Malaysian Applied Biology (2024) 53(4): 1-6
https://doi.org/10.55230/mabjournal.v53i4.3009

Research

Green Synthesis of *Chrysanthemum morifolium* Silver Nanoparticles and Evaluation of Its Antibacterial Activity

Lai Mun Leong[1], Ghim Hock Ong[2], Khye Er Loh[1]*

1. Department of Bioscience, Faculty of Applied Sciences, Tunku Abdul Rahman University of Management and Technology, Jalan Genting Kelang, 53300 Setapak, Kuala Lumpur, Malaysia
2. Faculty of Health and Life Sciences, INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia
*Corresponding author: lohke@tarc.edu.my

ABSTRACT

The increasing prevalence of microbial infections and antibiotic resistance has sparked interest in investigating the therapeutic potential of silver nanoparticles (AgNPs) as effective antimicrobial agents. The current study seeks to optimize the synthesis of AgNPs using *Chrysanthemum morifolium* (CM) extract and evaluate their antibacterial activity. Maximum synthesis of CM-AgNPs was achieved using 10 mM of $AgNO_3$ and 20 mg/mL of CM extract at a 6:4 ratio and 3 hr of incubation period at pH 11 and 40°C. The *Chrysanthemum morifolium*-synthesized silver nanoparticles (CM-AgNPs) displayed a spherical shape, with sizes ranging from 12 to 34 nm. The minimum inhibitory concentration (MIC) against *Pseudomonas mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were 0.0117, 0.0117, 0.0031, and 0.100 mg/mL, respectively. CM-AgNPs demonstrated notable antibiofilm activity of 49.26%, 87.31%, and 66.23% against *P. mirabilis*, *S. aureus*, and *K. pneumoniae*, respectively. These results indicate that CM-AgNPs possess antibacterial properties and hold promise as antimicrobial agents.

Key words: Antibacterial, antibiofilm, *Chrysanthemum morifolium*, minimum inhibitory concentration, silver nanoparticles

Article History
Accepted: 26 August 2024
First version online: 27 October 2024

Cite This Article:
Leong, L.M., Ong, G.H., Loh, K.E. 2024. Green Synthesis of *Chrysanthemum morifolium* silver nanoparticles and evaluation of its antibacterial activity. Malaysian Applied Biology, 53(4): 1-6. https://doi.org/10.55230/mabjournal.v53i4.3009

INTRODUCTION

The widespread application of nanomaterials and their unique physicochemical properties have drawn researchers' attention. Among the commercially available nano-sized materials, one of the most widely used nanomaterials is silver nanoparticles (AgNPs), owing to their diverse applications and physicochemical properties. According to the Nanotechnology Product Database (2022), AgNPs have been used in many industries, including textile, cosmetics, food, medicine, health care, construction, and optics. Besides, AgNPs play a significant role in nanotechnology, especially in the field of nanomedicine (Rafique *et al*., 2017). There is a rise in interest in the field of green nanotechnology, which involves the biosynthesis of AgNPs from various sources of medicinal plants that possess phytochemicals that can improve the biological action of AgNPs. This is because the secondary metabolites found in plant extracts are involved in the redox reactions during the formation of AgNPs (Chung *et al*., 2016). In addition, there are several benefits of employing plant extracts for the green synthesis of AgNPs, including the vast accessibility of plant sources, safety in handling, energy efficiency, environmental friendliness, and support for large-scale synthesis of AgNPs.

Chrysanthemum morifolium (CM) possesses several pharmacological properties, like antibacterial, anti-tumorigenesis, antipyretic, antioxidant, anti-inflammatory, anti-aging, and anti-hypertensive (Liang *et al*., 2021). These

properties are attributed to the abundance of phytochemical compounds, such as flavonoid and phenolic compounds. Previous research on AgNPs synthesized using *C. morifolium* has shown that it has antibacterial, antioxidant, antimicrobial, and anticancer activities (Kalishwaralal *et al*., 2010). However, the antibacterial action of *C. morifolium* extracts mediated AgNPs (CM-AgNPs) against biofilm-associated microorganisms is not well studied. Since *C. morifolium* contains significant amounts of phenolic and flavonoid compounds, synthesizing AgNPs from its extract may enhance the AgNPs' antibacterial properties. Therefore, the objective of this study was to investigate the antibacterial activity of CM-AgNPs against *S. aureus*, *P. mirabilis*, *K. pneumoniae,* and *P. aeruginosa*.

