Research Article

Biosynthesis and Characterization of Nanoparticles from *Cheilocostus speciosus* Rhizome Plant Extracts

Shalet Varghese, Baskaran. K*, Haseera. N, Nirmala Devi. N
Department of Biochemistry, Sree Narayana Guru College, Coimbatore, Tamilnadu, India.
***Corresponding author's E-mail:** baskar.bio86@gmail.com

Received: 14-07-2024; **Revised:** 28-10-2024; **Accepted:** 09-11-2024; **Published on:** 20-11-2024.

ABSTRACT

This study was aimed to analyze the anti-cancer activity of silver nanoparticles (AgNPs) synthesized using five different solvent extracts from the rhizome of *Cheilocostus speciosus*. Synergistic five different solvent extract of rhizome of *Cheilocostus speciosus* was used to biosynthesis of AgNPs. Characterization of AgNPs was performed using UV–visible spectroscopy, DLS and SEM analyses. Anti-cancer activity of AgNPs against SK-MEL-28 cells was tested using MTT assay. UV–Visible spectroscopy analysis indicated the surface plasmon resonance (SPR) sharp peak at 350–430 nm wavelength that corresponds to the production of AgNPs. SEM analysis showed that AgNPs are in a spherical shape with a size of 42–61 nm. Anti-cancer study reveals that AgNPs had shown cytotoxicity against SK-MEL-28 cells at the concentrations ranged from 25 to 500 µg/mL and IC50 at 150.8 µg/mL. This study concludes that AgNPs synthesized using rhizome of of *Cheilocostus speciosus* possesses potential anti-cancer activity.

Keywords: Silver nanoparticles, Characterizations, of *Cheilocostus speciosus*, cancer activity.

INTRODUCTION

Medicinal plants are being used for human health care management in traditional medicine for centuries. High demand in the products from medicinal plants increased the commercial production and formulation of various herbal-based cosmetics and nutritional supplements[1]. Zingiberaceae family has around 52 plant genera and more than 1300 plant species, and also, many of these plant products were in use for various human health benefits[2]. Some of the Zingiberaceae plants were reported for their therapeutic usage[3]. Among those, *Cheilocostus speciosus* are the most important medicinal plants, and they have possessed a variety of medicinal values. *Cheilocostus speciosus* is an aromatic plant, commonly known as turmeric. In Ayurveda and Siddha, the vegetal root of *Cheilocostus speciosus* has been used for the production of various pharmaceutical formulations for the different ailments, including wounds, acne, common cold, parasitic infections, urinary tract infection, and liver diseases[4]. This plant also possesses various important medicinal properties like antioxidant, anti-inflammatory, antibacterial, anti-human immunodeficiency virus properties[5,6]. Polyphenol curcumin is a vital phytocompound responsible for the pharmacodynamics action of *C. longa*.[7,8] Mainly, it has potential anti-cancer property against liver, pancreatic, colon, cervical, lung, brain, breast, and bone cancers.[9,10]

Another most important medicinal plant is *Cheilocostus speciosus*, generally called as Ginger. The rhizome of this plant is widely used as a nutraceutical and in the formulation of folk medicine. In Ayurvedic and Chinese traditional systems of medicines, *Cheilocostus speciosus* has being used for treating indigestion, arthritis, rheumatism, fever, and microbial infections[11]. Asgingerols, zingerone, shogaols, paradols, and gingerdiols are the major phytochemical constituents that have been reported for its antioxidant, anti-inflammatory, antihyperglycemic, immunomodulatory, anti-cancer, and cardioprotective properties[12, 13].

