Natural synthesis of silver nanoparticles from grape fruit juice and estimation of antimicrobial activity

Mehreen Fatima¹, Azra Quraishi¹, Maida Irfan²

1. University of Management and Technology, Lahore and University of the Punjab, Lahore, Pakistan
2. Faculty of Pharmacy, Punjab University College of Pharmacy, Lahore, Pakistan

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: https://doi.org/10.32350/BSR.0302.01.i

Abstract

Silver has been known for its antimicrobial activity for a very long time. Formulation of silver particles that range from 1-100nm in size makes it even more potent to induce antimicrobial effect. Green chemistry has started to become more frequent in the field of biochemical research. Silver nanoparticles synthesized from green synthesis method provide a cheap and environmental friendly method of nanoparticle preparation. The aim of the current study is green synthesis of silver nanoparticles using tomato juice as reducing and capping agent and evaluation of its antimicrobial activity. The stability and conformation of SNPs was determined by UV-visible spectroscopy. The antimicrobial activity of synthesized SNPs was determined against E.coli DH5α. Ultraviolet spectroscopic analysis offered peak at 400 nm that indicate the production of SNPs of adequate size. E.coli DH5α showed considerable decrease upon introduction of SNPs to the bacterial inoculum. Upon increasing the concentration of silver nanoparticles an increase in zone of inhibition was recorded. For 70µg/ml of SNPs, the zone of inhibition was 0.5 cm, while 0.6 cm, 0.7 cm and 0.7cm was recorded for 100µg/ml, 150µg/ml and 200µg/ml of SNPs respectively. The efficacy of antimicrobial activity of SNPs derived from tomato juice proves its potential use in pharmaceutical and medicinal industries for synthesis of nanomedicine.

Keywords: antimicrobial effect, green chemistry, grape fruit, nanomedicine, silver nanoparticles
1. Introduction

Nanotechnology has picked up quite a hype in the research society and has been used in multiple branches of research and technology [1]. Nano-biotechnology is an emerging field in biotechnology. The nanomedicine that is use of nanoparticles to form nanoformulations and nanocomposites for treatment of various diseases [2]. This field includes nanoscale doses of drugs that are targeted specifically to the site of action by using nanoparticles linked with site specific ligands [3].

Silver has been used of centuries for treatment of diseases and wounds. This is due to the antimicrobial nature of silver (Figure 1). Therefore, it is used in homeopathic and ayurvedic medicine to cure infections, infected wounds and sometimes it is used in ayurvedic medicine to treat blood diseases and poisoning [1].

Silver Nanoparticles (SNPs) are well known as antimicrobial and antibacterial materials. However, resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years [4]. This is a major challenge for the health care industry, and has been widely studied. Silver is characterized as a soft acid. There's a natural tendency of an acid to react with a base and during this case, a soft acid to react with a soft base [5]. The bacterial cells (E.coli) are majorly made from sulfur and phosphorus which are soft bases. The action of those nanoparticles on the cell can cause the synthesis of Reactive oxygen species to take place and subsequently cause apoptosis [6]. Another fact is that the DNA has sulfur and phosphorus as its major components. The nanoparticles can act on these soft bases and destroy the DNA which might definitely lead to cell death. [7]The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can cause problems within the DNA replication of the bacteria and thus terminate the microbes. It also affects the permeability and respiration of bacterial cells [8].
Nanoparticles release silver ions, which can have a further contribution to the bactericidal effect of SNPs. Silver nanoparticles can inhibit the activities of interferon gamma and tumor necrosis factor alpha which are involved in inflammation. Though these studies prove that silver nanoparticles are involved within the anti-inflammatory effects, the exact, precise mechanism of action remains to be determined. Grape fruit is an excellent candidate for green synthesis of silver nanoparticles as it has high amount of citric acid present in it. This citric acid reacts with silver nitrate to produce silver nanoparticles. [10]

Currently in this paper green method i.e. the production of silver nanoparticles from fruit extract, is being utilized to create a better and cheaper method for silver nanoparticle synthesis. The sour taste of grape fruit juice is due to having high amount of acids i.e. citric acid and ascorbic acid. The citric acid in turn reacts with silver nitrate and produces silver oxide nanoparticles that can be of fine size if proper spinning or sonication is provided to the reaction mixture. The silver nanoparticles are analyzed by spectrophotometric analysis and then antimicrobial activity of the synthesized nanoparticles is studied. E.coli strains were taken to study the antibacterial effect of the synthesized nanoparticles as it is a commonly found bacteria and it is necessary to evaluate the antibacterial activity on bacteria that are commonly present in environment before moving to resistant strains for study [11, 12].

