Characterization and Antibacterial Activity of Silver Nanoparticles Biosynthesized Using Leaves Extract of Artemisia sieberi and Calotropis procera

SALEH H. SALMEN1*, NADIAH MATLUQ ALKAMMASH1, TAHANI AWAD ALAHMADI2, SULAIMAN ALI ALHARBI1
1 Department of Botany and Microbiology, College of Science, King Saud University P.O. Box 2455.Riyadh-11451, Saudi Arabia
2 Department of Pediatrics, College of Medicine, King Saud University [Medical City], King Khalid University Hospital, PO Box 2925, Riyadh, 11461, Saudi Arabia

Abstract: The prevalence of antibiotic-resistant bacteria has increased recently leading to the need for novel, natural antibacterial agents such as plant-synthesized silver nanoparticles. Such synthesis is safe, cheap, rapid, non-toxic and environmentally friendly. In this study, characterization of biosynthesized silver nanoparticles from extracts of A. sieberi and C. procera was carried out using transmission electron microscopy, Fourier transform infrared and energy dispersive x-ray analysis. Spherical nanoparticles with an average size was ~10 nm for A. sieberi and ~14 nm for C. procera were synthesised; synthesis was most effective using A. sieberi. Antibacterial activity of silver nanoparticles was carried out using the agar-diffusion method and by determination of the minimum inhibitory concentration. Biosynthesized silver nanoparticles showed antibacterial activity against Staphylococcus aureus, MRSA, Salmonella typhimurium and Escherichia coli, with silver nanoparticles extracts from A. sieberi being the most antibacterial.

Keywords: A. sieberi, C. procera, plant extract, silver nanoparticles, antibacterial activity

1. Introduction

In recent years, the emergence of antibiotic-resistant bacteria has become a serious public health concern [1,2]. Antimicrobial agents are important in reducing the prevalence of multidrug resistant (MDR) strains in pathogenic bacteria [3]. Many medicinal plants are known to be a sources of natural antimicrobial compounds and can provide alternatives to antibiotics [4]. The synthesis of nanoparticles, particularly silver nanoparticles (AgNPs), has increased due to their varied application as antimicrobial agents and in, for example, biomedicine, optics and catalysis [5-9].

A number of medicinal plants are used in the green synthesis of antimicrobial nanoparticles, including, Aloe fleurentiniorum [10]; Green tea [11]; Indigofera oblongifolia [12]; Aloe vera, Portulaca oleracea and Cynodon dactylon [13]; Ribes nigrum [14]. Those medicinal plants are rich in bioactive compounds such as alkaloids, tannins, phenolic and flavonoids compounds, which have antimicrobial activities against some pathogenic microorganisms [15,16]. The genus Artemisia family asteraceae comprises approximately 300 species worldwide [17] and in Saudi Arabia is represented by four species (A. sieberi, A. monosperma, A. scoparia and A. judaica) [18-25]. Calotropis procera is a shrub of the family Asclepiadaceae which is distributed in Asia, Africa and South America [26]. It produces a milky white latex [27-29] which is stored in unique branching tubes called latex channels [30]; it is antiviral [31,32], antibacterial [33], antifungal [34] and possesses anticarcinogenic properties [35,36].

The biogenic synthesis of nanoparticles from plant extracts is rapid, cheap, safe and ecofriendly. Newly, biogenic synthesis of silver nanoparticles using some Artemisia species including, A. vulgaris [37,38], A. annua [39], A. monosperma [40], A. turcomanica [41], A. afra [42]. Here, we evaluate and compare the antibacterial effects and characterization of silver nanoparticles synthesized using extracts of A. sieberi and C. procera.

* email: ssalmen@ksu.edu.sa
2. Materials and methods

2.1. Materials

AgNPs were synthesized using *A. sieberi* and *C. procera* obtained from Riyadh, Saudi Arabia as described in our previous study [43].

2.2. Method

2.2.1. Characterization of AgNPs

Characterization of AgNPs synthesized using *A. sieberi* and *C. procera*, including UV–visible spectroscopy and scanning electron microscopy was conducted as described in our previous study [43]. Transmission electron microscopy (TEM) Analysis was used to observe the morphology and size of silver nanoparticles using a TEM, JEOL microscope JEM-1011). Fourier transform infrared (FTIR) Analysis was used to determine functional groups of plants extracts and synthesized silver nanoparticles using a Perkin-Elmer 1000 FTIR instrument (Waltham, MA, USA). Energy dispersive Xray (EDX) analysis was done by using Jeol SEM model JSM 6360A (Japan) in order determine the elemental composition of nanoparticles and the morphology.

