Research Article

Characterization and Assessment of Antibacterial Potential of Garlic Based Silver Nanoparticles

Mehwish Saleem[1*] and Amina Atiq[2]

[1]Lahore College for Women University, Lahore, Pakistan; [2]Islamia College Cooper Road, Lahore, Pakistan.

Abstract | Garlic nanoparticles are inexpensive, harmless, easy to use and eco-friendly. In this study, silver nanoparticles based on inexpensive garlic clove extract were created, and their all-around antibacterial effectiveness was assessed. To create garlic clove extract-based silver nanoparticles, 100 ml of distilled water was combined with 0.01g, 0.025g, and 0.05g extracts of both aqueous and ethanol to create 0.01%, 0.025%, and 0.05% extract solutions, respectively. All of these extracts were then mixed with silver nitrate to create a 0.01M solution of silver nitrate nanoparticles. UV-VIS was done for the characterization of the nanoparticles. Various bacterial strains were successfully combatted by garlic nanoparticles' strong antibacterial properties *(E. coli, Klebsiella pneumonia, Bacillus subtilis* and *Staphylococcus aureus)*. Additionally, Staphylococcus aureus showed the greatest sensitivity to garlic nanoparticles, with inhibitory zones of 19 mm, 17 mm, and 18 mm for an aqueous extract for aqueous extract and for ethanolic garlic extract was (19mm, 17mm and 15mm) *K. pneumonia* was the least susceptible, with inhibitory zones for aqueous and ethanolic garlic extracts of 10 mm, 9 mm, and 11 mm, respectively (10mm, 8mm and 10mm). The findings of the study point to the potential for supplementing antibiotic therapy with garlic, which may improve antibiotic efficacy.

Received | September 25, 2022; **Accepted** | October 31, 2022; **Published** | November 26, 2022
***Correspondence** | Mehwish Saleem, Lahore College for Women University, Lahore, Pakistan; **Email:** rafia_1@yahoo.com
Citation | Saleem, M., and A. Atiq. 2022. Characterization and assessment of antibacterial potential of garlic based silver nanoparticles. *Journal of Innovative Sciences,* 8(2): 250-255.
DOI | https://dx.doi.org/10.17582/journal.jis/2022/8.2.250.255
Keywords | Nanotechnology, Garlic, Silver nanoparticle, Antibiotic resistance, Pathogens

1. Introduction

A well-known medicinal herb known for its therapeutic and insecticidal capabilities is garlic (*Allium sativum*) (Ankri *et al.*, 1997; Chen *et al.*, 2018). Additionally, it has a lot of phytochemicals that shield people from illnesses (Kaur *et al.*, 2019). Similarly, silver is well known for its bactericidal and inhibitive properties (Shankar *et al.*, 2004). Antimicrobial agent resistance in bacteria has become a major global issue, increasing mortality in most cases (Ashour *et al.*, 2015). As a result, it has become essential to identify novel antibiotic alternatives (Azam *et al.*, 2012).

The wide range of uses for nanometals can be attributed to their high surface/ volume ratio (Ashour *et al.*, 2015) which makes them superior to conventional antibiotics (Wang *et al.*, 2017). Nanoparticles have additional antioxidant and antibacterial capabilities (Pasupuleti *et al.*, 2008). Plant extracts and phytochemicals may function as both stabilizing and reducing agents during the formation of nanoparticles (Akhtar *et* al., 2013; Mittal *et al*., 2013). In recent decades, one of the most widely studied materials has been silver nanoparticles (Saravanan *et al*., 2018). Additionally, they have minimal costs, little cytotoxicity, and little immunological reaction (Samuel *et al*., 2020).