MATERIALS AND METHODS

Preparation of CM extract

The flowers of *C. morifolium* were purchased from a traditional Chinese herbal store (GPS coordinates: 3.0875°N, 101.6451°E) and the authentication of the plant sample (voucher specimen: SK3139/17) was conducted at the Institute of Bioscience, Universiti Putra Malaysia. The dried flowers are ground into powder and sieved before extraction. The extraction method was adopted by Kandiah and De Silva (2021) with some modifications. Briefly, 20 g of CM flower powder was added to 300 mL of Milli-Q water and boiled for 10 min. The extract was then filtered through Smith Filter Paper (150 mm) and concentrated using a rotary evaporator, then lyophilized.

Green synthesis of silver nanoparticles

The parameters, including $AgNO_3$ to CM extract ratio, concentration of CM extract, concentration of $AgNO_3$, temperature, pH, and duration of incubation were optimized for rapid and maximal synthesis of CM-AgNPs with small size and monodisperse shape. The one-factor-at-a-time technique was used to determine the ideal parameters for the maximal synthesis of small and monodisperse AgNPs (Melkamu *et al*., 2022). The variables used to optimize the synthesis of AgNPs are shown in Table 1. The synthetic reaction was carried out in the dark to ensure a more controlled reduction process to obtain more uniform nanoparticles. The color shift showed the formation of nanoparticles and was further confirmed using UV-Vis spectroscopy.

Table 1. Parameters and variables used to optimize the green synthesis of AgNPs

Parameter	Variable
Ratio of $AgNO_3$ to CM extract	9:1, 8:2, 7:3, 6:4, and 5:5
CM extract concentration	10, 20, 30, 40, and 50 mg/mL
$AgNO_3$ concentration	2, 4, 6, 8, and 10 mM
pH of CM extract	pH 7, 9, and 10
Temperature	30, 40, 60 and 80°C
Duration	3, 6, 12, 24, and 48 hr

Characterization of CM-AgNPs

The CM-AgNPs formation was monitored by using a UV-Vis spectrophotometer (Thermo Scientific, Orion AquaMate 8100) at 300-700 nm (Arokiyaraj *et al*., 2014). The morphology, size and shape of synthesized CM-AgNPs was confirmed by field emission scanning electron microscopy (FESEM) (Hitachi, SU8220) at 5.0 kV of accelerating voltage, magnification of 100,000x and 100 nm scale (Kandiah & De Silva (2021)).

Broth microdilution assay

The bacterial strains of *Pseudomonas aeruginosa* (ATCC 10145), *Proteus mirabilis*, *Klebsiella pneumoniae* (ATCC 13883), and *Staphylococcus aureus* (ATCC 25923) were used in this study. The bacteria were cultured in Mueller-Hinton broth at 37°C in a shaker overnight. The bacterial broth was standardized to OD600 of 0.1 (1×10^8 CFU/mL) and diluted further to 1×10^6 CFU/mL. The stock solutions of CM extract, CM-AgNPs, and positive control for *S. aureus* (ampicillin), *P. aeruginosa* (tetracycline), *K. pneumoniae,* and *P. mirabilis* (chloramphenicol) were prepared. The treatment wells were added with 50 µL of the test sample and 50 µL of bacterial broth solution. After incubation overnight in a 37°C incubator, microbial growth was determined by adding the triphenyl tetrazolium chloride (TTC) dye by adding 10 µL of 20 mg/mL of TTC into each well and incubated for 30 min in the dark. The absorbance

of each well was also measured at 540 nm (Klancnik *et al*., 2010).

