Vol.66(spe): e23220989, 2023
https://doi.org/10.1590/1678-4324-ssbfar-2023220989
ISSN 1678-4324 Online Edition
II South-Brazilian Symposium on Pharmacognosy

Article - Health Science/Pharmacognosy

Silver Nanoparticles Green Synthesis from *Catharanthus roseus* Flowers and Effect on A549 Lung Cancer Cells

Marcela Novak Gumy[1]*
https://orcid.org/0000-0001-5476-5816

Carla Cristine Kanunfre[2]
https://orcid.org/0000-0002-2865-3084

Josiane de Paula Padilha[1]
https://orcid.org/0000-0002-2717-8867

Luíza Stolz Cruz[1]
https://orcid.org/0000-0001-6705-0967

Patrícia Mathias Döll Boscardin[1]
https://orcid.org/0000-0001-6092-043X

[1]Universidade Estadual de Ponta Grossa, Departamento de Ciências Farmacêuticas, Ponta Grossa, Paraná, Brasil; [2]Universidade Estadual de Ponta Grossa, Departamento de Biologia Geral, Ponta Grossa, Paraná, Brasil

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Jane Manfron Budel

Received: 19-Dec-2022; Accepted: 07-Aug-2023

*Correspondence: marcelagumy@gmail.com; Tel.: +55-42-998000619

HIGHLIGHTS

- The aqueous extract of *Catharanthus roseus* (L.) G Don flowers can successfully produce silver nanoparticles (AgNPs) in a simple and fast way.
- The syntethised AgNPs presented higher cytotoxic activity against A549 lung cancer cells compared to the *C. roseus* flower extract.

Abstract: Silver nanoparticles (AgNPs) have extensive applications in nanomedicine due to their physicochemical properties and interactions with biomolecules. One way of obtaining them is through green synthesis with plant extracts containing reducing and capping agents, such as *Catharanthus roseus* (L.) G Don. This plant has pharmacological applications due to its phytocompounds with biological activity. The *C. roseus* flowers are rich in alkaloids and phenolic compounds, and have the potential to synthesize AgNPs but have never been used with this purpose. In this study, AgNPs were synthesize with the aqueous extract from the *C. roseus* flowers, and their cytotoxicity evaluated on A549 lung tumor cells through MTT assay. The AgNP synthesis could be seen visually from the solution's color change and through UV-visible spectroscopy to detect a peak between 400 - 530 nm. FEG microscopy showed AgNPs with variable shapes and sizes. In vitro cytotoxicity assay against A549 cells showed that the *C. roseus* extract have low toxicity to the lineage, and AgNPs reduced half the viable cells at the 1.5% concentration, demonstrating high cytotoxicity. Therefore, AgNPs from *C. roseus* were successfully obtained and showed significant cytotoxic activity on A549 lung cancer cells.

Keywords: Green synthesis; nanotechnology; silver nanoparticles; sustainability; cytotoxicity.

INTRODUCTION

Nanomedicine is one of the fields where nanotechnology has extensive applications with nanomaterials and devices designed for diagnosis, treatment, pain relief, and overall preservation and improvement of

health. The application of silver nanoparticles (AgNPs) in nanomedicine is possible due to their physicochemical properties, interactions with biomolecules on both the surface and inside of the cells, size, and natural transport uptake mechanisms of the drug by the diseased cells [1-7].

The green synthesis of metallic nanoparticles is an eco-friendly alternative to the use of precursor chemicals and solvents like sodium borohydrate, ascorbate, hydrazine, and sodium citrate [2, 9]; and possible generation of toxic byproducts. This technique uses biological agents such as plants and microorganisms or their parts to synthesize nanoparticles both extracellular and intracellular. In this approach, plants extracts are a great resource for nanoparticles synthesis by providing natural reducing and capping agents such as monosaccharides, sapogenins, flavonoids, and other phytochemicals [7-11].

Catharanthus roseus (L.) G Don (Family: Apocynaceae) is an herbaceous plant known by her alkaloids vincristine and vimblastine with antineoplastic properties, besides other several active phytocompounds, such as organic acids, flavonoids, tannins, saponins, glycosides and terpenoids. Originated from Madagascar, this plant has now worldwide distribution, and is widely cultivated as an ornamental plant and herbal medicine for antibacterial, antifungal, wound healing, antiplasmodial, antioxidant, antiviral, and anticancer activities [5, 7, 10-12].

Reports have confirmed AgNPs synthesis using *Catharanthus roseus* (L.) G Don leaf [5] and root extracts [14], but the use of *C. roseus* flowers have not yet been reported. The *C. roseus* flowers contain the anthocyanidin pigment rosinidin and are especially rich in alkaloids and phenolic compounds (Table 1) [10, 16-21]. The phytochemical composition of *C. roseus* provides flowers with great potential to synthesize AgNPs.

Therefore, the present work aimed the green synthesis of silver nanoparticles (AgNPs) using *Catharanthus roseus* (L.) G Don aqueous flowers extract and cytotoxicity evaluation on A549 tumor cells.