2.2.2. Antibacterial activity

Bacteria The following bacteria were used from ATCC: *Staphylococcus aureus* (29213), MRSA (43300), *Salmonella typhimurium* (14028) and *Escherichia coli* (25922). Both species were inoculated on Nutrient Agar (Scharlau Microbiology, Spain) and incubated for 24 h at 37°C.

2.2.3. Agar-diffusion method

The Agar-diffusion Method was used to determine the *in vitro* antibacterial activity of AgNPs. Concentrations 1.6 and 4 mg/disk were tested against bacteria, which were inoculated onto Muller Hinton Agar (Scharlau Microbiology, Spain) using a swab. Disks containing the selected AgNP concentration were then transferred to the surface of the inoculated media and incubated at 37°C for 24 h; any zones were then measured (mm).

2.2.4. Minimum inhibitory concentration (MIC)

The MIC of AgNPs, was determined using the broth media dilution method using Muller Hinton broth inoculated with 100 μL of the bacterial cultures and incubated for 24 h at 37°C.

3. Results and discussions

3.1. Characterization of AgNPs

The biogenic synthesis of AgNPs was achieved as describe previously [43] and confirmed by UV-vis spectroscopy and scanning electron microscopy. Here, we also include characterization of the *A. sieberi* and *C. procera* extracts with FTIR, EDX and TEM.

3.2. FTIR spectra

FTIR spectra were conducted to characterize the synthesized AgNPs and to determine the presence of functional groups responsible to the biocomponents in extracts of *A. sieberi* and *C. procera* used in the preparation of AgNPs. FTIR spectra of *A. sieberi* extract and AgNPs produced by *A. sieberi* exhibited more functional bonds in the plant extract compared to synthesized nanoparticles (Figure 1a,b). A peak was observed at 3410 cm$^{-1}$ corresponding to the hydroxyl (O–H), while peaks at 2849 to 2917 cm$^{-1}$ correspond to C–H. The band found at 1629 cm$^{-1}$ is allocated to C=O of the amide groups and the bands at 1033 cm$^{-1}$ corresponding to C–O. On the other hand, FTIR spectrum of AgNPs by *A. sieberi* was observed the disappearance of all the bonds except the bond at 1602 cm$^{-1}$ corresponding to C=O of the amide groups. (Figure 2 a,b) exhibits the spectrum of *C. procera* plants and synthesized AgNPs. All the peaks shown are comparably similar to the *A. sieberi* plant peaks. Similarly, in several previous studies,
these peaks were recorded for different plant extracts used in biogenic AgNP synthesis. [10, 12, 37,40,41].

**Figure 1.** FTIR spectra of *A. sieberi* extract (A) and AgNPs produced using *A. sieberi* (B)

**Figure 2A.** FTIR spectra of *C. procera* extract
3.3. EDX analysis

EDX was used to observe elemental composition of nanoparticles of AgNPs synthesized using *A. sieberi* and *C. procera* extract (Figure 3a,b). The results showed strong signals at ~3 keV in silver (Ag) zone for AgNPs synthesized using *A. sieberi* and *C. procera* extracts. This signal at ~3 keV for AgNPs has been previously widely reported [44-46,10,12]. The appearance of signal at ~3 keV was as evidence of the presences of Ag elements in the AgNPs.

![Figure 3. EDX spectrum of AgNPs produced by *A. sieberi* (A) and *C. procera* (B)](image)

3.4. TEM analysis

Transmission electron microscopy (TEM) images (Figure 4a,b) indicating the size and morphology of the synthesized nanoparticles. TEM results show the spherical and monodisperse of AgNPs produced by *A. sieberi* and *C. procera* extracts. The average diameter of AgNPs was almost 10 nm for *A. sieberi* and 14 nm for AgNPs produced using the *C. procera* extract. The diameter of AgNPs produced here using *A. sieberi* are smaller compared to previous studies, which gave a size of 25 nm for leaf extract of *A. vulgaris* [37], 17 nm for *A.monosperma* [40] 22 nm for *A.turcomanica* leaf extract [41] and 20–90 nm for *A. annua* L. extract [39]. On the other hand, diameters of AgNPs produced by *C. procera* extracts were similar to results for *C.procera* reported by Mohamed et al. [47] and latex of *C. gigantea* L [48].
3.5. Antibacterial activity