Antibiofilm assay

The antibiofilm assay was conducted using the crystal-violet staining method. The bacteria, *S. aureus*, cultured in tryptic soy broth (TSB), as well as *P. mirabilis* and *K. pneumoniae* in Mueller-Hinton broth (MHB) were incubated at 37°C in a shaker overnight. The bacteria were inoculated in TSB and MHB supplemented with 2% glucose, respectively, and were standardized to OD600 of 0.1 (1×10^8 CFU/mL). Then, 100 µL of the bacterial broth was pipetted into a sterile flat 96-well polystyrene plate. The plate was incubated at 37°C. After 48 hr, the medium with planktonic bacteria was aspirated using a micropipette, and the wells were rinsed with phosphate-buffered saline twice. The treatment wells were added with 100 µL of CM-AgNPs and were left for 24 hr, after which the solution was removed and washed with PBS twice. The biofilm was fixed with 150 µL of 99% methanol for 20 min at room temperature. Then, 150 µL of 1% crystal violet was added into each well and incubated at room temperature for 15 min. After that, the wells were washed under running water and left to air dry. The remaining crystal violet was dissolved with 150 µL of 95% ethanol. The absorbance of the dissolved crystal violet was measured at 590 nm on a microplate reader (Lamret *et al*., 2021). The MICs of the CM-AgNPs were serially diluted and evaluated against *K. pneumoniae*, *S. aureus*, and *P. mirabilis*. The percentage of biofilm eradication was determined by the formula: Biofilm eradication (%) = [(OD growth control wells – OD treatment wells) / OD growth control wells] × 100%

Statistical analysis

The data are presented as mean ± standard deviation (n=3). Statistical analysis was performed using one-way analysis of variance (one-way ANOVA) and multiple comparisons using GraphPad Prism 8.0.

RESULTS AND DISCUSSION

One factor at a time technique was used to determine the ideal parameters for the maximal synthesis of small and monodisperse AgNPs. Color shift from light yellow to dark brown indicated the formation of AgNPs and the nanoparticles formed were further confirmed using UV-Vis spectroscopy. The findings showed that stable and uniformed sized silver nanoparticles can be produced under the optimized conditions of $AgNO_3$: CM (6:4), pH 11, 40°C, 3 hr incubation period using 20 mg/mL of CM extract, and 10 mM $AgNO_3$ (Figure 1). Numerous variables, including temperature, pH, metal concentration, plant extract concentration, and incubation time, can have an impact on the morphology, shape, and size of the AgNPs (Alharbi *et al*., 2022). It was found that the size of AgNPs can be controlled by altering important factors, including temperature, pH, substrate concentration, and time of exposure to the substrate (Alharbi *et al*., 2022). If these parameters are not optimized, it will eventually affect the biological activity of the AgNPs.

FESEM was used to determine the surface shape and size of the AgNPs produced. The FESEM micrograph (Figure 2) showed that the synthesized CM-AgNPs have high density and homogeneous spherical shape. The diameter size distribution of the CM-AgNPs was small, it ranged from 12 to 33 nm, with a mean of 21.13 ± 4.60 nm. This result suggested that the green synthesis of CM-AgNPs was optimized successfully. This is crucial because AgNPs' size guarantees that a sizable surface area will be in touch with bacterial effluent in subsequent antibacterial assays.

The CM-AgNPs exhibited antimicrobial activity against *P. aeruginosa*, *K. pneumoniae, P. mirabilis* (Gram-negative bacteria), and *S. aureus* (Gram-positive bacteria). The MIC of the CM-AgNPs against each tested bacterium was summarized in Table 2. The results also showed that CM extract alone was unable to inhibit bacterial growth at 1 mg/mL (not shown). The findings suggested the potential of CM-AgNPs as an antibacterial agent as it demonstrated a stronger antibacterial activity than its plant extract alone. Several studies have emphasized smaller size AgNPs have better bactericidal activity compared to larger AgNPs due to their large surface area-to-volume ratio, better binding surface, and ability to pass through cell walls or membranes, which allows them to directly affect intracellular components (Bruna *et al*., 2021). Phenolic and flavonoid compounds have been shown to have the ability to alter the membrane's permeability and lead to the destruction of the cell wall, thereby killing the bacteria due to cell lysis (Batubara *et al*, 2021). Therefore, the phytochemical compounds that played a vital role in synthesizing AgNPs can further enhance the antibacterial activity of AgNPs.