Table 1. Alkaloids and phenolic compounds on *C. roseus* flowers

Class	Compound Name	References
Alkaloids	14′,15′-Didehydrocyclovinblastine	[16-21]
	17-Deacetoxycyclovinblastine	
	17-Deacetoxyvinamidine	
	Cathachunine	
	Catharanthine	
	Catharoseumine	
	Clovinblastine	
	Cycloleurosine	
	Leurosidine	
	Leurosine	
	Methylvingramine	
	Ocyclovinblastine	
	Perivine,	
	Tricin (Flavones)	
	Vinamidine	
	Vindoline	
	Vingramine,	

Cont. Table 1

Phenolic Compounds	4-O-caffeoylquinic acid Hirsutidin-3-O-(6-O-p-coumaroyl) Hirsutidin-3-O-glucosides Isorhamnetin-3-O-(6-O-rhamnosyl-galactoside) Isorhamnetin-3-O-(6-O-rhamnosyl-glucoside) Kaempferol-3-O-(2,6-di-O-rhamnosyl-galactoside) Kaempferol-3-O-(2,6-di-O-rhamnosyl-glucoside) Kaempferol-3-O-(6-O-rhamnosyl-galactoside) Kaempferol-3-O-(6-O-rhamnosyl-glucoside) Malvidin-3-O-(6-O-p-coumaroyl) Malvidin-3-O-glucosides Petunidin-3-O-(6-Op-coumaroyl) Quercetin-3-O-(2,6-di-O-rhamnosyl-galactoside)	[14-17, 21]

MATERIAL AND METHODS

Preparation of *C. roseus* extract

Catharanthus roseus (L.) G Don, Apocynaceae, flowers were collected from Palmeira, Parana, Brazil (25° 26′ 3″ S, 49° 59′ 60″ W; 867 m above sea level). Representative samples can be found at the Federal University of Technology – Paraná of Dois Vizinhos Herbarium under the register DVPR000082. The fresh flowers were de-stemmed, displayed on kraft paper, then dried in a forced air circulation oven at 60º for 24 hours. The extract was prepared using 2.5 grams of the dried flowers in 100 mL distilled water; the mixture was heated at 80 ± 5°C for 20 minutes, and then filtered with a paper filter. The extract concentration was adjusted to 1%, 1.5%, 2%, and 2.5% with volumetric flasks.

Green synthesis of silver nanoparticles

1 mL *C. roseus* flower extract was mixed with 9 mL AgNO3 1mM. The solution was exposed to heat (70 ± 5°C) and UV-light (365 - 405 nm) for 10 minutes. The conversion of silver nitrate ($AgNO_3$) into silver nanoparticles (AgNPs) was accompanied by the solution color change and record absorbance at 200-800 nm using a UV-visible spectrophotometer (Genesys 10S UV-Vis) [22].

Characterization of green synthesized silver nanoparticles

AgNPs were characterized by Field Emission Gun-Scanning Electron Microscopy (FEG-SEM) (Mira 3 / Tescan), UV-visible spectrophotometry (Genesys 10S UV-Vis), Dynamic light scattering (DLS) with Polydispersion Index (PDI) and Zeta Pontential (Zetasizer Nano ZS90).

Cell Viability Assay

A549 cells were maintained at 37 °C under 5% CO2 and 100% humidity in DMEM and supplemented with 10% fetal calf serum and antibiotics (200 µl/mL penicillin G). After sufficient growth, the cells detached using trypsin, plated in 96-cluster well culture plates, and incubated for 24 h at 37 °C under 5% CO2. Later, the medium was removed and the cells exposed to AgNPs (from 0,1 to 50% v/v) and *C. roseus* flower extract (from 0,004 to 3% m/v) for 24 h (n=8 for each concentration). As negative control, only culture medium was used. After the incubation period the liquid was removed from each well and added MTT reagent, incubated for 1 h and then discarded. 100 µL of isopropanol was added and the absorbance was measured using a microplate reader at a wavelength of 570 nm [23].

RESULTS AND DISCUSSION

Green synthesis of silver nanoparticles

The silver nanoparticles (AgNPs) synthesis from the *Catharanthus roseus* (L.) G Don flower extract was seen visually from the graduating change of the solution's color from a yellowish transparent to reddish-brown presented in Figure 1. The formation of the AgNPs was monitored through UV-Visible spectral analysis recorded between 200-800 nm, and the appearance of a peak around 420 nm confirmed the synthesis (Figure 2). Different concentrations of extract tested (1%, 1.5%, 2%, 2.5%) produced similar results, therefore the smallest was chosen.