Fig. 1: Schematic of the mechanism that is utilized by silver nanoparticles to cause antimicrobial effect. [9]
2. Methodology

2.1 Synthesis of silver nanoparticles from grape fruit extract

Preparation of extract

1kg of grape fruit was obtained from local market and cleaned with water. The fruits (1Kg) were squeezed into the beaker. 250ml of the juice was then filtered using muslin cloth and centrifuged at 1000 rpm for 25 minutes. After centrifugation pellet was discarded and supernatant was collected.

Biosynthesis of SNPs

Silver nitrate was mixed with grape fruit extract in 1:4 ratio. It was transferred to 50 ml falcon tube and shaking was carried out at 150 rpm for 2 hours. With the appearance of black precipitates mixture was centrifuged at 10,000 rpm for 20 minutes. Supernatant was discarded and nanoparticles were dispersed in water. The particles were re- centrifuged under the same conditions. After this supernatant was discarded and particles were dispersed in 10 ml of water, stored at 4°C.

2.2 UV- visible spectroscopy and stability confirmation of silver nanoparticles

Reduction of SNPs was monitored by removing aliquots of reaction mixture periodically and diluted 12 times with deionized water. The absorption spectra at wavelength at 300-700nm against deionized water were recorded. Remaining aliquots were saved at 4°C for further analysis.

2.3 Determining the antimicrobial activity of silver nanoparticles

Optical density evaluation

250 ml of LB broth was prepared and taken in a separate flask. It was kept at 37°C for overnight culture development before inoculating the broth with E.coli DH5α cells (100 µl). On next day 1ml of culture was taken and added in 30 ml media. Incubate it at 37°C at 160 rpm. After 2-3 hours optical density was measured to be 0.6 at 600nm. The culture was kept at ice to stop further division. The culture was diluted at 1×10^5 cells/ml. The grown culture was transferred in 100ml LB media. The media was divided into 5 separate flasks (20 ml per flask). 200µg silver particles were added in four flasks. The flasks were placed in a shaking incubator at 37°C at 160 rpm. OD of flask 1 was measured after every 1 hour using LB media to autozero the spectrophotometer. After every 1 hour OD of the next flask was measured.
Measurement of zone of inhibition

LB agar was poured on Petri plate and allowed to solidify. Holes/ wells were punctured in LB agar by end of blue tip.

After the above process DH5-α culture was spread on agar plate. Nanoparticles were added into the specified holes in concentration of SNPs, 70, 100, 150 and 200ug/ml and incubation was carried out for 22 hours at 37 degree Celsius.

3. Results

3.1 UV-visible spectroscopy and stability confirmation of silver nanoparticles

Figure 2: UV-spectra of AgNPs

Silver nanoparticles were subjected to spectrophotometric analysis and gave peak absorbance at 400 nm (Figure 2). The peak absorbance indicates that proper sized silver nanoparticles are formed.

3.2 Determining the antimicrobial activity of silver nanoparticles

Table 1: measurement of optical density in response to silver nanoparticle concentration 70µg/ml

| Time (hour) | OD of control | OD of test sample |
|------------|---------------|------------------|
|            |               |                  |

Accepted
The graph (Figure 3.1) indicates that bacterial growth showed considerable decrease upon introduction of silver nanoparticles to the bacterial inoculum thus supporting the antimicrobial activity of the silver nanoparticles.

Table 2: Measurement of Optical Density in Response to Silver Nanoparticle Concentration 100µg/Ml

| Time (hour) | OD of control | OD of test sample |
|------------|---------------|-------------------|
| 0          | 0.0           | 0.0               |
| 1          | 0.9           | 1.0               |
| 2          | 1.245         | 1.3               |
| 3          | 1.32          | 1.31              |
3.2. Graph of optical density of sample and control with use of 100µg SNPs

The graph indicates that bacterial growth showed considerable decrease upon introduction of silver nanoparticles to the bacterial inoculum thus supporting the antimicrobial activity of the silver nanoparticles. Upon increasing the concentration of silver nanoparticles a greater decrease in bacterial growth was observed.