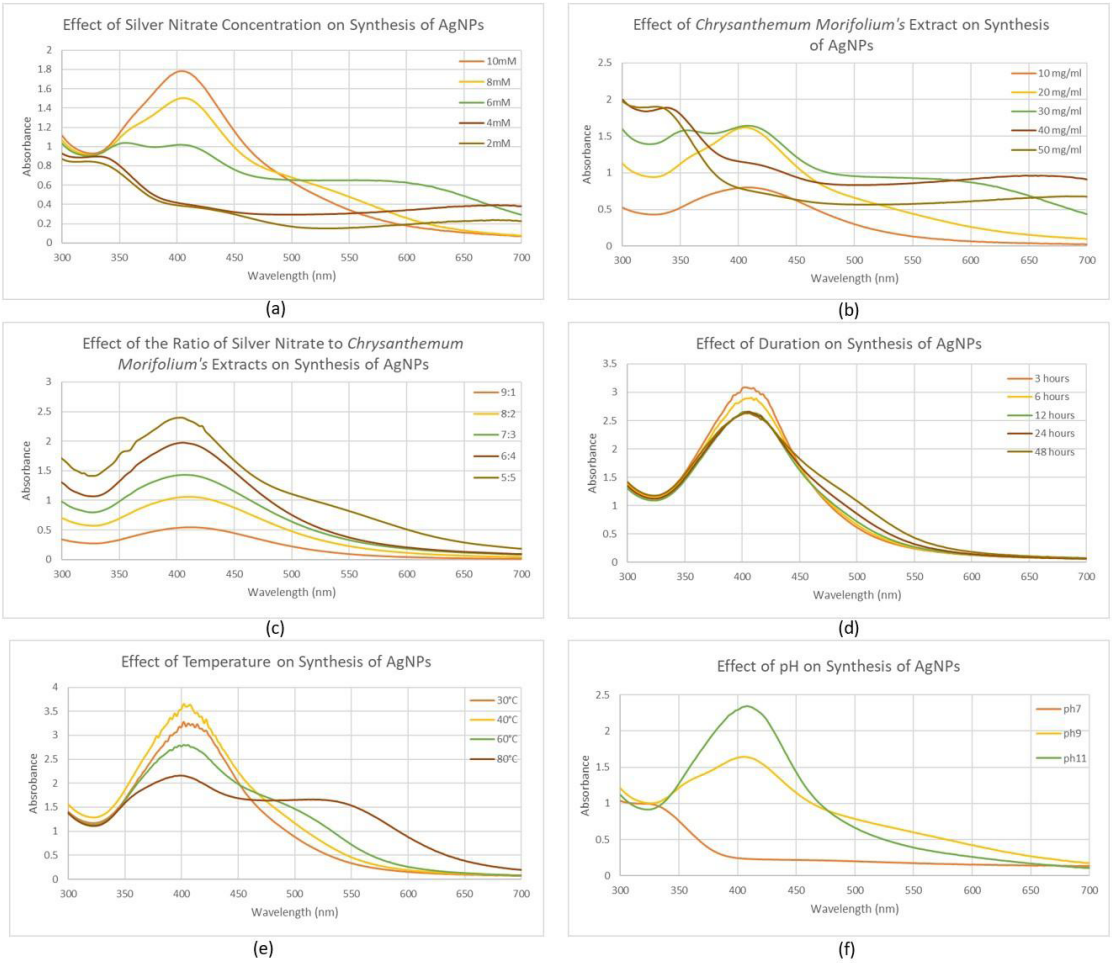

Fig. 1. UV-Vis spectra of CM-AgNPs with (a) varying concentrations of silver nitrate, (b) varying concentrations of CM extract, (c) varying ratios of silver nitrate to CM extract, (d) different reaction times, (e) different reaction temperature, and (f) varying pH of CM extract. CM, *Chrysanthemum morifolium*.