Garlic's properties include actions that are antibacterial, antioxidant, immunomodulatory, anti-cancer, anti-inflammatory, hypoglycemic, and cardiovascular (Arshad *et al*., 2020). Recently, drug delivery utilizing nanoparticles has advanced quickly (Farrag *et al*., 2019; Girish *et al*., 2019), and the potential biological uses of nanoparticles derived from garlic have become more apparent. Furthermore, a wide variety of bacterial pathogens are not as commonly used as silver-based nanoparticles (Gu *et al*., 2013). As a result, there has been a lot of interest in the biosynthesis of silver nanoparticles, which is particularly intriguing for plants and could be a significant alternative in the treatment and control of infection by human diseases (Wang *et al*., 2017). Due to their outstanding qualities that make them suitable for various applications, many types of garlic extracts and their separated bio actives are used as an ingredient in the biogenesis of nanoparticles (Hussein *et al*., 2017; Kim *et al*., 2007; Jakobsen *et al*., 2012).

2. Materials and Methods

The garlic was purchased at a local market in Lahore. Garlic aqueous extracts were made using the maceration method. Garlic ethanolic extract was also made by dissolving 10g of sample in 100 mL of ethanol. For 24 hours, the suspension was shaken on a flask shaker at 280 rpm (Abbas *et al*., 2022). 0.01g, 0.025g and 0.05g aqueous and ethanol extracts were mixed with 100 mL of distilled water to make 0.01%, 0.025%, and 0.05% extract solutions, respectively (Table 1, Figure 1). The extracts were then mixed with silver nitrate to make silver nitrate nanoparticles (Figure 2). Furthermore, streptomycin was used as a positive control as a standard antibiotic, and a simple silver nitrate solution was used as a negative control to compare the antimicrobial results (Tahir *et al*., 2020).

2.1 Characterization

The size of the silver nanoparticles conjugated with garlic was determined using the BT-90 nano laser particle size analyzer. The 4 cm cuvette was briefly filled with nanoparticle solution to determine particle size. Triplicate runs of freshly manufactured samples were performed, and the findings were considered as average measurements (Salem *et al*., 2018). The diagnostic peaks of garlic and garlic coupled with silver were examined using UV-17000. To determine the particle nature of the AgNPs, an XRD analysis (X-ray diffractometer) was executed. The chemical formation of prepared NPs in LUMS was evaluated using (Fourier transform infrared) analysis (Tahir *et al*., 2020).

Table 1: Groups of garlic silver nanoparticles for antibacterial activity.

S. No.	Groups
1	0.01M Silver Nitrate Solution
2	Antibiotic Streptomycin
3	0.01% Aqueous Extract + 0.01M Silver Nitrate
4	0.025% Aqueous Extract + 0.01M Silver Nitrate
5	0.05% Aqueous Extract + 0.01M Silver Nitrate
6	0.01% Ethanolic Extract + 0.01M Silver Nitrate
7	0.025% Ethanolic Extract + 0.01M Silver Nitrate
8	0.05% Ethanolic Extract + 0.01M Silver Nitrate

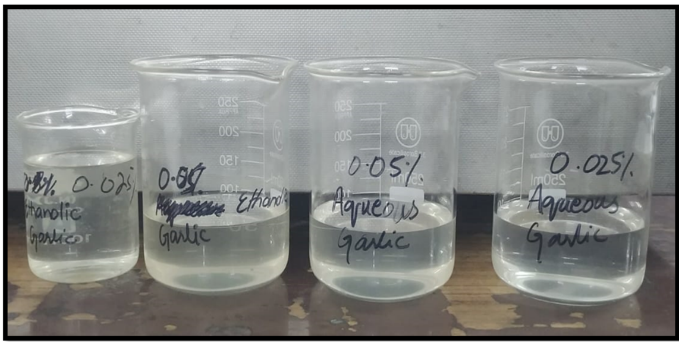

Figure 1: Before addition of silver nitrate.

Figure 2: After addition of silver nitrate.

2.2 Culture media preparation

To prepare a culture media for bacterial growth, 7.4g nutrient agar and 2.1g agarose gel were mixed in 250ml of distilled water in a flask that was placed on a hot plate for 10-15 minutes. For sterilization, the culture media was autoclaved for 1 hour. The sterilized culture media was poured into Petri dishes that were placed in laminar air flow to solidify. After solidification the Petri plates were incubated in incubator (A. Polok-Kowalska, S. Kowalski Typ; CLN 32 STD S/N; CN32SF 140474) at 37º C for 24 hours (Fahmy and Mamdouh, 2018).