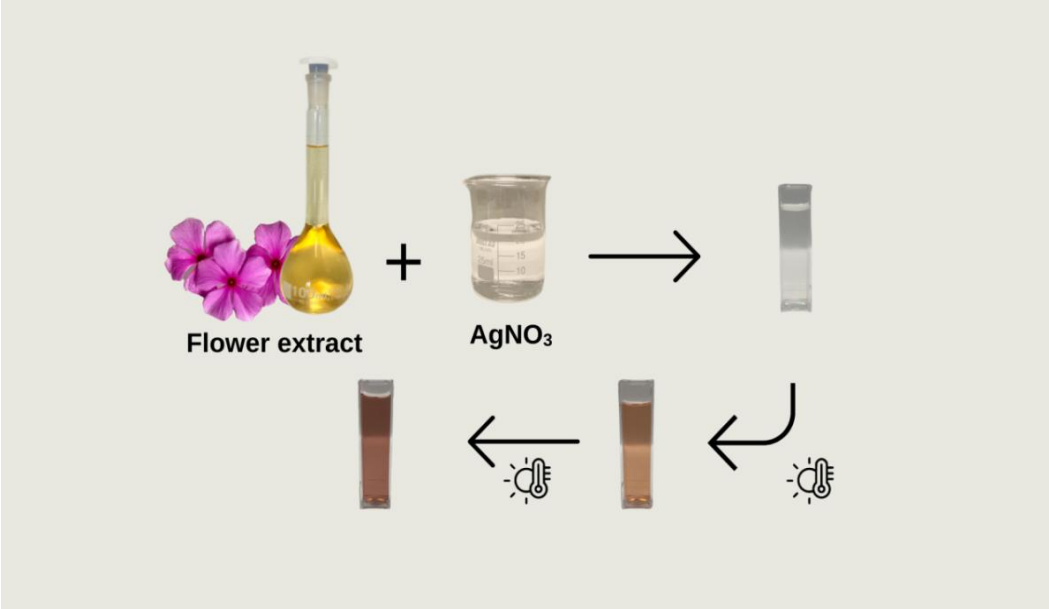

Figure 1. Process of AgNPs synthesis with *C. roseus* flower extract and AgNO using UV-light and heat evidencing graduate solution's color change yellowish transparent to reddish-brown that indicated the successful synthesis.

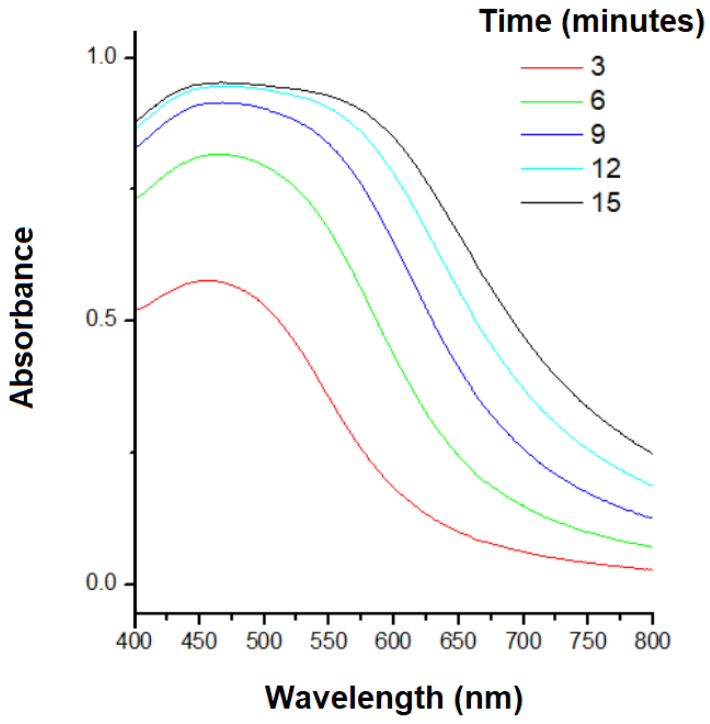

Figure 2. UV-Vis absorption spectra of AgNPs synthesized from *C. roseus* flowers.

As reported in the literature [1, 8, 24, 25], the color change from transparent to a brownish color and UV-visible spectroscopy techniques used to track the formation of AgNPs through an increase in the absorbance between 400 and 530 nm is possible due to the surface plasmon resonance of the nanometal [26]. These techniques are often used as an indicator of the formation of nanoparticles. The AgNP synthesis was made using heat, UV-light, and UV-light and heat simultaneously; during the process was observed a faster change of color with UV-light plus heat indicating an optimization of the AgNP formation. Through UV-Vis spectroscopy was possible to track the complete synthesis in approximately 10 min (Figure 2).

The biosynthesis mechanism of AgNPs by green synthesis is not fully understood but likely related to the chemical composition of the extracts, in which metabolites such as monosaccharides, sapogenins, polyphenols, and others might convert silver ions to elemental silver, and they might act as stabilizing agents for the nanoparticles as well [5-10]. Studies have shown that proteins might also be involved in the synthesis process [25].

The diagram presented in Figure 3 illustrates a possible synthesis mechanism of AgNPs accomplished in four main steps. It starts with the reduction of silver ion (Ag^{+}) to molecular silver (Ag^{0}), followed by a nucleation process. The third step consists of growth with coalescing metallic silver atoms into AgNPs, and finally, the fourth step is the stabilization that prevents further growth and agglomeration and might involve the extract metabolites.