Medicinal plants-based synthesis of nanoparticles has various biological benefits since they have no toxic chemicals and biological compounds as capping agents[14]. Plant-based synthesized AgNPs are having strong antimicrobial activity and they are widely used as an ingredient in the pharmaceutical industry for preparation of human health care medicines[15, 16]. Biological synthesis of NPs is an environmental friendly method than chemical, electrochemical[17], radiation[18], photochemical methods.[19] The biological and medicinal properties of NPs are mostly determined by various physical properties like particle size, structure, and crystallinity composition. AgNPs are interacting with cells and regulate active and passive cellular responses. AgNPs also cause DNA damage and chromosomal aberrations at a low concentration without toxicity, especially no genotoxicity effects on human cells[20-22]. Since the usage of AgNPs as a drug carrier in cancer treatment has recently gained considerable attention[23]. In this study, AgNPs synthesized using synergistic five different extracts of the rhizome of *Cheilocostus speciosus* were used for analyzing *in vitro* anti-cancer activity against (**SK-MEL28**) cells.

MATERIALS AND METHODS

Characterization of nanoparticles

In order to understand their physicochemical characteristics, several analytical techniques are employed for nanoparticle characterization. UV-Visible spectroscopy allows the measurement of absorbance and provides information about the size and shape of nanoparticles. Scanning Electron Microscopy (SEM) offers high-resolution

imaging, enabling visualization of nanoparticle morphology and surface structure. Dynamic Light Scattering (DLS) provides size distribution and measures the hydrodynamic diameter of nanoparticles in solution. Together, these techniques enable comprehensive characterization of nanoparticles, facilitating their understanding and utilization in diverse applications.

UV Spectroscopy analysis

The initial characterization of the silver Nanoparticles (AgNPs) was performed using UV-Visible spectroscopy. The process involved monitoring the reduction of silver ions to nanoparticle form by measuring the UV-Visible spectra of diluted samples. A dilution factor of 20 was applied, where the sample was mixed with Millipore water. The UV-Visible spectra of the AgNPs solution were recorded using a UV-Vis spectrophotometer (Thermofischer, USA) within the wavelength range of 200 to 700nm. The respective plant extracts were considered as a blank reference to adjust the baseline for accurate measurements. The measurements were repeated after 10min, 20min, 30min, 40min, 50min time interval during synthesis.

Dynamic Light Scattering (DLS) Study

Dynamic Light Scattering (DLS) is a widely used technique for studying the size distribution and hydrodynamic properties of Nanoparticles in solution. It provides valuable information about the particle size, size distribution, and stability of nanoparticles, including their agglomeration or aggregation behavior. DLS is a non-invasive and non-destructive technique that measures the fluctuations in the intensity of scattered light caused by the Brownian motion of Nanoparticles in solution. The hydrodynamic diameter of stable biosynthesized AgNPs were determined by Particle Size Analyzer (Malvern Zetasizer Nano ZS). It helps to determine the hydrodynamic diameter of dispersed nanoparticle.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a powerful technique used for the characterization of nanoparticles. It provides detailed information about the morphology, size, shape, and surface structure of Nanoparticles at higher solution. The first step in SEM analysis is the preparation of the nanoparticle sample. The Nanoparticles suspended in liquid medium was drop-casted on a 1cm x1cm glass slide and dried. The samples were then placed in sputtering chamber for gold sputter coating. The prepared sample was then placed inside the sample chamber in a Scanning Electron Microscope (Jeol JSM6390LA) where it is subjected to a high-energy electron beam. The SEM chamber is maintained under vacuum to ensure proper electron beam interaction with the sample. The electron beam scans across the sample surface, and interactions between the beam and the Nanoparticles result in various signals, including secondary electrons, back scattered electrons, and characteristic X-rays. The secondary electrons and back scattered electrons are collected by detectors, and the resulting signal is used to generate an image of the nanoparticles. The images obtained from SEM provide information about the size, shape, distribution, and surface features of the nanoparticles.