2.3 Antimicrobial assay

The agar well diffusion method was used to assess the antibacterial activity of garlic extract attached NPs against *E. coli, S. aureus, B. subtilis,* and *K. pneumoniae* (He *et al.*, 2017). Petri plates containing solidified culture media were transferred to a laminar air flow chamber, and each Petri plate was divided into four wells. Then, to observe antibacterial activity, each well was labelled with a name. The antibiotic streptomycin was poured into the first well in a volume of 10:1 (as a positive control). The second well was filled with 60µl of silver solution (negative control). The remaining two wells were pored with 60µl of ethanolic and hot water extracts containing similar concentrations of silver-based nanoparticles. In three separate Petri plates, a 0.01 M solution of 0.01%, 0.025%, and 0.05% garlic aqueous extract and ethanolic extract based nanoparticle solution was poured (He *et al.*, 2017).

3. Results and Discussion

3.1 UV-visible spectrophotometer

UV-visible (UV-Vis) spectrophotometry is the most crucial and simple method for verifying the creation of nanoparticles. Using a Shimadzu (Kyoto, Japan) UV-Vis spectrophotometer model 1800 with a wavelength between 190 and 800 nm. The absorbance spectra of numerous substances were captured. Garlic AgNPs suspension is a deep dark brown color. As a result, it is possible to forecast particle size and stability using absorbance peaks. The absorbance maximum of smaller Ag NPs is around 400 nm, and it rises with particle size before disappearing once the particle size reaches the nanoscale (Figure 3). Our research revealed that green produced Ag NPs had their highest absorbance at 408 nm. Ag NPs made by the green method have a limited size distribution, with smaller particles predominating, as shown by a narrow absorption peak at 408 nm.

3.2 Antibacterial activity

The negative control's growth inhibition zone against bacterial strains (*E. coli, S. aureus, K. pneumoniae* and *B. subtilis*) was 5mm, 5mm, 12mm, and 9 mm, respectively. The growth inhibition zone of aqueous garlic extract against *E. coli* was 10mm, 10mm, and 13mm at 0.01%, 0.025%, and 0.05%, respectively. 9mm, 10mm and 12mm at 0.01%, 0.025%, and 0.05%. The growth inhibition zone of aqueous garlic extract against *K. pneumoniae* was 10mm, 9mm and 11mm at 0.01%, 0.025%, and 0.05%, respectively, and 10mm, 8mm, and 10mm at 0.01%, 0.025%, and 0.05% (Figure 5).