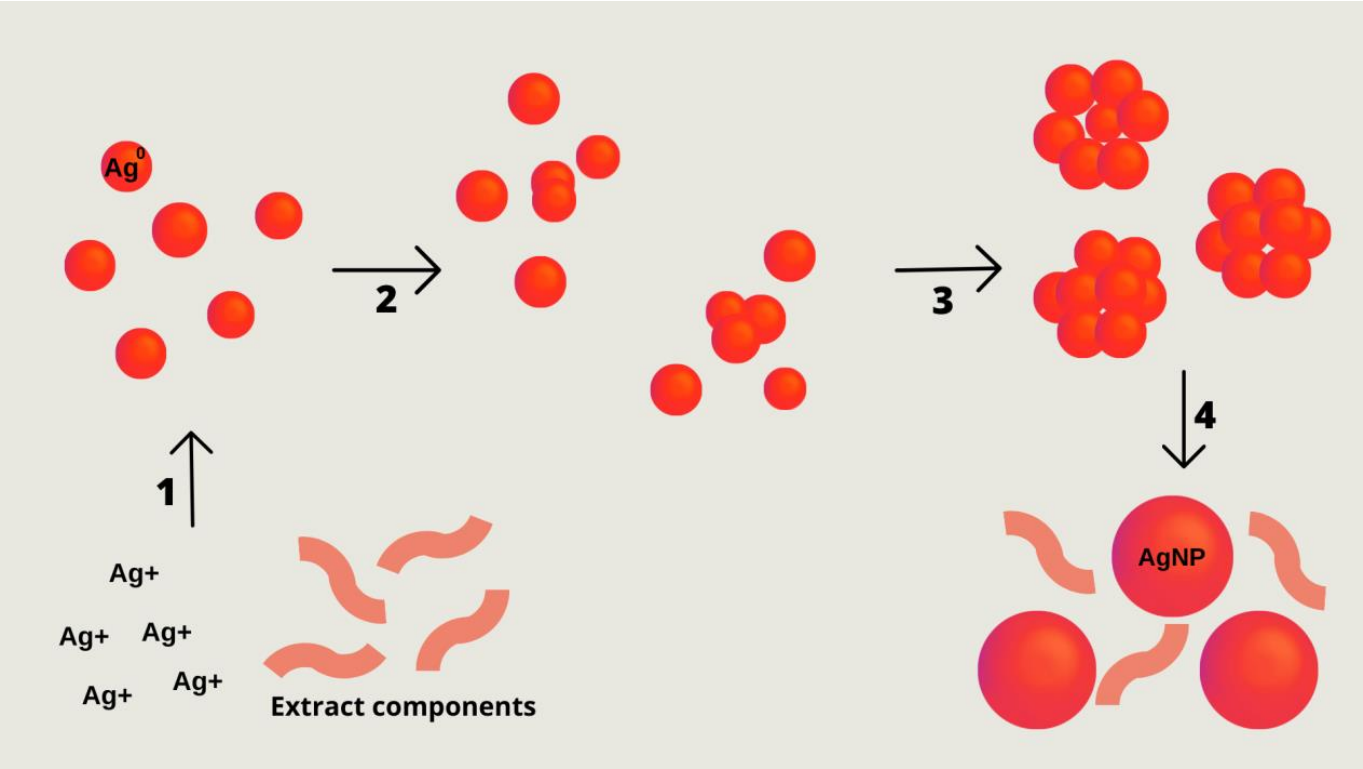

Figure 3. The schematic diagram represents a possible mechanism of AgNPs synthesis from *C. roseus* flower extract. (1) Reduction of Ag+ to Ag0 by extract components; (2) Nucleation of molecular silver, starting the AgNP formation; (3) Growth with coalescing metallic silver atoms into AgNPs; (4) Stabilization of the AgNP by extract components and physical forces.

Characterization of green synthesized silver nanoparticles

FEG-SEM confirm the synthesis of the AgNP through photomicroscopy and inform about their morphology, size, and distribution. The results obtained showed nanoparticles with diameter ranging from 70 to 100 nm and different shapes such as spherical, rod, triangle, rhombic, and prism-like structures (Figure 4). The different AgNPs sizes and shapes can be the result of a number of factors such as precursor concentration, extract composition, time of incubation, pH, temperature, as well as method of preparation [9, 27-29].

