus Aspergillus Fumigatus,” *Colloids Surfaces B: Biointerfaces* 2 **(47)**, 160-164.

[4] Dean, P., B., Thomas, L., K., Eric, C., John, D., (2006), “Two-Layer Nanosphere Lithography”, *OPT 463, Fall Semester*.

[5] Gulrajani, M., L. 2004, "Cold plasma an environment friendly textile processing medium", *Asian Dyer*, 39-41.
[6] Hulteen, J., C., Treichel, D., A., Smith, M., T., Duval, M., L., Jensen, T., R., Van Duyne, R., P. (B1999), "Nanosphere Lithography: Size-Tunable Silver Nanoparticle and Surface Cluster Arrays", *J. Phys. Chem.* 103, 3854-3863.
[7] Ip, M., Lui, S., L., Poon, V., K., Lung, M., I., Burd A. (2006) "Antimicrobial activities of silver dressings: an in vitro comparison", *J. Medical Microbiol* 55, 59- 63.
[8] Jose, R., M., Jose, L., E., Alejandra, C., Katherine, H., Juan, B., K., Jose, T., R., Miguel, J., Y. (2005), "The bactericidal effect of silver nanoparticles", *Nanotechnology* 16, 2346- 2353.
[9] Lok, C., N., Ho, C., Chen, M., R., He, Q., Y., Yu, W., Y., Sun, H. Tam, P., K., H., Chiu, J., F., Che, C., M. (2007), "Silver nanoparticles: partial oxidation and antibacterial activities" *J. Biol. Inorg. Chem.* 12, 527-534.
[10] Mallikarjuna, K., Sushma N. J., Narasimha, G., Manoj, L., Raju, B. D. P. (2012), "Phytochemical fabrication and characterization of silver nanoparticles by using Pepper leaf broth", *Arabian Journal of Chemistry*, Article in press.
[11] Nithya, G., N., Hema, S., Balaji, S. (2011), "Biosynthesis of silver nanoparticle and its antibacterial activity", Scholars Research Library 3 **(2)**, 377-380.
[12] Parashar, V., Prashar, R., Sharma, B., Pandey, A., C. (2009), "Parthenium leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization" *Digest journal of nanomaterials and biostructures* 4 **(1)**, 45-50.
[13] Qin Y. (2004), "Novel antimicrobial fibres", *Textile Magazine* 2 **(31)**, 14-17.
[14] Roy, N., Barik, A. (2010), "Green Synthesis of Silver Nanoparticles from the Unexploited Weed Resources", *International Journal of Nanotechnology and Applications* 4 **(2)**, 95- 101.
[15] Saifuddin, N., Wong C., W., Nur yasumira, A, A. (2009) "Rapid Biosynthesis of Silver Nanoparticles Using Culture Supernatant of Bacteria with Microwave Irradiation", *E-Journal of Chemistry* 6 **(1)**, 61-70.
[16] Vedpriya, A. (2010), "Living systems: Eco-Friendly Nanofactories", *Digest Journal of Nanomaterials and Biostructures* 5, 9-21.
[17] Veera, B., N., Rama, K., Manisha, R., D., Jahnavi A., Karunakar, R., K., Pratap Rudra, M., P. (2012), "Green Synthesis and characterization of Silver nanoparticles from Cajanus cajan leaf extract and its antibacterial activity", *International Journal of Nanomaterials and Biostructures* 2 **(3)**, 39- 43.
[18] Wasif, A., I., Laga, S., K. (2009) "Use of nano silver as antimicrobial agent for cotton", *AUTEX Research Journal* 9 **(1)**, 5-13.
[19] Wen-Ru, L., Xiao-Bao, X., Qing-Shan, S., Hai-Yan, Z., & You-Sheng O-Y., Yi-Ben, C. (2010) "Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli", *Applied Microbiology and Biotechnology* 85, 1115–1122.

Table (1) shown the inhibition zones for each bacteria with a different concentration.

Concentration of silver nanoparticlew from pepper (µl/disc)	**Zone of Inhibition (mm)**			
	E. coli	*Staphylococcus* sp.	*Bacillus* sp	*Pseudomonas* sp.
100	7	7.5	7	8
200	8	8	8.5	9
300	8.5	9.5	9	10
400	9	10	11	10.5
500	10	11	13	15

Figure (1) shown Plasmon peak of silver nanoparticles reduced by pepper.