Table 3: Measurement of Optical Density in Response to Silver Nanoparticle Concentration 150µg/

| Time (hour) | OD of control | OD of test sample |
|------------|---------------|-------------------|
| 0          | 0.252         | 0.252             |
| 1          | 0.445         | 0.258             |
| 2          | 0.534         | 0.152             |
| 3          | 0.591         | 0.062             |
| 4          | 0.598         | 0.061             |
3.3. Graph of optical density of sample and control with use of 150µg SNPs

The graph indicates that bacterial growth showed considerable decrease upon introduction of silver nanoparticles to the bacterial inoculum thus supporting the antimicrobial activity of the silver nanoparticles. Upon increasing the concentration of silver nanoparticles a greater decrease in bacterial growth was observed.

Table 4: Measurement of Optical Density in Response to Silver Nanoparticle Concentration 200µg/Ml

| Time (hour) | OD of control | OD of test sample |
|------------|---------------|-------------------|
| 0          | 0.38          | 0.38              |
| 1          | 1.1           | 0.33              |
| 2          | 2.1           | 0.30              |
| 3          | 2.6           | 2.8               |
| 4          | 3.8           | 0.26              |
3.4. Graph of optical density of sample and control with use of 200µg SNPs

At 0 hour, OD of the control and test sample remains the same but with the increase in time duration, OD of the test sample decreases with respect to OD of control. This is because control contains only the bacterial culture with no silver nanoparticles added and with the passage of time bacteria pass through exponential phase but test sample contains silver nanoparticles in addition to culture that inhibit the bacterial growth.

3.4. Measurement of zone of inhibition

![Image of zone of inhibition](image)

**Fig 4: Zone of Inhibition of Silver Nanoparticles (positive control)**

Increasing the concentration of silver nanoparticles in agar plate, the zone of inhibition increases in plate and bacterial growth decreases.

**Table 5: Measurement of Zone Of Inhibition in Response to Silver Nanoparticle Concentration.**

| No. | Concentration of SNPs (µg/ml) | Distance (cm) |
|-----|-------------------------------|---------------|
| 1   | 70                            | 0.4           |
| 2   | 100                           | 0.7           |
| 3   | 150                           | 0.8           |
For 70µg/ml, zone of inhibition was 0.4, while 0.7, 0.8 and 0.8cm for 100µg/ml, 150µg/ml and 200µg/ml respectively. It shows that by increasing the concentration of silver nanoparticles in agar plate culture, zone of inhibition increases by decreasing bacterial growth.

4. Discussion

Nanoparticles provide a promising forum for enhanced action of chemicals, medicine, drugs, compounds etc. to the site of action and provide a platform to specifically target the required site without hindering any other system in the organism in which the chemical effect is being analyzed [13].

Multifunctional nanomedicine allows for the targeted delivery and the molecular diagnosis of the cancer cells and is emerging as an extensively integrated platform for simultaneous treatment and monitoring of cancer without depending on multiple methods for diagnosis and treatment [14].

Silver nanoparticles provide a forum for creating nanomedicine to treat blood infections, infections that are located at specific sites where normal drugs cannot be delivered, treatment of brain infections etc. [15]. As this study has proved that green method provides a promising method for silver nanoparticle preparation and these silver nanoparticles are quite effective antimicrobial agents. Thus in future antibiotics can be formed using silver nanoparticle formulations.

Recently plant extract has been used for synthesis of silver nanoparticles in which the process of photonanosynthesis is used to obtained fine sized silver nanoparticles without the use of heavy machinery or chemicals [16].

Edible fruits have also been reported to be used for green synthesis of silver nanoparticles. In these fruits papaya, orange, kiwi and grape fruit have been reported to have given good titer of silver nanoparticles due to high concentration of citric acid in these fruits. Antimicrobial activity of these nanoparticles have been reported against *E.coli* and *pseudomonas* strains as well as some fungal strains [17].

