Current Trends in Biotechnology and Pharmacy
Vol. 17 (1) 621 - 626, Jan 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2023.1.3

Synthesis and Biological Potential of Sliver Nanoparticles of *Euphorbia helioscopia* L.

Mehr-un-Nisa[1], Saleem Jan[1*], Yar Muhammad Khan[2], and Zeeshan Hussain Shah[3], Abul Kalam Azad[4*], Ashok Kumar Balaraman[4]

[1] Department of Chemistry, University of Science and Technology, Bannu, KP, Pakistan
[2] Department of Biotechnology, University of Science and Technology, Bannu, KP, Pakistan
[3] Department of Biological Sciences, Gomal University, D.I.Khan
[4] Faculty of Pharmacy, MAHSA University, Bandar Saujana Putra, 42610 Jenjarom, Selangor, Malaysia

*Corresponding Author : saleem_jann@hotmail.com,

Abstract

Silver Nanoparticles of extract of *Euphorbia helioscopia* L. were synthesized and their biological potential was determined. Effect of pH on the synthesis of silver nanoparticles, extract amount and time studies were performed. The antibacterial activity of nanoparticles of *Euphorbia helioscopia* at various concentrations against certain bacterial strains was determined and concentration of 100 µl was found to have good antibacterial result as compared to other concentrations. Similarly, the antioxidant potential of silver nanoparticles at various concentrations was also determined and it was found that the sliver nanoparticles at concentration 100 µg/ml showed good result as compared to ascorbic acid, while at concentration 75 µg/ml showed moderate result and the remaining concentrations exhibited weak activities.

Keywords: Antibacterial, Antioxidant, *Euphorbia helioscopia* L., Nanoparticles

Introduction

Natural products are used for treatment of various human diseases throughout the world and various medicinal plants have absolutely been considered by human beings since ancient times (1,4). Currently, the use of nanoparticle for this purpose is emerging. These are the particles having size between 2,500 and 100 nanometers including organic polymers and vesicles which are widely used around drug delivery (5, 6). Plant extract also shows a significant bioactivity against fungal infection (7). Many physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles (8). As the application of these physical and chemical methods are limited due toxicity of compounds, so an alternate and feasible method to synthesize silver nanoparticles is to synthesis by using microbes and plants. Silver nanoparticles are most effective due to their good antimicrobial efficacy against bacteria, viruses, and other eukaryotic micro-organisms (9, 10). They are used as antimicrobial agents, in textile industries, for water treatment, sunscreen lotions etc. (11, 12). Studies have already reported the successful biosynthesis of silver nanoparticles by various plants (13, 14). We also planned to synthesis and find biological potential of sliver nanoparticles of extract of *Euphorbia helioscopia* (15). *E. helioscopia* is a smooth annual plant with an erect, stout stem and belongs to family Euphorbiaceae which has about 2000 species distributed in tropical regions of the world (16).

Materials and Methods

Instrumentation

Absorption of the synthesized

nanoparticles was determined by using double beam UV-vis spectrophotometer.

Plants collection and identification

The plant material (Whole) was collected from Landi Dack regions of District Bannu, Khyber Pakhtunkhawa, Pakistan. The plant material was identified (Fig. 1) by a botanist, Dr Faizan Ullah at Department of Botany University of Science and Technology Bannu.

Fig. 1 Plant *Euphorbia helioscopia* L.

Extract preparation

The plant was dried under shade. The dried plant material was extracted with 80% aqueous methanol at room temperature. The mixture obtained was filtered by using filter paper and funnel. The filtrate was dried by using rotary evaporator to get initial crude material (8).

Solution preparation for silver nanoparticles

0.5 g of crude extract was dissolved in 100 ml methanol. The solution was taken in the shaker for 3 hours to mix it well. After that, the solution was filtered and was used for the synthesis of Ag-NPs

Synthesis of silver nanoparticles

For the synthesis of AgNPs, 1 ml of $AgNO_3$ solution (0.01 M) was taken in 10 ml graduated cylinder and was diluted up to 10 ml with deionized water. This solution was transferred to conical flask and NaOH was added to this solution drop wise to adjust the pH of $AgNO_3$ at 9. At this pH, 0.2 ml extract solution was added into the flask. The color of solution became light brownish yellow. This was the indication of the synthesis of silver nanoparticles. The color of the solution completely changed to yellow. The spectrum was recorded using UV-visible spectrophotometer which appeared at 414 nm which was the characteristic peak of Sliver nanoparticles (8).