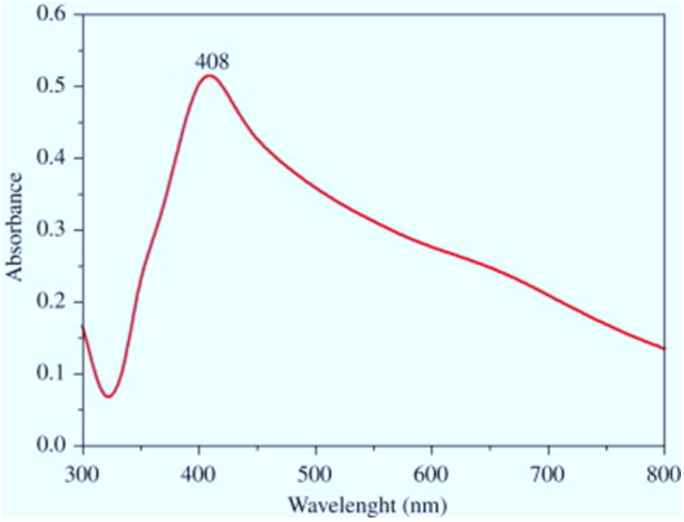

Figure 3: UV–vis spectrum of garlic coated Ag NPs

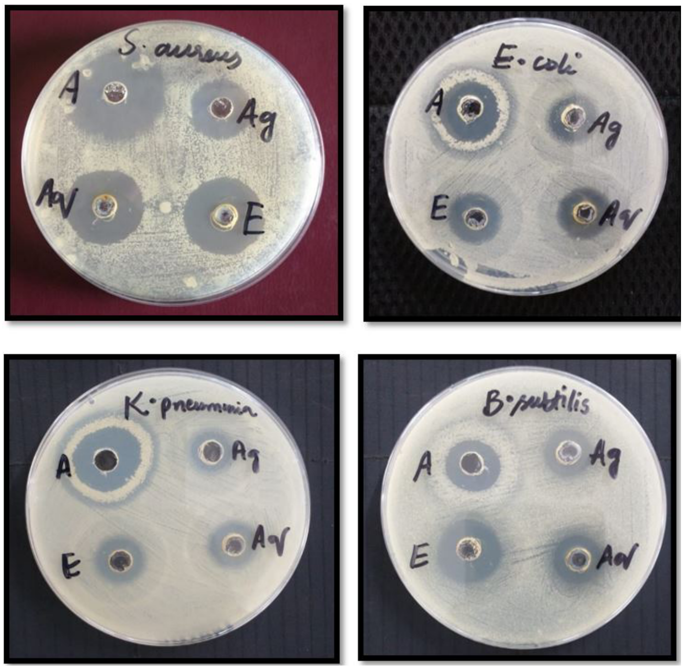

Figure 4: Growth of inhibition zone at 0.05% garlic conjugated silver nanoparticles.

The growth inhibition zone of aqueous garlic extract against *B. subtilis* was 9mm, 9mm, and 10mm at 0.01%, 0.025%, and 0.05%, respectively, and 11mm, 13mm, and 9mm at 0.01%, 0.025%, and 0.05%. When compared to other bacterial strains, G-AgNPs had a greater effect on *S. aureus.* The zone of inhibition of

Table 2: Zone of inhibition of experimental trial against for different strains of bacteria by using agar well diffusion method.

	E. coli	*K. pneumoniae*	*S. aureus*	*B. subtilis*
Positive control	14mm	11mm	16mm	12mm
Negative control	5mm	5mm	12mm	9mm
0.01% AGE + 0.01M $AgNO_3$	10mm	10mm	19mm	9mm
0.025% AGE+ 0.01M $AgNO_3$	10mm	9mm	17mm	9mm
0.05% AGE + 0.01M $AgNO_3$	13mm	11mm	18mm	10mm
0.01% EGE + 0.01M $AgNO_3$	9mm	10mm	19mm	11mm
0.025% EGE+ 0.01M $AgNO_3$	10mm	8mm	17mm	13mm
0.05% EGE+ 0.01M $AgNO_3$	12mm	10mm	15mm	9mm

aqueous garlic extracts against *S. aureus* was 19mm, 17mm, and 18mm at 0.01%, 0.025%, and 0.05%, respectively, and 19mm, 17mm, and 15mm at 0.01%, 0.025%, and 0.05%, respectively (Table 2, Figure 4).

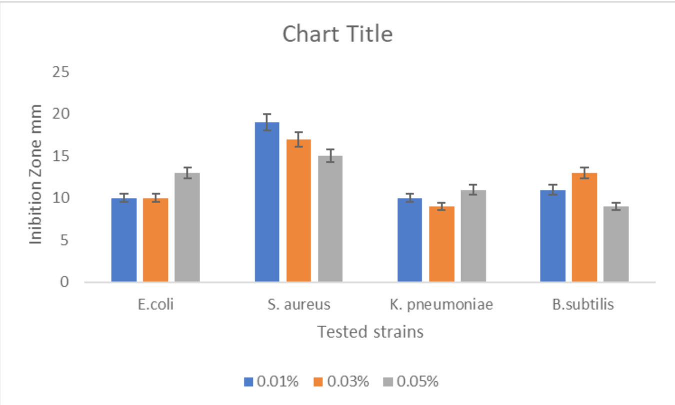

Figure 5: Zone of inhibition of different bacterial strains by using agar well diffusion method (0.01%, 0.025% and 0.05%).