The Agar-diffusion Method was used to determine the antibacterial activity of AgNPs against some pathogenic bacteria (Table 1). In general, all bacteria were inhibited by AgNPs produced by *A. sieberi* and *C. procera* extracts and Gram-positive bacteria showed the most marked effect. Bacterial inhibition was directly related to AgNPs concentration where inhibition zones increase with increasing AgNPs concentration (1.6 to 4 mg). AgNPs synthesized by *A. sieberi* showed greater antibacterial activity than those synthesized using *C. procera*. Inhibition zones values (mm) of *Staphylococcus aureus* and MRSA ranged from 9 to 14 mm for AgNPs synthesized using *A. sieberi* from 8 to 10 mm for AgNPs synthesized by *C. procera*. Inhibition zones values (mm) of *Salmonella typhimurium* and *Escherichia coli* ranged from 7 to 13 mm for AgNPs synthesized using *A. sieberi* and 7 to 9 mm for AgNPs synthesized using *C. procera*. MIC values are shown in Table 2. The results confirmed that bacterial inhibition for AgNPs synthesized using *A. sieberi* was more marked than those synthesized using *C. procera* [10, 12, 13, 37, 38].

### Table 1. Inhibition zone (mm) of AgNPs against some pathogenic bacteria at concentrations of 1.6 and 4 mg

| Bacteria      | AgNPs of *A. sieberi* | AgNPs of *C. procera* |
|---------------|-----------------------|-----------------------|
|               | 1.6 mg                | 4 mg                  | 1.6 mg | 4 mg |
| *S. aureus*   | 9                      | 14                    | 8      | 9    |
| MRSA          | 9                      | 14                    | 8      | 10   |
| *S. typhimurium* | 7                    | 13                    | 7      | 9    |
| *E. coli*     | 7                      | 12                    | 6      | 7    |

### Table 2. MIC values of AgNPs against pathogenic bacteria at (mg/L)

| Bacteria      | AgNPs of *A. sieberi* | AgNPs of *C. procera* |
|---------------|-----------------------|-----------------------|
| *S. aureus*   | 0.75                  | 1.5                   |
| MRSA          | 0.375                 | 0.75                  |
| *S. typhimurium* | 0.187               | 0.75                  |
| *E. coli*     | 0.375                 | 0.75                  |
4. Conclusions

The aim of this study was to characterize and determine the antibacterial effects of silver nanoparticles biosynthesized using extracts of *A. sieberi* and *C. procera*. Additionally, we used various methods, including transmission electron microscopy, fourier transform infrared, energy dispersive x ray to characterize silver nanoparticles. The results confirmed the synthesis of spherical nanoparticles with an average size was ~10 nm for *A. sieberi* and ~14 nm for *C. procera*. Tests for antibacterial activity showed that the synthesized nanoparticles showed antibacterial activity against *Staphylococcus aureus*, MRSA, *Salmonella typhimurium* and *Escherichia coli*, with silver nanoparticles from extracts of *A. sieberi* proving to be more antibacterial than those obtained from *C. procera*.

Acknowledgments: This project was supported by Researchers Supporting Project number (RSP-2020/230), King Saud University, Riyadh, Saudi Arabia.