In studies carried out for silver nanoparticles formulated by green synthesis it was observed that upon TEM studies the size of these nanoparticles varies from a range of 20-50nm. This is considered a good size range to obtain good nanoparticle efficiency [18].

Gold nanoparticles have also been reported to be synthesized along with silver nanoparticles using pomegranate peels. These peels are used for green synthesis of these nanoparticles and have provided with good titer. The gold nanoparticles along with silver nanoparticles are then used for nanomedicine synthesis mostly for cancer treatment [19].

Further the silver nanoparticles can be conjugated with other antimicrobial molecules and this with cause two fold antimicrobial effect and will increase the treatment efficiency [20]. Thus, silver
nanoparticles is still an unexplored branch of nanobiotechnology that can be further studies to produce better and more efficient antibacterial and antifungal medications [21].

Green synthesis is the future of nanomaterial production and will be applied on a large scale for nanomaterial production in the near future. This procedure provides good sized effective nanoparticle production with expenditure of minimal resources thus for nanomaterial production and nanomedicine formulation in medicine industry this procedure provides a cheap and promising option [22].

5. Conclusion

Plant mediated synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and plant biotechnology. This green route of silver nanoparticle synthesis has many advantages such as, ease with which process can be scaled up, economic viability and eco-friendliness. The use of easily available and cheap fruits can be used by nanotechnology processing industries. This study concluded that silver nanoparticles can be synthesized easily using green method and can be used for antimicrobial treatments and research. Silver nanoparticles were synthesized using vitamin C fruit juice. Temperature, concentration of silver nitrate and reducing agents affect particle size and yield of silver nanoparticles. It is natural, renewable and low-cost bio-reducing agent. Due to the potent activities of Ag-NPs, they can be promissingly used in treating infectious pathogens and preventing microbial infections. This study demonstrated an excellent disinfectant ability of Ag-NPs for the prevention of bacterial growth. Silver nitrate was used as a base and grape fruit juice was used as reducing as well as stabilizing agent.

Acknowledgement

Funding

No funding was received for this study

Conflict of interest

Authors declare no conflict of interest.

References

1. Gurunathan S., Park J.H., Han J.W., Kim J.H. Comparative assessment of the apoptotic potential of silver nanoparticles synthesized by Bacillus tequilensis and Calocybe indica in MDA-MB-231 human breast cancer cells: Targeting p53 for anticancer therapy. Int. J. Nanomed. 2015;10:4203–4222. doi: 10.2147/IJN.S83953.
2. Gurunathan S., Han J.W., Kim E.S., Park J.H., Kim J.H. Reduction of graphene oxide by resveratrol: A novel and simple biological method for the synthesis of an effective anticancer nanotherapeutic molecule. Int. J. Nanomed. 2015;10:2951–2969. doi: 10.2147/IJN.S79879.

3. Mukherjee P., Ahmad A., Mandal D., Senapati S., Sainkar S.R., Khan M.I., Renu P., Ajaykumar P.V., Alam M., Kumar R., et al. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. Nano Lett. 2001;1:515–519. doi: 10.1021/nl0155274

4. R. Renuka, K. Renuka Devi, M. Sivakami, T. Thilagavathi, R. Uthrakumar, K. Kaviyarasu: Biosynthesis of silver nanoparticles using phyllanthus emblica fruit extract for antimicrobial application. Biocatalysis and Agricultural Biotechnology, Volume 24, 2020, 101567, ISSN 1878-8181, doi: 0.1016/j.bcab.2020.10156710.

5. Li W.R., Xie X.B., Shi Q.S., Zeng H.Y., Ou-Yang Y.S., Chen Y.B. Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. Appl. Microbiol. Biotechnol. 2010;8:1115–1122. doi: 10.1007/s00253-009-2159-5

6. Sapsford K.E., Tyner K.M., Dair B.J., Deschamps J.R., Medintz I.L. Analyzing nanomaterial bioconjugates: A review of current and emerging purification and characterization techniques. Anal. Chem. 2011;83:4453–4488. doi: 10.1021/ac200853a.