The current study confirms that antibacterial property of ethanolic extract is far better that aqueous extract. Several studies have been published that support our findings that an ethanolic (30%) extract of fermented black garlic has higher antibacterial activity against 11 bacterial strains than an aqueous extract that causes oral diseases. This extract inhibited the growth of more than 90% of salivary bacteria in both short and long incubations (Vlachojannis *et al*., 2018). Water extract of garlic Toluene extract has been reported to reduce the mortality of Caenorhabditis elegans from *P. aeruginosa* infections and clean the lungs of *P. aeruginosa* infected mice by modulating inflammation (Bapat *et al*., 2018).

Furthermore, garlic extracts are said to be effective against both gram-positive and gram-negative bacteria, such as *B. subtilis, S. aureus, E. coli* and *K. pneumoniae* (Gabriel *et al*., 2022). According to our findings, green-produced Ag NPs had the highest absorbance at 408 nm. The green approach produces Ag NPs with a restricted size distribution, with smaller particles predominating, as evidenced by a narrow absorption peak at 408 nm. According to this paper, nano-particles have a brown color (Shafea *et al*., 2021). The absorbance spectrum of the silver nanoparticles prepared in the reaction mixture was obtained using UV-Vis analysis, with the highest peak occurring at about 433 nm (Vijayakumar *et al*., 2019).

Conclusions and Recommendations

According to considerable studies, organosulfur compounds from garlic have potent antibacterial properties against a variety of bacteria, including MDR strains. The effectiveness of garlic extracts against Gram-positive and Gram-negative bacteria such *E. coli, K. pneumoniae, B. subtilis* and *S. aureus* has also been reported. Garlic and its components have been studied extensively for their antibacterial properties, but more recent gaps in our knowledge must be filled before we can use them as antibacterial agents in clinical settings.

Novelty Statement

Addition of garlic based silver nanoparticles proved an alternative to replace antibiotics, hence proved an effective step to combat diseases.

Author's Contribution

Mehwish Saleem designed the study, wrote and edit the paper, paid publication fee. Amina Atiq performed the experimental work.

Conflict of interest

The authors have declared no conflict of interest.

References

Abbas, M., Hussain, T., Iqbal, J., Rehman, A., Zaman, M.A., Jilani, K., and Nazir, A., 2022. Synthesis of silver nanoparticle from *Allium sativum* as an eco-benign agent for biological applications. *Polish Journal of Environmental Studies,* 31: 533-538. https://doi.org/10.15244/pjoes/135764

Akhtar, M.S., Panwar, J., and Yun, Y.S., 2013. Biogenic synthesis of metallic nanoparticles by plant extracts. *ACS Sustainable Chemistry and Engineering*, 1(6): 591-602. https://doi.org/10.1021/sc300118u

Ankri, S., and Mirelman, D., 1999. Antimicrobial properties of allicin from garlic. *Microbes and Infection*, 1(2): 125-129. https://doi.org/10.1016/S1286-4579(99)80003-3

Ankri, S., Miron, T., Rabinkov, A., Wilchek, M., and Mirelman, D. 1997. Allicin from garlic strongly inhibits cysteine proteinases and cytopathic effects of Entamoeba histolytica. *Antimicrobial agents and chemotherapy*, 41(10): 2286-2288.

Arshad, M., Rahman, A., Qayyum, A., Hussain, K., Khan, M.A., Hussain, T., and Iqbal, M., 2020. Environmental applications and bio-profiling of Tribulus terrestris: An ecofriendly approach. *Polish Journal of Environmental Studies*, 29(4): 2981-2986. https://doi.org/10.15244/pjoes/111240

Ashour, A.A., Raafat, D., El-Gowelli, H.M., and El-Kamel, A.H., 2015. Green synthesis of silver nanoparticles using cranberry powder aqueous extract: Characterization and antimicrobial properties. *International Journal of Nanomedicine*, 10: 7207. https://doi.org/10.2147/IJN.S87268

Azam, A., Ahmed, A.S., Oves, M., Khan, M.S., Habib, S.S., and Memic, A., 2012. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: A comparative study. *International Journal of Nanomedicine*, 7: 6003. https://doi.org/10.2147/IJN.S35347