References

1. BOUCHER, H.W., TALBOT, G.H., BRADLEY, J.S., EDWARDS, J.E., GILBERT, D., RICE, L.B., SCHELD, M., SPELLBERG, B., BARTLETT, J. Clin Infect Dis. 48, no.1, 2009, p.1.
2. GIAMARELLOU, H. Int. J. Antimicrob. Agents, 36, 2010, p.S50.
3. BHATIA, R., NARAIN, J.P. Indian J Med Res. 132, 2010, no.5, p.482.
4. IWU, M.W., DUNCAN, A.R., OKUNJI, C.O. J. Janick, Ed., ASHS Press, Alexandria, Virginia, 1999.
5. SONG, J.Y., KIM, B.S. Bioprocess Biosyst Eng. 32, 2009, p.79.
6. JYARAJ, M., RAJESH, M.R., ARUN, ALI, D.M., SATHISHKUMAR, G., SIVANANDHAN, G., DEV, G. K., MANICKAVASAGAM, M., PREMKUMAR, K., THAJUDDIN, N., GANAPATHI. A. Colloid Surf. B-Biointerfaces, 102, 2013, p.708.
7. JYARAJ, M., SATHISHKUMAR, G., SIVANANDHAN, G., ALI, D. M., RAJESH, M., ARUN, R., KAPILDEV, G., MANICKAVASAGAM, M., THAJUDDIN, N., PREMKUMAR, K., GANAPATHI, A., Colloids Surf B: Biointerfaces. 106, 2013.p.86.
8. OKAFOR, F., JANEN, A., KUKHTAREVA, T., EDWARDS, V., CURLEY, M. Int. J Environ. Res. Public. Health.10, no.10, 2013.p.5221.
9. TRAN, H.V., TRAN, L.D., BA, C.T., VU, H.D., NGO, T.N., PHUM, D.G., NGUYEN, P.X. Colloids and Surfaces A: Physicochem. Eng. Aspects 360, 2010 p. 32.
10. SALMEN, S., ALHARBI, S.A. Green Chem. Lett. Rev. 13, no.1, 2020. p.1.
11. WALLACE, R.R., MILENA, T.P., BRUNA DE ARAÚJO, L. Appl. Surf. Sci., 463, 2019. p. 66.
12. SALMEN, S.H., ALWHIBI, M.S., ALHARBI, S.A. Appl. Ecol. Environ. Res., 17, no.6, 2019, p.12869.
13. TARAD, A.A., ALHARBI, S.A., SALMEN, S.H., WAINWRIGHT, M., Biotechnol. Biotechnol Equip, 31, no.2, 2017, p. 411.
14. DOBRUCKA, R., KACZMAREK, M., DLUGASZEWSKA, J. Adv. Nat. Sci. Nanosci. Nanotechnol. 9, 2018. 025015.
15. DURAIANDIYAN, V., AYYANAR, M., IGNACIMUTHU, S. BMC Complement Altern Med. 6, 2006. p.1.
16. DJEUSSI, D.E., NOUMEDEM, J.A.K., SEUKEP, J.A., FANKAM, A.G., VOUKENG, I.K., TANKEO, S. B., NKOETE, A.L., KUETE, V. BMC Complement Altern Med. 13, no. 164, 2013, p.1.
17. GUETAT, A., AL-GHAMDI, F.A. OSMAN, A.K. Nat. Prod. Res., 31, 2016, p.598.
18. WILLOUGHBY, J.A., SR, J.A., SUNDAR, S.N., CHEUNG, M., TIN, A. S., MODIANO, J., FIRESTONE, G.L. J. Biol. Chem. 284, 2009.p.2203.
19. ARAB, H.A., RAHBARI, S., RASSOULI, A., MOSLEMI, M.H., KHOSRAVIRAD, F. Trop. Anim. Health Prod. 38, 2006. p.497.
20. ROMERO, M.R., SERRANO, M.S., VALLEJO, M., EFFERTH, T., ALVAREZ, M., MARIN, J., J. Planta. Medica, 72, 2006. p.1169.
21. NEGABAN, M., MOHARRAMIPOUR, S., SEFIDKON, F. J. Asia-Pacific Entom.., 9, 2006. p.381.
22. RUSTAIYAN, A., NAHREVANIAN, H., KAZEMI, M. Pharmacogn. Mag., 5, 2009, p.1.
23. VERDIAN-RIZI, M.R. Pharm. Res. 1, 2009, p. 21.
24. PETRETTO, G.L., CHESSA, M.A. PIANA, A., MASIA, M.D., FODDAI, M., CULEDDU, N., AFIFI, F. PINTORE, G. Nat. Prod. Res. 27, no.19, 2013, p.709.