Antibacterial activity

Four bacterial strains i.e. *E. coli, S. epidermis, S. aureus, K. pneumoniae* were used in antibacterial activity. Agar solution was used as a medium and 4.2 g of the suspended agar nutrient was treated with 150 ml of distilled H_2O at pH 9. Papered media, pettri plates, cotton swabs, saline solution, test tube were sterilized using autoclave at 121 ^{0}C for time 15 mints. After autoclaving media was poured into petri plates. Media was allowed to solidify for 1 hr. The wells in the center were assigned for antibiotic 0.015 m Erythromycin as standard. All concentration of NPs and standard was papered in DMSO. All concentration and standard were added in respective wells. Petri plates were kept in incubator for 24 hrs at 37 ^{0}C. Approximately each hole was detecting for clear inhibition zones. DMSO (1 ml) was used for same circumstance for each organism as under control. Inhibition zone of each zone was measured with the help of scale. Inhibition zones were calculated as means of each bacterial strain (16,17).

Antioxidant activity

A little change was done in the standard protocol described by Choi *et al*., 2002, for the evaluation of silver nanoparticles in DPPH free radical scavenging activities (18, 19). Different concentrations of silver nanoparticles solution were taken in the append drops with values 25,50,75 and 100 µg/ml and was mixed with the 0.5 ml of 2 mM DPPH solution and all the append drops were kept in dark to avoid the light interference. The absorbance of the samples was observed at 517 nm with a control append drop of methanol. Ascorbic acid solution was used as a standard was used in the same concentration as that of the samples. The following formula was used for the calculation of

free radical inhibition percentage.

[Control absorbance - sample absorbance]/
control absorbance x 100

Results and Discussion

pH effect on the synthesis of silver nanoparticles

The effect of pH on the synthesis of AgNPs was calculated in the range from 7-11. All samples showed good absorption and gave intense spectra but the sample having pH 9 gave maximum absorption and showed the most intense peak at 414 nm (Fig. 2). While the peaks at pH 7, 8, 10, 11 were below the peak of pH 9. So, the pH 9 was found suitable for synthesis of AgNPs from the extract of *E. helioscopia*.

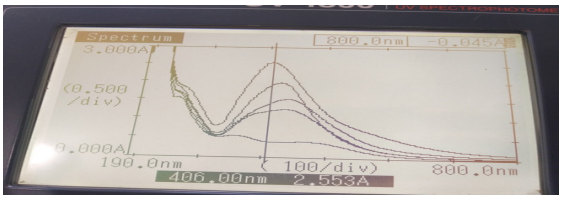

Fig. 2 pH study of silver nanoparticles of *Euphorbia helioscopia* L.

Extract amount study

The absorbance of AgNPs at different amount of extract was noted by using UV Vis spectrophotometer. The absorbance increased as the amount of plant extract increased, and maximum absorbance was noted at 0.2 ml extract (Fig. 3). On further increase to 0.3 ml, the absorbance was decreased, which indicated that at 0.2 ml plant extract, the synthesis of AgNPs reached to a maximum level. By increasing the plant extract, all the Ag were reduced and there was decreased in SPR intensity and decreased in absorption spectrum occurred.

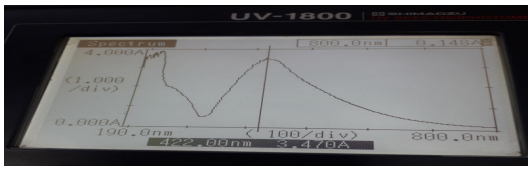

Fig. 3 Concentration study of silver nanoparticles of *Euphorbia helioscopia* L.

Stability of nanoparticles

There was regular increase in the synthesis of agNPs from 0 hour to 264 hours. After that the AgNPs get stable and there was no further increase in the absorbance. The spectra taken at 288 hours appeared at the same position as of the 264 hours which indicated that the AgNPs get stable and there is no more synthesis of Ag NPs (Fig. 3).

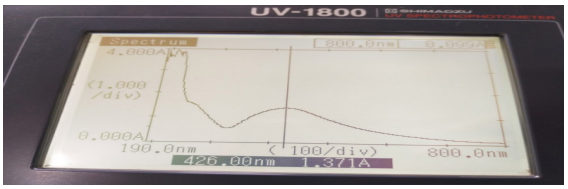

Figure-3: SPR band of silver nanoparticles of *Euphorbia helioscopia* L.

Antibacterial activity

The antibacterial activity exhibited by NPs of *Euphorbia helioscopia* against the tested bacterial strains namely *E.coli, S epidermas, S.areus and K pneumoniae* in which concentration of 100 µl showed good antibacterial result as compared to other concentrations (Table 1). *Similar study found that the silver doped copper oxide nanoparticles have potential antibacterial activity [22]. In*

Table 1 Antibacterial Activity of different fractions of Euphorbia helioscopia

Name of Bacterium	25 µl	50 µl	75 µl	100 µl	Standard (cm)
E. coli	0.9	0.7	0.8	1.5	4.0
S. epidermis	0.7	0.9	0.8	1.1	3.5
S. areus	0.6	0.9	1.0	1.2	3.5
K. pneumonia	0.8	0.8	1.1	1.3	4.0

addition, synthesis of silver nanoparticles using *Euphorbia wallichii* leaf extract showed antibacterial action against citrus canker causal agent and antioxidant potential (23, 24).