Bapat, R.A., Chaubal, T.V., Joshi, C.P., Bapat, P.R., Choudhury, H., Pandey, M., and Kesharwani, P., 2018. An overview of application of silver nanoparticles for biomaterials in dentistry. *Materials Science and Engineering*, 91: 881-898. https://doi.org/10.1016/j.msec.2018.05.069

Bouqellah, N.A., Mohamed, M.M., and Ibrahim, Y., 2019. Synthesis of eco-friendly silver nanoparticles using Allium sp. and their antimicrobial potential on selected vaginal bacteria. *Saudi Journal of Biological Sciences*, 26(7): 1789-1794. https://doi.org/10.1016/j.sjbs.2018.04.001

Chen, C., Liu, C.H., Cai, J., Zhang, W., Qi, W.L., Wang, Z., and Yang, Y., 2018. Broad-spectrum antimicrobial activity, chemical composition and mechanism of action of garlic (*Allium sativum*) extracts. *Food Control*, 86: 117-125. https://doi.org/10.1016/j.foodcont.2017.11.015

Fahmy, S.A., and Mamdouh, W., 2018. Garlic oil loaded PLGA nanoparticles with controllable size and shape and enhanced antibacterial activities. *Journal of Applied Polymer Science*, 135(16): 46133. https://doi.org/10.1002/app.46133

Farrag, H.A., Hosny, A.E.D.M., Hawas, A.M., Hagras, S.A., and Helmy, O.M., 2019. Potential efficacy of garlic lock therapy in combating biofilm and catheter-associated infections; experimental studies on an animal model with focus on toxicological aspects. *Saudi Pharmaceutical Journal*, 27(6): 830-840. https://doi.org/10.1016/j.jsps.2019.05.004

Gabriel, T., Vestine, A., Kim, K.D., Kwon, S.J., Sivanesan, I. and Chun, S.C. 2022. Antibacterial Activity of Nanoparticles of Garlic (Allium sativum) Extract against Different Bacteria Such as Streptococcus mutans and Poryphormonas gingivalis. *Applied Sciences*, 12(7): 3491.

Girish, V.M., Liang, H., Aguilan, J.T., Nosanchuk, J.D., Friedman, J.M., and Nacharaju, P., 2019. Anti-biofilm activity of garlic extracts loaded nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, 20: 102009. https://doi.org/10.1016/j.nano.2019.04.012

Gu, X., Wu, H., and Fu, P., 2013. Allicin attenuates inflammation and suppresses HLA-B27 protein expression in ankylosing spondylitis mice. *BioMed Research International*, 90(2): 439-446. https://doi.org/10.1155/2013/171573

He, H., Tao, G., Wang, Y., Cai, R., Guo, P., Chen, L., and Xia, Q. 2017. In situ green synthesis and characterization of sericin-silver nanoparticle composite with effective antibacterial activity and good biocompatibility. *Materials Science and Engineering*: C, 80: 509-516.

Hussein, H.J., Hameed, I.H., and Hadi, M.Y., 2017. A review: Anti-microbial, Anti-inflammatory effect and cardiovascular effects of garlic: *Allium sativum*. *Research Journal of Pharmacy* *and Technology*, 10(11): 4069-4078. https://doi.org/10.5958/0974-360X.2017.00738.7

Jakobsen, T.H., van Gennip, M., Phipps, R.K., Shanmugham, M.S., Christensen, L.D., Alhede, M., and Givskov, M., 2012. Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. *Antimicrobial Agents and Chemotherapy*, 56(5): 2314-2325. https://doi.org/10.1128/AAC.05919-11

Kaur, M., Mehta, A., Bhardwaj, K.K., and Gupta, R., 2019. Phytochemical analysis, antimicrobial and antioxidant activity assessment of orange peels. *Journal of Global Biosciences*, 8(3): 6062-6072.