***In-vitro* cytotoxic activity of AgNPs by MTT assay**

The cytotoxicity activity of AgNPs was performed using MTT assay (24) against **SK-MEL28** cells. In this method, 200 µL **SK-MEL28** cells, roughly 1×10^4 cells/well, were seeded into 96 well plates and permitted to achieve confluence cell growth. After cells were completely attached to the well, culture media was discarded. Further, 100 µL of various concentrations such as 25, 50, 100, 250, and 500 µg/mL of AgNPs were added and incubated for 24 h and followed for 48 and 72 h. After that, freshly prepared MTT [5 mg/mL of phosphate buffer solution] was added and incubated at 37 °C for 4–6 h.

RESULTS AND DISCUSSIONS

Biosynthesis of nanoparticles

Biosynthesis of Nanoparticles was studied for different extracts namely, ethanol, methanol, water, ethyl acetate and chloroform. However, yellow to brown red color transformation was visible only in the case of plant extract in methanol. The plant extract in ethanol yielded a resultant solution which was moderately brown in color. Therefore, further experiments were proceeded with only using methanol-based extract.

UV Spectroscopy analysis

The AgNP synthesis with 5mM initial silver nitrate was analyzed by UV over 50 min. The AgNP synthesized from methanol extract yielded consistent trend in UV–spectroscopy when measured at 10, 20, 30, 40 and 50 min. A stable growing dumb bell shaped curve peaking was observed over the time upon synthesis at 440 nm. This study acts as a confirmation for synthesis. The dumb bell shaped peak formation was formed in the case of AgNPs synthesized from ethanolic extract as well as water extract. However, their peak formation was not stable over the time tested.

Table 1: Physical properties of biosynthesized AgNP solution

Extract	Color	Clarity	Stability (48 hrs)
Ethanol	Yellow - brown	Clear	No visible aggregation
Water	Moderately Yellow - brown	Clear to cloudy	Low level aggregation
Methanol	Red brown	Clear	No visible aggregation
Ethyl acetate	Yellow	Moderately cloudy	High level aggregation
Chloroform	Yellow	Moderately cloudy	High level aggregation

Figure 1: AgNPs Methanol extract

Figure 2: AgNPs Ethanol extract

Figure 3: AgNPs chloroform extract

Figure 4: AgNPs ethyl acetate extract

Figure 5: AgNPs water extract

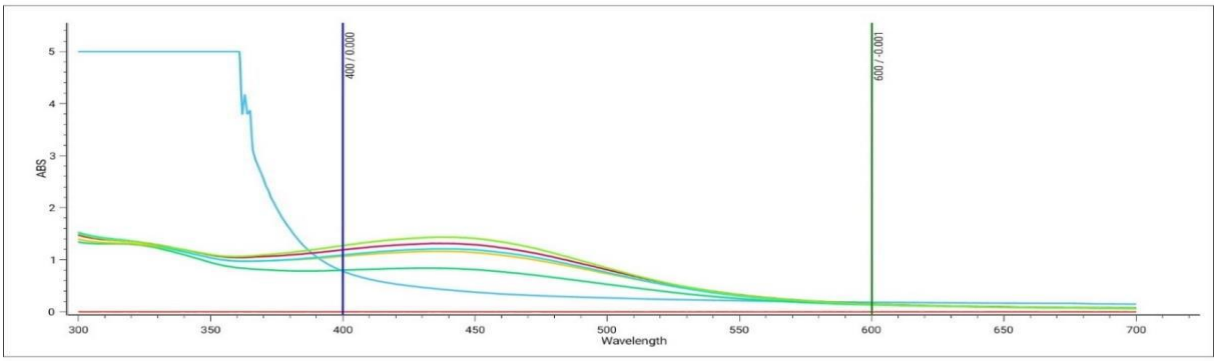

Figure 6: UV-Spectrometer analysis of Methanol extract synthesized AgNPs (Redrawn for better understanding [Blue line: absorbance curve for plant extract]