7. Li C.Y., Zhang Y.J., Wang M., Zhang Y., Chen G., Li L., Wu D., Wang Q. In vivo real-time visualization of tissue blood flow and angiogenesis using Ag2S quantum dots in the NIR-II window. Biomaterials. 2014;35:393–400. doi: 10.1016/j.biomaterials.2013.10.010.

8. Chernousova S., Epplle M. Silver as antibacterial agent: Ion, nanoparticle, and metal. Angew. Chem. Int. Ed. 2013;52:1636–1653. doi: 10.1002/anie.201205923.

9. Burdușel A-C, Gherasim O, Grumezescu AM, Mogoantă L, Ficai A, Andronescu E. Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview. Nanomaterials. 2018; 8(9):681. https://doi.org/10.3390/nano8090681

10. Sondi I., Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria. J. Colloid Interface Sci. 2004;275:177–182. doi: 10.1016/j.jcis.2004.02.012.
11. Gurunathan S., Kalishwaralal K., Vaidyanathan R., Venkataraman D., Pandian S.R., Muniyandi J., Hariharan N., Eom S.H. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids Surf. B Biointerfaces. 2009;74:328–335. doi: 10.1016/j.colsurfb.2009.07.048.

12. Pleus R. Nanotechnologies-Guidance on Physicochemical Characterization of Engineered Nanoscale Materials for Toxicologic Assessment. ISO; Geneva, Switzerland: 2012.

13. Li L., Hu J., Yang W., Alivisatos A.P. Band gap variation of size- and shape-controlled colloidal CdSe quantum rods. Nano Lett. 2001;1:349–351. doi: 10.1021/nl015559r.

14. Sharma V.K., Yngard R.A., Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv. Colloid Interface. 2009;145:83–96. doi: 10.1016/j.cis.2008.09.002.

15. Lin P.C., Lin S., Wang P.C., Sridhar R. Techniques for physicochemical characterization of nanomaterials. Biotechnol. Adv. 2014;32:711–726. doi: 10.1016/j.biotechadv.2013.11.006.

16. Borase, H.P., Salunke, B.K., Salunkhe, R.B. *et al.* Plant Extract: A Promising Biomatrix for Ecofriendly, Controlled Synthesis of Silver Nanoparticles. *Appl Biochem Biotechnol* 173, 1–29 (2014). https://doi.org/10.1007/s12010-014-0831-4

17. Antony V. Samrot, P. Raji, A. Jenifer Selvarani, P. Nishanthini, Antibacterial activity of some edible fruits and its green synthesized silver nanoparticles against uropathogen – Pseudomonas aeruginosa SU 18, Biocatalysis and Agricultural Biotechnology, Volume 16, 2018, Pages 253-270, https://doi.org/10.1016/j.bcab.2018.08.014.

18. Lakshmanan G., Sathiaseelan A., Kalaichelvan P.T., Murugesan K., Plant-mediated synthesis of silver nanoparticles using fruit extract of Cleome viscosa L.: Assessment of their antibacterial and anticancer activity, Karbala International Journal of Modern Science, Volume 4, Issue 1, 2018, Pages 61-68, https://doi.org/10.1016/j.kijoms.2017.10.007

19. Ahmad N., Sharma S. and Rai R., Advanced Material Letters, VBRI press, 2012, DOI: 10.5185/amlett.2012.5357
20. Murdock R.C., Braydich-Stolle L., Schrand A.M., Schlager J.J., Hussain S.M. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol. Sci. 2008;101:239–253. doi: 10.1093/toxsci/kfm240.

21. Maedeh Sadat Mohseni, Mohammad A. Khalilzadeh, Moein Mohseni, Fariba Zamani Hargalani, Muhammad Ibrahim Getso, Vahid Raissi, Omid Raiesi, Biocatalysis and Agricultural Biotechnology, Volume 25,2020,101569,ISSN 1878-8181,doi: 1016/j.bcab.2020.101569

22. Bianca Moldovan, Luminiţa David, Marcela Achim, Simona Clichici, Gabriela Adriana Filip, A green approach to phytomediated synthesis of silver nanoparticles using Sambucus nigra L. fruits extract and their antioxidant activity, Journal of Molecular Liquids, Volume 221, 2016, Pages 271-278, https://doi.org/10.1016/j.molliq.2016.06.003.