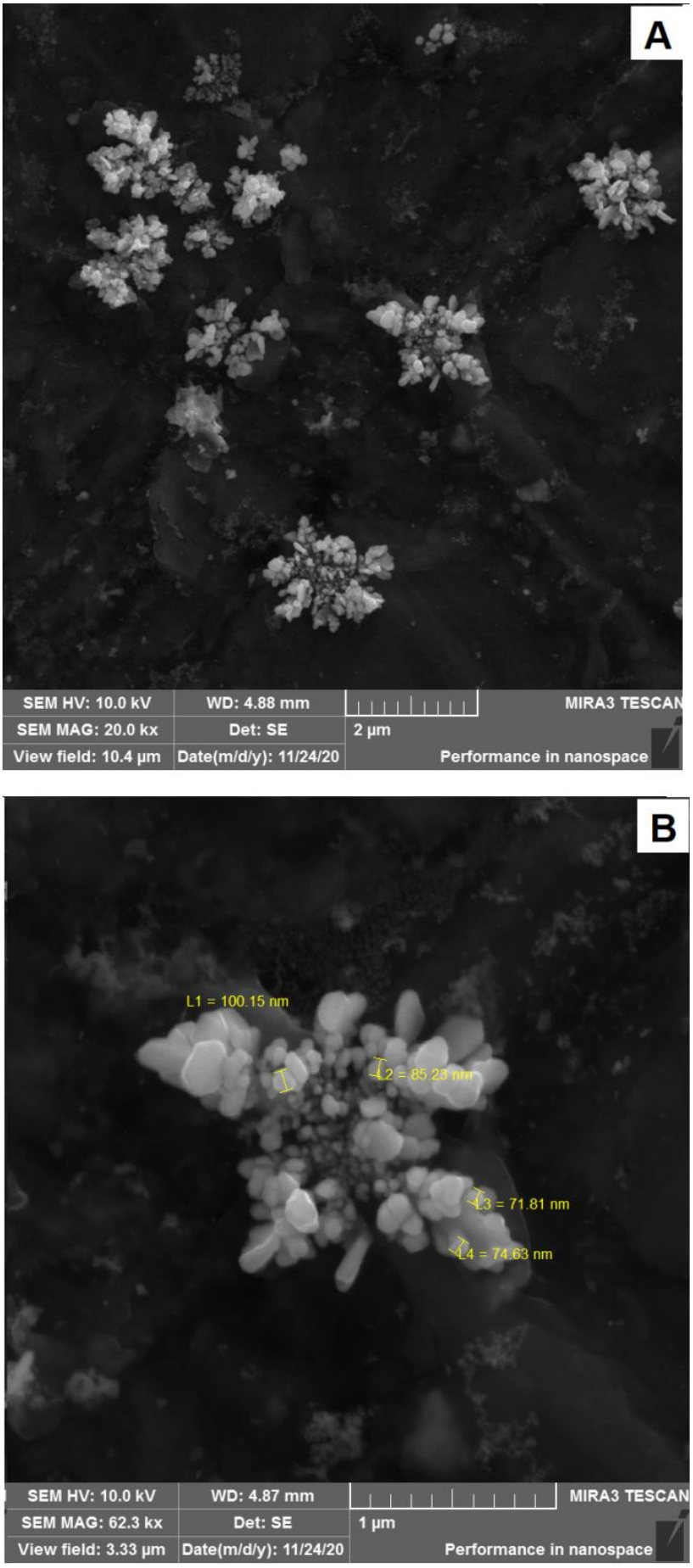

Figure 4. FEG microscopy of the synthesized AgNPs. (A) x20.0 k/scale. (B) x62,3 k/scale.

To confirm the hydrodynamic diameter of nanoparticles in solution and size distribution profile, the Dynamic Light Scattering (DLS) and Polydispersion Index (PDI) was used. The Figure 5 shows one of the DLS and PDI results, where it is observed only one population of AgNPs with average size of 158,4 nm and PDI of 0,210. After three repetitions the average size was 161 nm (± 31,05) and PDI of 0,230 (± 0,016). The ideal value for the DLS is under 1 micrometer and for the PDI under 0,300 [30, 31]. Therefore, the results confirm the synthesis of homogeneous nanoparticles.

The zeta potential is related to the stability of the synthesized AgNPs. The zeta potential - 16,6 mV (± 2,15) was verified by using water as dispersant (Figure 6). The negative potential value shown by the AgNPs could be due to the extract components that contain negative charge. The ideal values to affirm the AgNPs electro stability should be around – 30 mV or + 30 mV [9, 30, 32]. Nonetheless the zeta potential alone is not enough to determine the overall stability, and other factors such as the presence of stabilizing agents in the solution have to be taken under consideration.

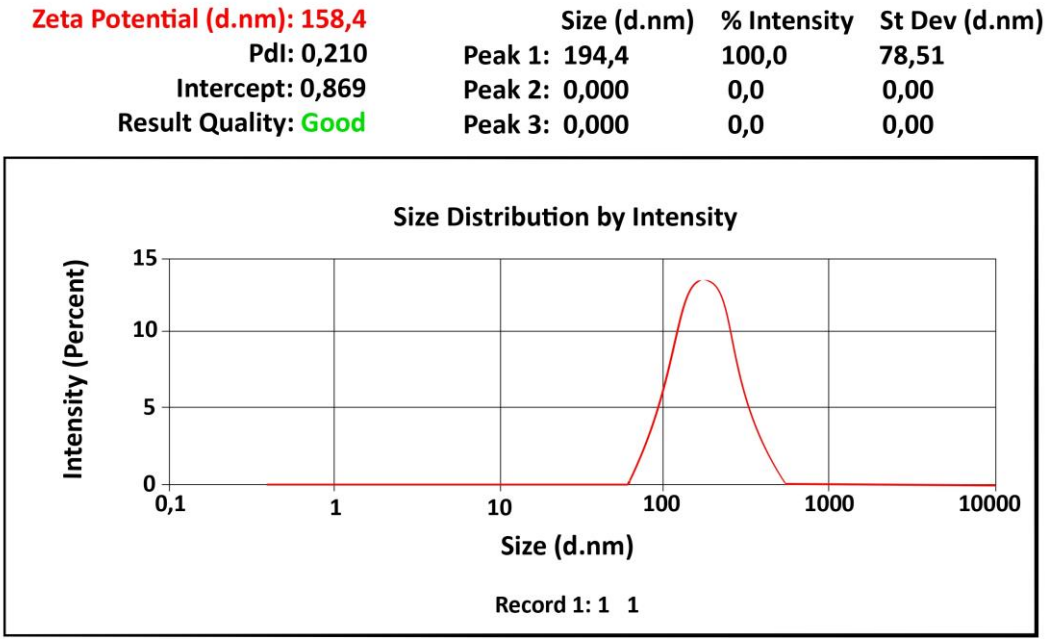

Figure 5. DLS and PDI of the synthesized AgNPs.