Kim, J.S., Kuk, E., Yu, K.N., Kim, J.H., Park, S.J., Lee, H.J. and Cho, M.H., 2007. Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, 3(1): 95-101. https://doi.org/10.1016/j.nano.2006.12.001

Mittal, A.K., Chisti, Y., and Banerjee, U.C., 2013. Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, 31(2): 346-356. https://doi.org/10.1016/j.biotechadv.2013.01.003

Tahir, M.H., Saleem, F., Ali, S., Ain, Q.U., Fazal, A., Summer, M., and Murtaza, G., 2020. Synthesis of sericin-conjugated silver nanoparticles and their potential antimicrobial activity. *Journal of Basic Microbiology*, 60(5): 458-467. https://doi.org/10.1002/jobm.201900567

Oves, M., Aslam, M., Rauf, M.A., Qayyum, S., Qari, H.A., Khan, M.S. and Ismail, I.M., 2018. Antimicrobial and anticancer activities of silver nanoparticles synthesized from the root hair extract of Phoenix dactylifera. *Materials Science and Engineering C*, 89: 429-443. https://doi.org/10.1016/j.msec.2018.03.035

Pasupuleti, V.R., Prasad, T.N.V.K.V., Shiekh, R.A., Balam, S.K., Narasimhulu, G., Reddy, C.S. and Gan, S.H., 2013. Biogenic silver nanoparticles using Rhinacanthus nasutus leaf extract: Synthesis, spectral analysis, and antimicrobial studies. *International Journal of Nanomedicine*, 8: 3355. https://doi.org/10.2147/IJN.S49000

Salem, D.S., Sliem, M.A., El-Sesy, M., Shouman, S.A. and Badr, Y., 2018. Improved chemo-photothermal therapy of hepatocellular carcinoma using chitosan-coated gold nanoparticles. *Journal of Photochemistry and Photobiology B Biology*, 182: 92-99. https://doi.org/10.1016/j.jphotobiol.2018.03.024

Samuel, M.S., Jose, S., Selvarajan, E., Mathimani, T. and Pugazhendhi, A., 2020. Biosynthesized silver nanoparticles using Bacillus amyloliquefaciens; Application for cytotoxicity effect on A549 cell line and photocatalytic degradation of p-nitrophenol. *Journal of Photochemistry and Photobiology B Biology*, 202: 111642. https://doi.org/10.1016/j.jphotobiol.2019.111642

Saravanan, M., Barik, S.K., Mubarak A.D., Prakash, P. and Pugazhendhi, A., 2018. Synthesis of silver nanoparticles from *Bacillus brevis* (NCIM 2533) and their antibacterial activity against pathogenic bacteria. *Microbial Pathogenesis*, 116: 221-226. https://doi.org/10.1016/j.micpath.2018.01.038

Shafea, T.M.M., and Mahmoud, K.M., 2021. Green synthesis, characterization and antimicrobial activity of silver nanoparticles using locally growing *Allium sativum* extract. *Zanco Journal of Pure and Applied Sciences*, 33(1): 35-41. https://doi.org/10.21271/ZJPAS.33.1.5

Shankar, S.S., Rai, A., Ahmad, A., and Sastry, M., 2004. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *Journal of Colloid and Interface Science*, 275(2): 496-502. https://doi.org/10.1016/j.jcis.2004.03.003

Vijayakumar, S., Malaikozhundan, B., Saravanakumar, K., Durán-Lara, E.F., Wang, M.H., and Vaseeharan, B., 2019. Garlic clove extract assisted silver nanoparticle Antibacterial, antibiofilm, antihelminthic, anti-inflammatory, anticancer and ecotoxicity assessment. *Journal of Photochemistry and Photobiology B: Biology*, 198 (9): 111558. https://doi.org/10.1016/j.jphotobiol.2019.111558

Vlachojannis, C., Chrubasik-Hausmann, S., Hellwig, E., Vach, K., and Al-Ahmad, A., 2018. Activity of preparations from Spilanthes oleracea, propolis, Nigella sativa, and black garlic on different microorganisms involved in oral diseases and on total human salivary bacteria: A pilot study. *Pathology,* 32(9): 1992–2001. https://doi.org/10.1002/ptr.6129

Wang, L., Hu, C., and Shao, L., 2017. The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International Journal of Nanomedicine*, 12: 1227. https://doi.org/10.2147/IJN.S121956