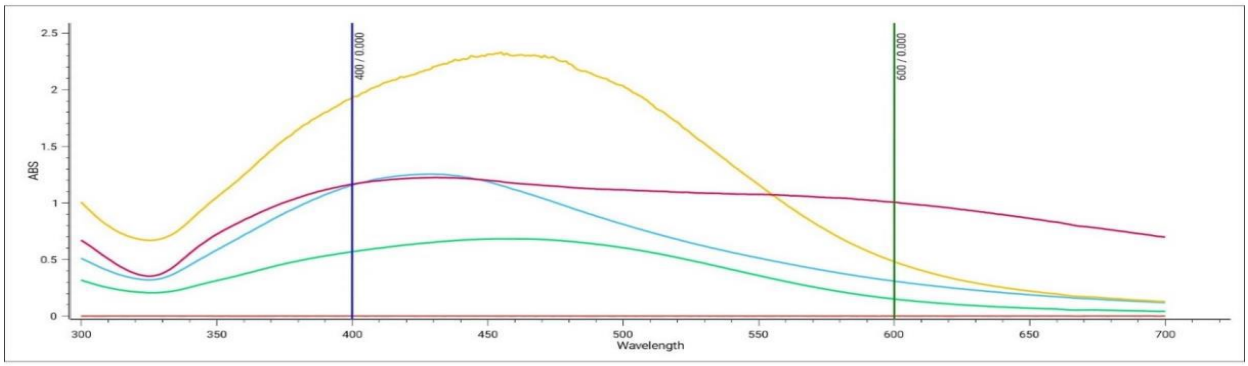

Figure 7: UV-Spectrometer analysis of Ethanol extract synthesized AgNPs

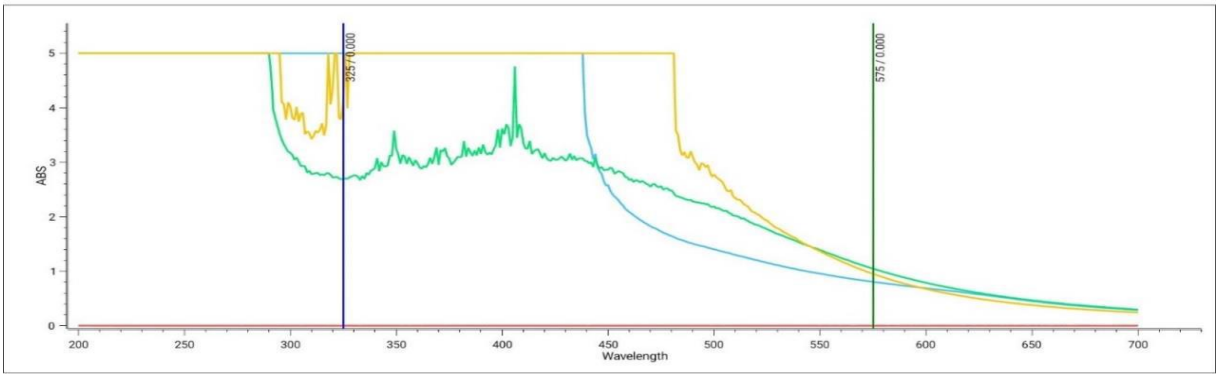

Figure 8: UV-Spectrometer analysis of Chloroform extract synthesized AgNPs

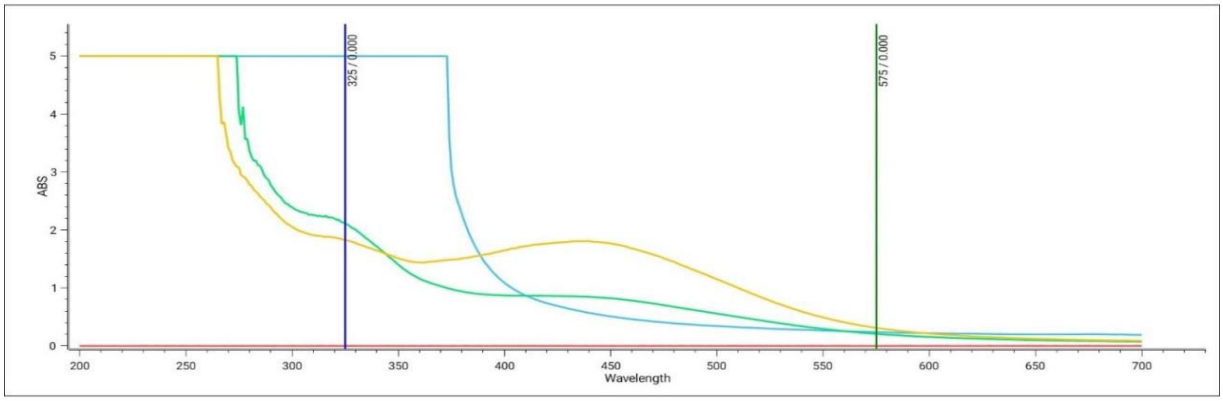

Figure 9: UV-Spectrometer analysis of Water extract synthesized AgNPs

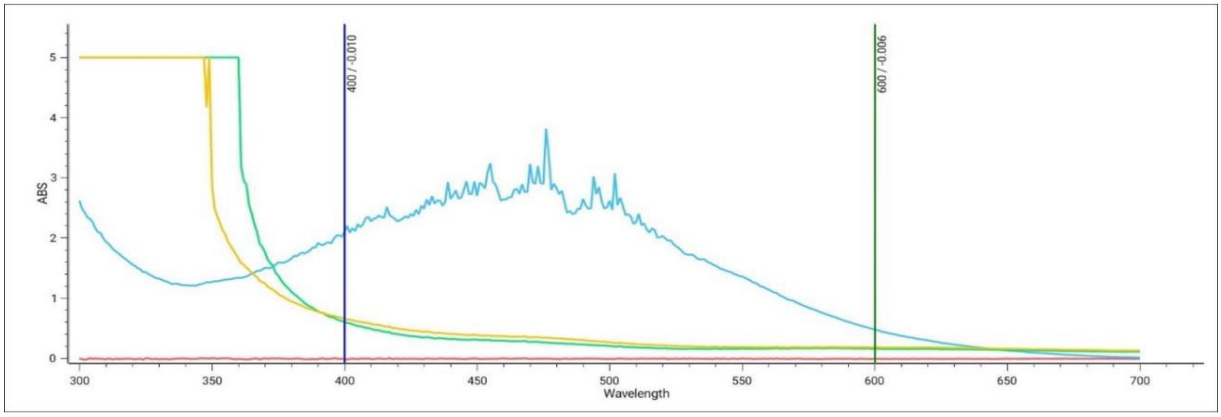

Figure 10: UV-Spectrometer analysis of Ethyl acetate extracts synthesized AgNPs

The hydrodynamic diameter of AgNP synthesized from methanol extract was measured based on the physical characteristics identified and UV-Visible spectrum analysis. The mean hydrodynamic diameter (Z-average) value was found to 109.6 nm. Dynamic light scattering experiments helped to characterize the prepared Ag NP for the size of nanoparticles in terms of hydrodynamic diameter and ascertain the colloidal stability of the solution. The dynamic light scattering results confirmed the size. The Polydispersity index value was measured to 0.321. The value of Polydispersity Index (PDI) may vary from 0.01 (mono dispersed particles) to 0.5-0.7, whereas, PDI Index value > 0.7 indicated broad particle size distribution of the formulation. The particle size and particle size distribution are very critical factors for performance evaluation of nanoparticles.

Dynamic Light Scattering (DLS) study

In a DLS study, a laser beam is directed at the nanoparticle suspension, and the scattered light is collected at different angles. The fluctuations in the scattered light intensity are analyzed using the principles of autocorrelation to extract information about the particle size and size distribution. By measuring the time-dependent fluctuations, DLS can determine the diffusion coefficient of the nanoparticles, which is directly related to their hydrodynamic size. DLS is particularly useful for nanoparticles with sizes ranging from a few nanometers to several hundred nanometers.