25. ZAFAR HAIDER, S., MANINDRA, M., ANDOLA, H.C. Pharmacognosy Res. 6, 2014, p.257.
26. MASCOLO, N., SHARMA, R., JAIN, S.C., CAPASSO, F. J. Ethnopharmacol., 22, 1988, p.211.
27. IQBAL, Z., LATEEF, M., JABBAR, A., MUHAMMAD, G., KHAN, M.N. J. Ethnopharmacol. 102, 2005, p. 256.
28. SAADABI, A.M.A. ALI, N.M.H. MOHAMMED, I., ALSAFI, F.N., MUSTAFA, H.B. Res. J. Medi. Sci. 6, 2012, p.13.
29. RAMOS, M.V., AGUIAR, V.V., MELO, M.M., MESQUITA, R.O., SILVESTRE, P.P., OLIVEIRA, J.S., OLIVEIRA, R.B., MACEDO, N.R., ALENCAR, N.N. J Ethnopharmacol. 111, 2007, p.115
30. MAHajan, R.T., BADGUJAR, S.B. Ethnobotanical Leaflets, 12, 2008, p. 1145.
31. PANDEY, B.P. Plant anatomy, S. Chand Limited, New Delhi, 2001, p. 57.
32. OLIVEIRA, J.S., COSTA-LOTUFO, L.V., BEZERRA, D.P., ALENCAR, N.M., MARINHO-SSILVA, A.B., DIAS, A.M., CRUZ, V.R. Int J. Nanomedicine 11, 2016, p. 1835.
33. ISHNAVA, K.B., CHAUHAN, J.B, GARG, A.A., THAKKAR, A.M., Saudi J. Biol. Sci., 19, 2012, p. 87.
34. DE FREITAS, C.T., NOGUEIRA, F.S., VASCONCELOS, I.M., OLIVEIRA, J.A., DOMONT, G.B., RAMOS, M.V. Plant Physiol. Bioch., 49, 2011, p.738.
35. SILVA, M.C., DA SILVA, A.B., TEIXEIRA, F.M., SOUSA, P.D., RONDON, R.M., JÚNIOR, J.H. Asian Pac J Trop Med. 3, 2010, p.332.
36. SAMY, R.P., RAJENDRAN, P., LI, F., ANANDI, N.M., STILES, B.G., IGNACIMUTHU, S. PLoS One, 7, no. 12, 2012, p.e48514.
37. RASHEED, T., BILAL, M., IQBAL, H., LI, C. Colloid Surf. B-Biointerfaces, 1, no.158. 2017, p.408.
38. KUMARARAJA, G., SUNDARAGANAPATHY, R., CONSTANTINE, I., VIJAYALAKSHMI, V., RAHIM, S.F.A. J. Pharm. Sci. Res. 11, 2019, p. 2558.
39. AGHAJANYAN, A., GABRIELYAN, L., SCHUBERT, R., TRCHOUNIAN, A. AMB Express. 10, no.66, 2020, p. 1.
40. ELSHARKAWY, E.R., “Oriental J. Chem. 34, 2018, p. 1420.
41. MOUSAVI, B., TAFVIZI, F., BOSTANABAD, S.Z. Artificial Cells, Nanomedic.Biotech.46, 2018, p. S499.
42. ELEMIKE, E.E., ONWUDIWE, D.C., EKENNIA, A.C., JORDAAN, A. IET Nanobiotech. 12, 2018, p.722.
43. ALKAMMASH, N.M. Biosci. Biotechnol. Res. Asia., 14, 2017, p. 521.
44. NADAGOUAD, M.N., SPETH, T.F., VARMA, R.S. Acc. Chem. Res. 44, no.7, 2011, p. 469.
45. KHAN, M., KHAN, S.T., KHAN, M., ADIL, S.F., MUSARRAT, J., AL-KHEDHAIRY, A., AL-WARTHAN, A., SIDDQUI, M.R.H., ALKHATHLAN, H.Z., Int J Nanomedicine. 9, 2014.p.3551.
46. SOLEHI, S., SHANDIZ, S.A.S., GHANBAR, F., DARVISH, M.R., ARDESTANI, M. S., MIRZIAIE, A., JAFARI, M. Int J Nanomedicine. 11, 2016, p.1835.
47. MOHAMED, N.H., ISMAIL, M.A., ABDEL-MAGEED, W.M., SHOREIT, A.A.M. Asian Pac J Trop Biomed. 4, no. 11, 2014, p.876.
48. RAJKUBERAN, C., SUDHA, K., SATHISHKUMAR, G., SIVARAMAKRISHNAN. S. Spectroc. Acta Pt. A-Molec. Biomolec. Spectr.136, 2015, p. 924

Manuscript received: 29.11.2020