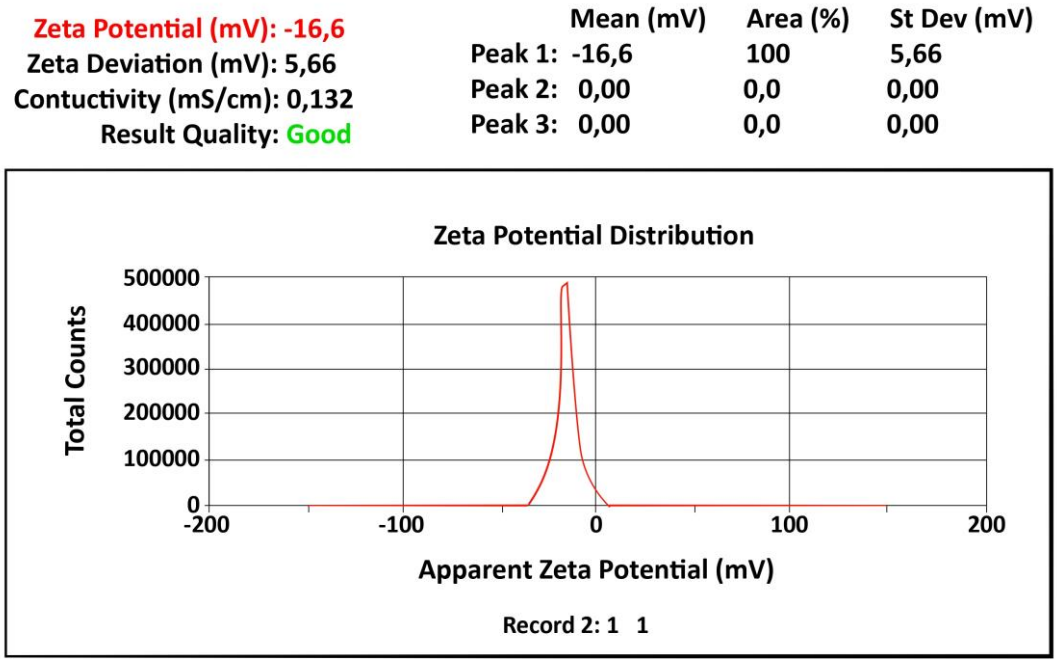

Figure 6. Zeta potential of the synthesized AgNPs.

Cell Viability Assay

In vitro cytotoxicity studies against A549 cells were performed with different concentrations of *C. roseus* extract and AgNPs with MTT assay to measure cell viability and proliferation. The obtained results are presented in Figure 7. It is observed that none of the extract flower concentrations tested in this study exhibited a reduction of viable cells equal or superior to 50%, indicating low cytotoxicity from aqueous extract of *C. roseus* flowers in the given conditions on A549 cellular lineage.

The results for the AgNPs showed a concentration-dependent decrease in the cell viability until 2,5%, with a decreasing percentage of viable cells observed as the concentration of AgNPs increased. A reduction of half the viable cells was observed around 1.5% of AgNPs, indicating high cytotoxicity. Others studies about the effects of green synthesized AgNPs on lunger cancer cells also demonstrated high cytotoxic activities [7, 34].

AgNPs are mainly known for their potent antibacterial activity [4, 6], but also possess cytotoxic activity and potential as agents for cancer therapy, as shown by Yesilot and Aydin [35] in a review article. These activities are possible due to the AgNPs' small size that enables them to enter the cells and capacity to reduce the activity of antioxidant enzymes from mitochondrial respiratory chain, production of free radicals, and disruption of the oxidative state of the cells leading to their death by oxidative stress [9, 34, 35].

The small size of AgNPs also implies a large surface area that allows the coordination of a vast number of ligants with diagnosis and therapeutic finalities that can be explored for medical applications, giving AgNPs great potential as caring agents for targeted and controlled release of drugs, markers and others [6]. But further studies assessing potential efficacy, biosafety, and biodistribution of AgNPs are necessary.

Therefore this study demonstrated that the synthesis of silver nanoparticles from *C. roseus* is possible, simple, fast, and ecofriendly. The AgNPs presented higher cytotoxic activity against A549 lung cancer cells than the extract in the tested concentrations, meaning that this activity is mainly attributed to the AgNPs and not the extract components.