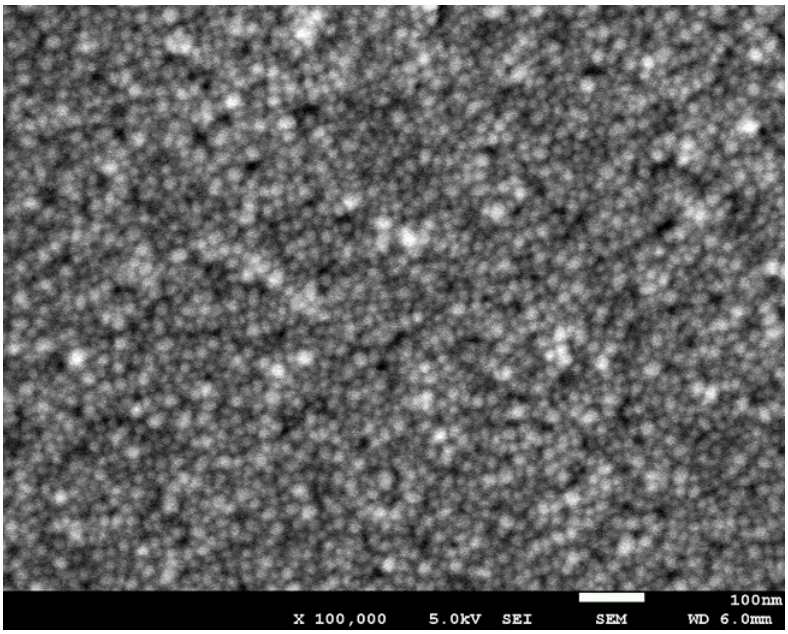

Fig. 2. Field Emission Scanning electron microscope (FESEM) image of CM-AgNPs taken under magnification of 100,000x and 5.0 kV accelerating voltages. Bar = 100 nm.

Table 2. Minimum inhibitory concentration (MIC) and antibiofilm activity of CM-AgNPs

Bacteria strain	Minimum inhibitory concentration (µg/mL)	Antibiofilm activity (%)
K. pneumoniae	93.75 [a]	66.24 ± 1.37 [b]
S. aureus	11.7 [b]	87.31 ± 0.37 [a]
P. aeruginosa	3.9 [c]	-
P. mirabilis	11.7 [b]	49.26 ± 2.88 [c]

Means with different superscript letters show significant differences ($p \leq 0.05$).

The antibiofilm activity of the CM-AgNPs was conducted against *K. pneumoniae*, *S. aureus,* and *P. mirabilis*. It was demonstrated that all three bacteria were inhibited by the CM-AgNPs at respective MIC and also at its subsequent concentration of two-fold dilution (Figure 3). This is because the small size of the nanoparticles enables them to permeate into the three-dimensional structure of biofilm and increase the surface area of AgNPs with microorganisms, both of which boost the antibacterial impact. According to Park *et al*. (2010), AgNPs inactivation of biofilm development is thought to be due to biosorption.

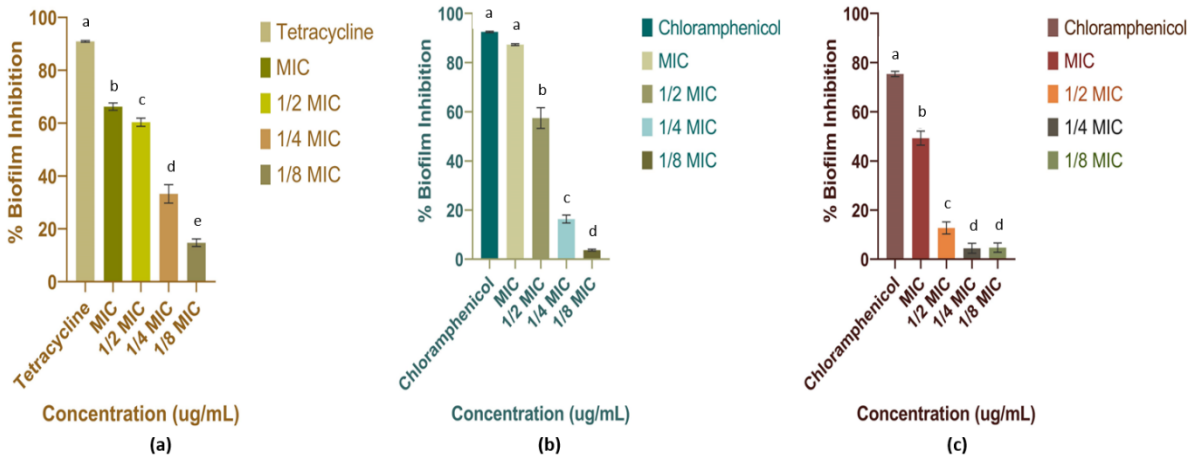

Fig. 3. The percentage of biofilm inhibition against (a) *Klebsiella pneumoniae*, (b) *Staphylococcus aureus,* and (c) *Proteus mirabilis* with CM-AgNPs at concentrations of MIC, ½ MIC, ¼ MIC, and ⅛ MIC and positive control. Bars with different superscript letters show significant differences ($p \leq 0.05$).

CONCLUSION

Stable and uniformed-sized silver nanoparticles can be produced using 10 mM of $AgNO_3$ and 20 mg/mL of CM extract at a 6:4 ratio and 3 hr of incubation period at pH 11 and 40°C. The CM-AgNPs synthesized showed antibacterial and antibiofilm activity, which suggests its potential as a novel antimicrobial agent.