Antioxidant activity

The various concentrations of nanoparticles of *E. helioscopia* showed antioxidant results as compared with standard (Ascorbic acid). 100 µg/ml concentration showed good result as compared to ascorbic acid while 75 showed moderate result and the the remaining concentrations exhibited weak activities (Table 2).

% scavenging = absorption of control– absorption of fraction / absorption of control × 100

Similar study reported that this plant extract exhibited potential antioxidant activity (26).

Table 2 Antioxidant activity at different concentrations of silver nanoparticles of E. helioscopia

Concentration (µg/ml)	Ab AgNPs	% of silver nanoparticles	Ab ascorbic acid	% of ascorbic acid
25	0.51	43.93	0.25	72
50	0.48	47.93	0.24	73
75	0.36	59.35	0.23	74
100	0.30	65.31	0.19	79

Conclusion

Silver Nanoparticles of extract of *Euphorbia helioscopia* were synthesized and their biological potential was determined. Effect of pH on the synthesis of silver nanoparticles was studied which resulted resulted that pH 9 was most suitable for synthesis. The antibacterial activity of nanoparticles of *Euphorbia helioscopia* at various concentrations against certain bacterial strains was determined and concentration of 100 µl was found to have good antibacterial result as compared to other concentrations. Similarly, the antioxidant potential of silver nanoparticles at various concentrations was also determined and it was found that the sliver nanoparticles at concentration 100 µg/ml showed good result as compared to tandard, ascorbic acid, while at concentration 75 µg/ml showed moderate result and the remaining concentrations exhibited weak activities.

Funding: This research work was collaboratively funded by the faculty of pharmacy, MAHSA University, Bandar Saujana Putra, 42610 Jenjarom, Selangor, Malaysia and University of Science and Technology, Bannu, KP, Pakistan.

Data Availability Statement: Data will be available upon request.

Acknowledgments: Authors would like to acknowledge to Department of Chemistry, University of Science and Technology, Bannu, Khyber Pakhtunkhwa, Pakistan for laboratory facilities and the faculty of pharmacy, MAHSA University, Malaysia.

References

1. Azad, A. K., Awang, M., & Rahman, M. M. (2012). Phytochemical and microbiological evaluation of a local medicinal plant Bacopa monnieri (l.) Penn. International Journal of Current Pharmaceutical Review and Research, 3(3), 66-78.

2. Singh, J. S. The biodiversity crisis: a multifaceted review. *Current Science*. 2002; 638-647.

3. Hamilton AC. Medicinal plants, conservation and livelihoods. *Biodivers Conserv*. 2004, 13; 1477-517.

4. Halberstein RA. Medicinal plants: historical and coss-cult uralusage patterns. *Ann Epidemiol.* 2005, 15; 686-9 9.

5. Roy, A., Singh, V., Sharma, S., Ali, D., Azad, A. K., Kumar, G., & Emran, T. B. (2022). Antibacterial and Dye Degradation Activity of Green Synthesized Iron Nanoparticles. Journal of Nanomaterials, 2022.

6. Jiale H, Liqin L, Qingbiao Li, Daohua S, Yuanpeng W, Yinghua L, Ning H, Kun Y, Xin Y, Huixuan W, Wenta W, Wenshuang L."Continuous-flow biosynthesis of silver nanoparticles by lixivium of sundried Cinnamomum camphora leaf in tubular microreactors." *Industrial & engineering chemistry research* 47.16 (2008): 6081-6090.

7. Shah, M. K. A., Azad, A. K., Nawaz, A., Ullah, S., Latif, M. S., Rahman, H., ... & Abdel-Daim, M. M. (2021). Formulation development, characterization and antifungal evaluation of chitosan NPs for topical delivery of voriconazole in vitro and ex vivo. Polymers, 14(1), 135.

8. Azad, A. K., Azizi, W. S., Ismail, A. F. H., Abbas, S. A., Uddin, J., & Labu, Z. K. (2019). Phytochemical and toxicity evaluation of traditional herb: Lagerstroemia speciosa L.(Banaba) by MCF-7 cell line and brine shrimp lethality bioassay. Bangladesh Pharmaceutical Journal, 22(1), 45-49.