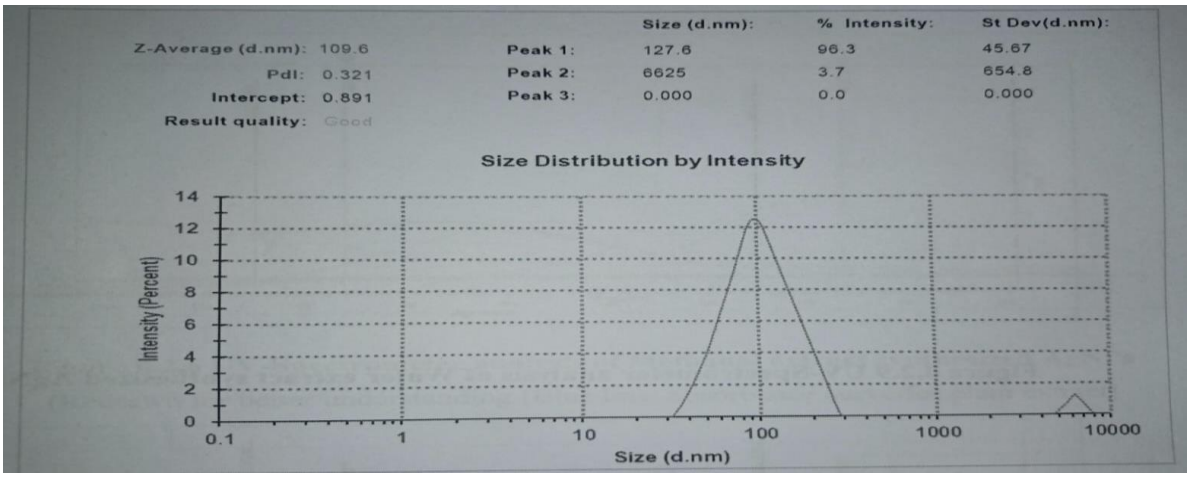

Figure 11: DLS spectra indicating z-average diameter of nanoparticles from methanol extract.

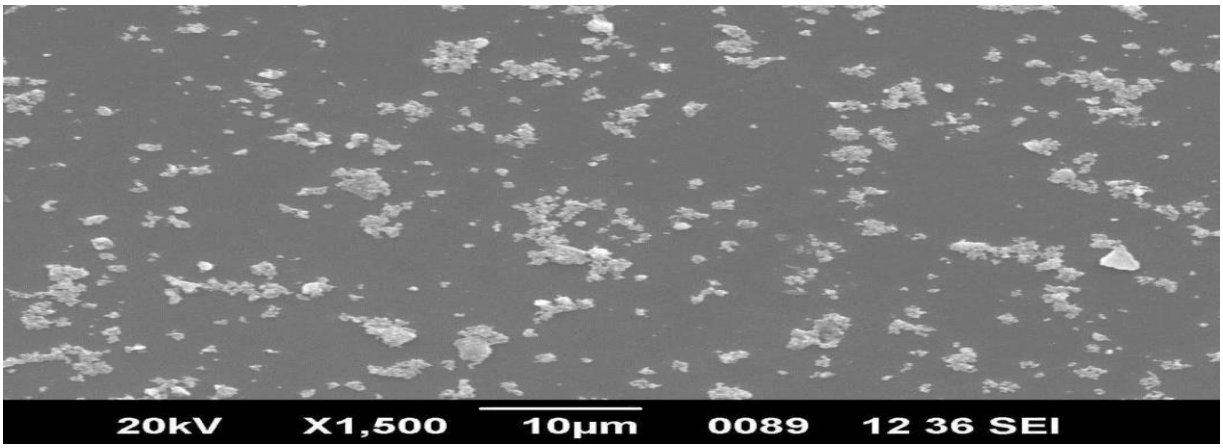

Figure 12: SEM image of AgNP synthesized from Methanolic extract of *Cheilocostus* rhizome