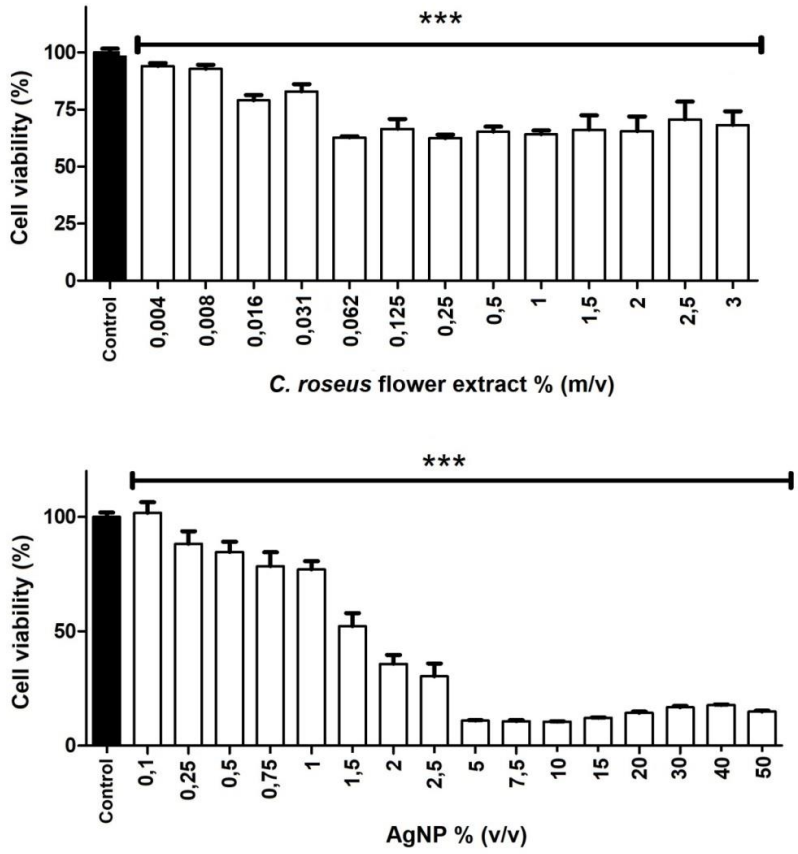

Figure 7. Cell viability of A549 lunger cancer human cells after treatment with *C. roseus* extract and AgNPs at 24 h determined via MTT reduction assay. Results presented as mean ± standard error of mean (n=8) and analysed using one-way ANOVA followed by Tukey's post hoc test of significance $p < 0.001$ (***) compared to negative control.

CONCLUSION

The present study showed that the aqueous extract of *Catharanthus roseus* (L.) G Don flowers successfully produce silver nanoparticles (AgNPs) in a simple, fast, and ecofriendly process; with high cytotoxic activity against A549 lung cancer cells. Therefore, the AgNPs obtained from the aqueous extract of *Catharanthus roseus* (L.) G Don flowers demonstrate to be a potential candidate for further studies evolving cancer therapy.

Funding: This research received no external funding.
Acknowledgments: State University of Ponta Grossa (UEPG); C-Labmu (UEPG); Araucaria Foundation; CNPq for providing instruments, facilities and other items used in this study, and for the incentives for research and scientific production in Brazil.
Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Chandrasekharan S, Chinnasamy G, Bhatnagar S. Sustainable phyto-fabrication of silver nanoparticles using *Gmelina arborea* exhibit antimicrobial and biofilm inhibition activity. Sci Rep. 2022 Jan; 12(1): 156.
2. Hasan KMF, Xiaoyi L, Shaoqin Z, Horváth PG, Bak M, Bejó L, et al. Functional silver nanoparticles synthesis from sustainable point of view: 2000 to 2023 – A review on game changing materials. Heliyon. 2022 Dec; 8 (12).
3. Jadoun S, Arif R, Jangid NK, Meena R. Green synthesis of nanoparticles using plant extracts: a review. Environ Chem Lett. 2021 Feb; 19, 355–74.
4. Keshari AK, Srivastava A, Chowdhury S, Srivastava, R. Antioxidant and Antibacterial Property of Biosynthesized Silver Nanoparticles. Nanomed Res J. 2021 Jan; 6 (1):17-27.
5. Lakkim V, Reddy MC, Pallavali RR, Reddy KR, Reddy CV, Inamuddin, et al. Green Synthesis of Silver Nanoparticles and Evaluation of Their Antibacterial Activity against Multidrug-Resistant Bacteria and Wound Healing Efficacy Using a Murine Model. Antibiotics (Basel). 2020 Dec; 9(12):902.
6. Lee SH, Jun BH. Silver Nanoparticles: Synthesis and Application for Nanomedicine. Int. J. Mol. Sci. 2019 Feb; 20 (4):865.
7. Jha M, Shimpi NG. Green synthesis of zero valent colloidal nanosilver targeting A549 lung cancer cell: in vitro cytotoxicity. J Genet Eng Biotechnol. 2018 Jun; 16(1): 115–24.
8. Kanchana P, Hemapriya V, Arunadevi N, Sundari SS, Chung M, Prabakaran M. Phytofabrication of silver nanoparticles from *Limonia acidissima* leaf extract and their antimicrobial, antioxidant and its anticancer prophecy. Journal of the Indian Chemical Society. 2022 Oct; 99 (10):100679.
9. Gontijo LAP, Raphael E, Ferrari DPS, Ferrari JL, Lyon LP, Schiavon MA. pH effect on the synthesis of different size silver nanoparticles evaluated by DLS and their size-dependent antimicrobial activity. Revista Matéria. 2020; 25 (04).
10. Durán N, Rolim WR, Durán M, Fávaro WJ, Seabra AB. [Nanotoxicology of siver nanoparticles: toxicity in animals and humans]. Quím. Nova. 2019 Feb; 42 (2).
11. Singh C, Anand SK, Upadhyay R, Pandey N, Kumar P, Singh D, et al. Green synthesis of silver nanoparticles by root extract of *Premna integrifolia* L. and evaluation of its cytotoxic and antibacterial activity. Mater Chem Phys, 2023 Mar; 297:127413.
12. Acharjeea S, Kumarb R, Kumar N. Role of plant biotechnology in enhancement of alkaloid production from cell culture system of *Catharanthus roseus*: A medicinal plant with potent anti-tumor properties. Ind Crop Prod. 2022 Feb; 176:114298.
13. Kumar S, Singh B, Singh R. *Catharanthus roseus* (L.) G. Don: A review of its ethnobotany, phytochemistry, ethnopharmacology and toxicities. J Ethnopharmacol. 2022 Feb; 284:114647.
14. Das A, Sarkar S, Bhattacharyya S, Gantait S. Biotechnological advancements in *Catharanthus roseus* (L.) G. Don. Appl Microbiol Biotechnol. 2020 Jun; 104 (11): 4811-4835.
15. Subha V, Ravindran E, Kumar ABH, Renganathan S. Bactericidal effect of silver nanoparticles from aqueous root extracts of *Catharanthus roseus*. Int J Nanoparticles. 2019 Jan; 11 (4): 294.
16. Chaturvedi V, Goyal S, Siddiqui MM, Meghani M, Patwekar F, Patwekar M, et al. A Comprehensive Review on *Catharanthus roseus* L. (G.) Don: Clinical Pharmacology, Ethnopharmacology and Phytochemistry. J. Pharmacol. Res. Dev. 2022 Sep; 4 (2).
17. Mohammad FS, Patwekar M, Patwekar F. Are plant-derived flavonoids the emerging anti-coronavirus agents? Theranostics and Pharmacol Sci. 2021 Apr; 4 (2).
18. Lee ON, Ak G, Zengin G, Cziáky Z, Jekó J, Rengasamy KRR, et al. Phytochemical composition, antioxidant capacity, and enzyme inhibitory activity in callus, somaclonal variant, and normal green shoot tissues of *Catharanthus roseus* (L) G. Don, Molecules. 2020 Oct; 25(21):4945.
19. Rajeev R, Kumar M. Biochemical profile of *Vinca rosea* Linn (*Catharanthus roseus* G. Don). J Biotechnol Biochem. 2020 Oct; 6(5):13-26.