9. Babar, Z. M., Azizi, W. M., Ichwan, S. J., Ahmed, Q. U., Azad, A. K., & Mawa, I. (2019). A simple method for extracting both active oily and water soluble extract (WSE) from Nigella sativa (L.) seeds using a single solvent system. Natural product research, 33(15), 2266-2270.

10. Azad, A. K., Laboni, F. R., Rashid, H., Ferdous, S., Rashid, S. S., Kamal, N., ... & Islam Sarker, Z. (2020). In vitro evaluation of Cuscuta reflexa Roxb. for thrombolytic, antioxidant, membrane stabilizing and antimicrobial activities. Natural product research, 34(16), 2394-2397.

11. Sharma V.K., Ria A.Y. and Lin Y., *Advances in Colloid and Interface Science*, 2009, 145; 83-96.

12. Sameena, V. P., & Thoppil, J. E. (2022). Green synthesis of silver nanoparticles from Euphorbia and its biological activities. Nanotechnology for Environmental Engineering, 1-16.

13. Nadaf, S. J., Jadhav, N. R., Naikwadi, H. S., Savekar, P. L., Sapkal, I. D., Kambli, M. M., & Desai, I. A. (2022). Green Synthesis of Gold and Silver Nanoparticles: Updates on Research, Patents, and Future Prospects. OpenNano, 100076.

14. Fuloria, S., Yusri, M. A. A., Sekar, M., Gan, S. H., Rani, N. N. I. M., Lum, P. T., ... & Fuloria, N. K. (2022). Genistein: A Potential Natural Lead Molecule for New Drug Design and Development for Treating Memory Impairment. Molecules, 27(1), 265.

15. Balakrishnan, V., Thangaraj, K., Palani, M., & Vaiyapuri, M. (2022). Green synthesis of copper oxide nanoparticles using Euphorbia hirta leaves extract and its biological applications. Inorganic and Nano-Metal Chemistry, 52(6), 809-818.

16. Govaerts R, Frodin DG, Radcliffe-Smith A, Carter S. World checklist and bibliography of *Euphorbiaceae* (with Pandaceae), Royal Botanic Gardens, Kew. 2000

17. Chang WC, Sei CK, Soon SH, Bong KC, Hye JA, Min YL, Sang HP, Soo KK. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science,* 2002*;* 163, 1161-1168.

18. Asghar, A. A., Akhlaq, M., Jalil, A., Azad, A. K., Asghar, J., Adeel, M., ... & Abdel-Daim, M. M. (2022). Formulation of ciprofloxacin-

loaded oral self-emulsifying drug delivery system to improve the pharmacokinetics and antibacterial activity. Frontiers in Pharmacology, 13.

19. Mounyr B, Moulay S, Saad K, Ibnsouda. Methods for *in vitro* evaluating antimicrobial activity: A review. *J. Pharmaceutical Analysis*, 2016; 6, 71-79.

20. Balchaa, E. K., Maricab, A., Zelekea, T. D., Murthya, H. C. A., Royc, A., Ravikumard, C. R., & Azade, A. K. (2022). Allium sativum extract mediated synthesis of Ag/Zn bimetallic nanostructures for antibacterial application. Digest Journal of Nanomaterials And Biostructures, 17(3), 1057-1072.

21. Nasir, N. N., Sekar, M., Fuloria, S., Gan, S. H., Rani, N. N. I. M., Ravi, S., ... & Fuloria, N. K. (2022). Kirenol: a potential natural lead molecule for a new drug design, development, and therapy for inflammation. Molecules, 27(3), 734.

22. *Preethi, D. R. A., & Philominal, A. (2022). Antimicrobial and antiurolithiatic activities of pure and silver doped copper oxide nanoparticles using Moringa Oleifera leaf extract on struvite urinary stones. Applied Surface Science Advances, 12, 100351.*

23. Arif, M., Ullah, R., Ahmad, M., Ali, A., Ullah, Z., Ali, M., ... & Sher, H. (2022). Green Synthesis of Silver Nanoparticles Using Euphorbia wallichii Leaf Extract: Its Antibacterial Action against Citrus Canker Causal Agent and Antioxidant Potential. Molecules, 27(11), 3525.

24. Iskandar, B., Lukman, A., Syaputra, S., Al-Abrori, U. N., Surboyo, M. D., & Lee, C. K. (2022). Formulation, characteristics and anti-bacterial effects of Euphorbia hirta L. mouthwash. Journal of Taibah University Medical Sciences, 17(2), 271-282.

25. Jaouadi, R., Elimem, M., Sayari, N., Kalboussi, M., Lahfef, C., Hammadi, J., & Rouz, S. Antioxidant and insecticidal properties of Euphorbia helioscopia L. Aqueous extracts.