Cell viability assay (MTT assay)

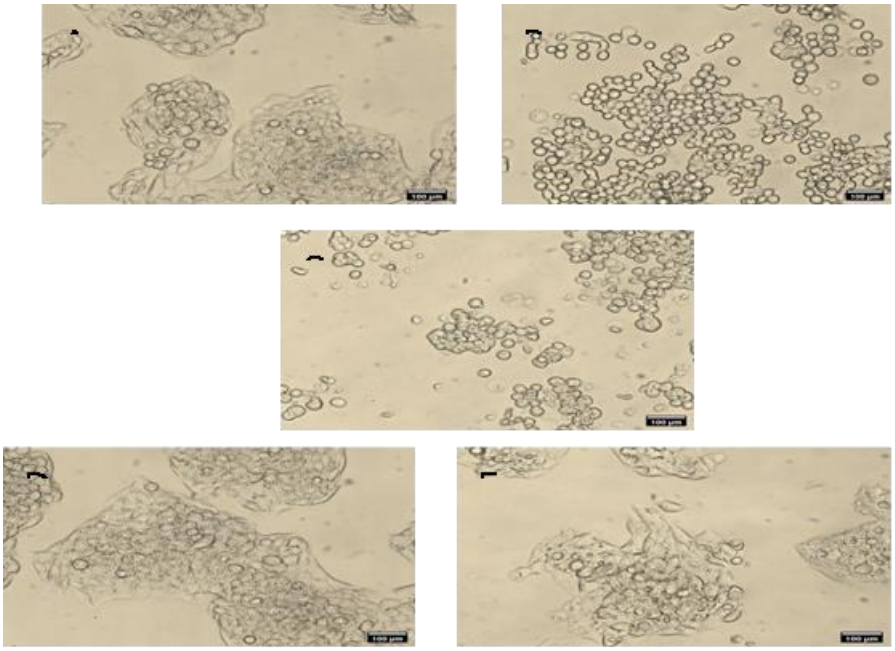

Figure 13: % cell viability values of given sample, REM with different concentrations against SK-MEL28 after the treatment period of 72 hrs.

A. Microscopy image of untreated SK-MEL28 cells
B. Microscopy image of Doxorubicin treated SK-MEL28 cells
C. Microscopy image of 1: 1 AgNP treated SK-MEL28 cells
D. Microscopy image of 1: 10 AgNP treated SK-MEL28 cells
E. Microscopy image of 1: 100 AgNP treated SK-MEL28 cells

Scanning Electron Microscopy (SEM) study

The scanning electron microscope image shows the presence of nanoparticles uniformly distributed. From the size bar it is indicative that the size of the particles was below 1000 nm. This result corroborated with what observed in DLS study. The slight aggregation appeared may be due to the particle settling upon drying the sample.

CONCLUSION

In conclusion, the biosynthesis of silver nanoparticles (AgNPs) from *Cheilocostus* plant extracts, specifically in water, methanol, and ethanol, demonstrated varying degrees of success. The color change from yellow to dark brown indicated the formation of AgNPs, with methanol extract showing the most promising results. Characterization of the nanoparticles through UV-Vis spectroscopy, Dynamic Light Scattering (DLS), and Scanning Electron Microscopy (SEM) provided valuable insights into their physicochemical properties. The UV-Vis spectra exhibited stable and distinctive peaks for AgNPs synthesized from methanol, suggesting a reliable synthesis process. DLS analysis confirmed a mean hydrodynamic diameter of 109.6 nm for methanol-synthesized AgNPs, with a Polydispersity Index indicative of a relatively narrow size distribution. SEM images further supported the uniform distribution of nanoparticles, although slight