ETHICAL STATEMENT

Not applicable.

ACKNOWLEDGEMENTS

This study was supported by Tunku Abdul Rahman University of Management and Technology Publication Incentive (Vote head number: 86000-4537).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Alharbi, N.S., Alsubhi, N.S. & Felimban, A.I. 2022. Green synthesis of silver nanoparticles using medicinal plants: Characterization and application. Journal of Radiation Research and Applied Sciences, 15(3): 109-124. https://doi.org/10.1016/j.jrras.2022.06.012

Arokiyaraj, S., Valan Arasu, M., Vincent, S., Prakash, N.U., Choi, S.H., Oh, Y.K., Choi K.C. & Kim,

K.H. 2014. Rapid green synthesis of silver nanoparticles from *Chrysanthemum indicum* L. and its antibacterial and cytotoxic effects: An in vitro study. International Journal of Nanomedicine, 9: 379-388. https://doi.org/10.2147/IJN.S53546

Batubara, R., Nelly, I. & Affandi O. 2021. GC-MS analysis of young and mature wild agarwood leaves (*Aquilaria malaccensis* Lamk) and its antioxidant potential, IOP Conference Series Earth and Environmental Science, 912(1): 012038. https://doi.org/10.1088/1755-1315/912/1/012038

Bruna, T., Maldonado-Bravo, F., Jara, P. & Caro, N. 2021. Silver nanoparticles and their antibacterial applications. International Journal of Molecular Sciences, 22(13): 7202. https://doi.org/10.3390/ijms22137202

Chung, I.M., Park, I., Kim, S.H., Thiruvengadam, M. & Rajakumar, G. 2016. Plant-mediated synthesis of silver nanoparticles: Their characteristic properties and therapeutic applications. Nanoscale Research Letters, 11: 40. https://doi.org/10.1186/s11671-016-1257-4

Kalishwaralal, K., BarathManiKanth, S., Pandian, S.R.K., Deepak, V. & Gurunathan, S. 2010. Silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Colloids and Surfaces B: Biointerfaces, 79(2): 340-344. https://doi.org/10.1016/j.colsurfb.2010.04.014

Kandiah, M. & De Silva, L.N. 2021. Microwave-assisted ecofriendly silver nanoparticle synthesis by varieties of *Chrysanthemum morifolium* Ramat: Assessing their antioxidant, photocatalytic and antibacterial activities. Journal of Metals, Materials and Minerals, 31(4): 51-61.

Klancnik, A., Piskernik, S., Jersek, B. & Mozina, S.S. 2010. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. Journal of Microbiological Methods, 81(2): 121-126. https://doi.org/10.1016/j.mimet.2010.02.004

Lamret, F., Varin-Simon, J., Velard, F., Terryn, C., Mongaret, C., Colin, M., Gangloff, S.C. & Reffuveille, F. 2021. *Staphylococcus aureus* strain-dependent biofilm formation in bone-like environment. Frontiers in Microbiology, 12: 714994. https://doi.org/10.3389/fmicb.2021.714994

Liang, W.L., Gong, D. & Zhang, W.K. 2021. The composition of chrysanthemum extracts and their pharmacological functions. STEMedicine, 2(5): 2021. https://doi.org/10.37175/stemedicine.v2i5.69

Melkamu, Z., Jeyaramraja, P.R. & Paulos, T. 2022. Optimization of the synthesis of silver nanoparticles using the leaf extract of *Ocimum sanctum* and evaluation of their antioxidant potential. Nano Express, 3(3): 035006. https://doi.org/10.1088/2632-959X/ac8fac

Nanotechnology Products Database, 2022. https://product.statnano.com/ (accessed 22.8.2022)

Park, E.J., Yi, J., Kim, Y., Choi, K. & Park, K. 2010, Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. Toxicology in Vitro, 24(3): 872-878. https://doi.org/10.1016/j.tiv.2009.12.001

Rafique, M., Sadaf, I., Rafique, M.S. & Tahir, M.B. 2017. A review on green synthesis of silver nanoparticles and their applications. Artificial Cells, Nanomedicine, and Biotechnology, 45: 1272-1291. https://doi.org/10.1080/21691401.2016.1241792