20. Kumar S, Singh A, Kumar B, Singh B, Bahadur L, Lal M. Simultaneous quantitative determination of bioactive terpene indole alkaloids in ethanolic extracts of *Catharanthus roseus* (L.) G. Don by ultra high performance liquid chromatography–tandem mass spectrometry. J. Pharm. Biomed. 2018 Mar; 151:32-41.
21. Pham HNT, Sakoff JA, Vuong QV, Bowyer MC, Scarlett CJ. Screening phytochemical content, antioxidant, antimicrobial and cytotoxic activities of *Catharanthus roseus* (L.) G. Don stem extract and its fractions. Biocatal Agric Biotechnol. 2018 Oct; 16:405-11.
22. Justus B, Arana AFM, Gonçalves MM, Boscardin PMD, Kanufre CC, Budel JM, et al. Characterization and Cytotoxic Evaluation of Silver and Gold Nanoparticles Produced with Aqueous Extract of *Lavandula dentata* L. in Relation to K-562 Cell Line. Braz Arch Biol Technol. 2019; 62:e19180731.
23. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983 Dec; 65(1-2):55-63.
24. Mohamed TK, Widdatallah MO, Ali MM, Alhaj AM, Elhag DA. Green Synthesis, Characterization, and Evaluation of the Antimicrobial Activity of *Camellia sinensis* Silver Nanoparticles. J Nanotechnol. 2021 Oct; 2021.
25. Cataldo F. Green synthesis of silver nanoparticles by the action of black or green tea infusions on silver ion. Eur Chem Bull. 2014 Jan; 3:280-9.
26. Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. Langmuir. 1996 Feb; 12(3):788–800.
27. Jain S, Mehata MS. Medicinal plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. Sci rep. 2017 Nov; 7(1):15867.
28. Mukherji S, Bharti S, Shukla G, Mukherji S. Synthesis and characterization of size- and shape-controlled silver nanoparticles. Phys Sci Rev. 2018 Aug; 4(1).
29. Zielonka A, Klimek-Ocha M. Fungal synthesis of size-defined nanoparticles. Adv Nat Sci: Nanosci Nanotechnol. 2017 Aug; 8(4).
30. Khane Y, Benouis K, Albukhaty S, Sulaiman GM, Abomughaid MM, Ali AA, et al. Green Synthesis of Silver Nanoparticles Using Aqueous *Citrus limon* Zest Extract: Characterization and Evaluation of Their Antioxidant and Antimicrobial Properties. Nanomaterials. 2022 Jun; 12 (12).
31. Kulikouskaya V, Hileuskaya K, Kraskouski A, Kozerozhets I, Stepanova E, Kuzminski I, et al. Chitosan-capped silver nanoparticles: A comprehensive study of polymer molecular weight effect on the reaction kinetic, physicochemical properties, and synergetic antibacterial potential. SPE Polymers. 2022 Feb; 3(2):77-90.
32. Alshameri AW, Owais M, Altaf I, Farheen S. *Rumex nervosus* mediated green synthesis of silver nanoparticles and evaluation of its in vitro antibacterial, and cytotoxic activity. OpenNano. 2022 Nov; 8.
33. Priya RS, Geetha D, Ramesh PS. Antioxidant activity of chemically synthesized AgNPs and biosynthesized *Pongamia pinnata* leaf extract mediated AgNPs – a comparative study. Ecotoxicol Environ Saf. 2016 Dec; 134:308-18.
34. Venugopal K, Rather HA, Rajagopal K, Shanthi MP, Sheriff K, Illiyas M, *et al.* Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of *Syzygium aromaticum*. J Photochem Photobiol B. 2017 Feb; 167:282-9.
35. Yesilot S, Aydin C. Silver Nanoparticles; A New Hope In Cancer Therapy? East J Med. 2019; 24(1):111-6.