aggregation was observed due to sample drying. Overall, the study establishes the potential of *Cheilocostus* plant extracts, particularly in methanol, for the eco-friendly synthesis of stable silver nanoparticles with promising characteristics for various applications.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front. Pharmacol. 2014; 10: 4–177.
2. Kress WJ, Prince LM, Williams KJ. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. Am. J. Bot. 2002; 89(10):1682–1696.
3. Basak S, Sarma GC, Rangan L. Ethnomedical uses of Zingiberaceous plants of Northeast India. J. Ethnopharmacol. 2010;132(1):286–296.
4. Chainani-Wu N. Safety and anti-inflammatory activity of curcumin: a component of tumeric (*Curcuma longa*) J. Altern. Complement. Med. 2003; 9(1):161–168.
5. Araújo CC, Leon LL. Biological activities of *Curcuma longa* L. Mem. Inst. Oswaldo. Cruz. 2011; 96(5):723–728.
6. Boaz M, Leibovitz E, Dayan YB, Wainstein J. Functional foods in the treatment of type 2 diabetes: olive leaf extract, turmeric and fenugreek, a qualitative review. Funct. Foods. Health. Dis. 2011;1(11):472–481.
7. Zhang X, Chibli H, Kong D, Nadeau J. Comparative cytotoxicity of gold–doxorubicin and InP–doxorubicin conjugates. Nanotechnology. 2012; 23(27):103–275.
8. Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. Eur. J. Cancer. 2005; 41(13):1955–1968.
9. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. Life. Sci. 2006; 78(18):2081–2087.
10. Bar-Sela G, Epelbaum R, Schaffer M. Curcumin as an anti-cancer agent: review of the gap between basic and clinical applications. Curr. Med. Chem. 2010;17(3):190–197.
11. Darvesh AS, Aggarwal BB, Bishayee A. Curcumin and liver cancer: a review. Curr. Pharm. Biotechnol. 2012; 13(1):218–228.
12. Shehzad A, Lee J, Lee YS. Curcumin in various cancers. BioFactors. 2013; 39(1):56–68.
13. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr. 2005; 81(1):215–217.
14. Garima S, Riju B, Kunal K, Ashish RS, Rajendra PS. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. J Nanopart Res. 2011;13(7):2981–2988.
15. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am. J. Clin. Nut. 2004; 79(5):727–747.
16. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food. Chem. Toxicol. 2008; 46(2):409–420.
17. Nicoll R, Henein MY. Ginger (*Zingiber officinale* Roscoe): a hot remedy for cardiovascular disease? Int. J. Cardiol. 2009; 131(3):408–409.
18. Li Y, Tran VH, Duke CC, Roufogalis BD. Preventive and protective properties of *Zingiber officinale* (ginger) in diabetes mellitus, diabetic complications, and associated lipid and other metabolic disorders: a brief review. Evid. Based. Complement. Altern. Med. 2012; 12(1):516–870.
19. Rhode J, Fogoros S, Zick S, Wahl H, Griffith KA, Huang J, Liu JR. Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. BMC. Complement. Altern. Med. 2007; 7(1):44.
20. Zhang X.-F, Liu Z.-G, Shen W, Gurunathan S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. Int. J. Mol. Sci. 2016; 17:15–34.
21. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. Int. J. Mol. Sci. 2016;17(9):15–34
22. Gurunathan S, Qasim M, Park C, Yoo H, Kim JH, Hong K. Cytotoxic potential and molecular pathway analysis of silver nanoparticles in human colon cancer cells HCT116. Int. J. Mol. Sci. 2018; 19(8):22–69.
23. Brahmbhatt M, Gundala SR, Asif G, Shamsi SA, Aneja R. Ginger phytochemicals exhibit synergy to inhibit prostate cancer cell proliferation. Nutr. Cancer. 2013; 65(2):263–372.
24. Kleemann W. Random-field induced antiferromagnetic, ferroelectric and structural domain states. Inter. J. Modern. Phy. B. 1993;7(13):24–69.