Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : https://ijpsdronline.com/index.php/journal

Research Article

Green Synthesized *Moullava spicata* (Dalz.) Nicolson Leaf Extract Mediated Silver Nanoparticles Potentiate Antioxidant and Anticancer Activity in Human Bone Marrow Neuroblastoma Cancer Cells

Rangavitala P, Taranath T C*

Environmental Biology and Green Nanotechnology Laboratory, P. G. Department of Studies in Botany, Karnatak University, Dharwad, Karnataka, India.

ARTICLE INFO

Article history:

Received: 23 May, 2024

Revised: 04 July, 2024

Accepted: 11 July, 2024

Published: 30 July, 2024

Keywords:

Green synthesis, *Moullava spicata*, Silver nanoparticles, Hydroacetonic extract, Antioxidant activity, Anticancer activity.

DOI:

10.25004/IJPSDR.2024.160415

ABSTRACT

Moullava spicata (Dalz) Nicolson is an effective therapeutic plant of the Western Ghat forests of India. Using hydroacetonic *M. spicata* leaf extract (MsLE) as a novel environmentally benign resource for the green synthesis of AgNPs. Synthesized *M. spicata* leaf extract silver nanoparticles (MsLE-AgNPs) were explored for antioxidant and anticancer activity. The development of a brownish color in the reaction mixture signified the AgNPs synthesis. The spectroscopic analysis of synthesized MsLE-AgNPs were accomplished through UV-visible spectrophotometer, fourier transform infrared (FTIR) spectroscopy, powder X-ray diffraction (XRD), high-resolution transmission electron microscopy (HR-TEM), and dynamic light scattering (DLS). The antioxidant potentiality was done by total phenolic content (TPC), total flavonoid content (TFC), and DPPH assay. Anticancer potentiality was evaluated by MTT assay against human bone marrow neuroblastoma (SH-SY5Y) cancer cell lines and induction of apoptosis was checked in SH-SY5Y cells. The absorption maxima of AgNPs at 405 nm was validated by UV-vis spectroscopy. FTIR demonstrated the contribution of chemical groups in both the reduction and stabilization process of AgNPs. Powder XRD patterns validated crystallinity. HR-TEM images showed spherical-shaped and ~10 to 20 nm sized AgNPs. The zeta potential of -28.4 mV and 68.6 nm hydrodynamic size of MsLE-AgNPs was confirmed by the DLS spectrum. The TPC and TFC were 134.76±0.096 mg GAE/100 mL and 32.38 ± 0.12 µg QE/mL, respectively. Dose-dependent DPPH radical scavenging assay showed a 95.89±0.016% inhibition. In MTT assay MsLE-AgNPs exhibited 13.88±0.015% cell viability and induced 74.68% apoptosis in cancer cells. The current results confirmed the potentiality of MsLE-AgNPs for antioxidant and anticancer applications.

INTRODUCTION

Nanotechnology is a specialized area of science handling the manipulation of nanomaterials along with physicochemical properties. These nanomaterials hold significant value in the realm of nanotechnology and owing to its unique nano-characteristics between 1 to 100 nm. The variation of surface-to-volume ratio, physicochemical and biological properties give broad opportunities to convene the prerequisites in diverse fields for biomedical research.[1] The nanoparticles have been connected to various fields like optics, biology, catalysis, medicine, and pharmaceutical research. The traditional physical and chemical routes for synthesizing nanoparticles are toxic to the environment, technically challenging, and more expensive.[2] However, the production of metallic nanoparticles requires potentially harmful chemicals, has low material conversion rates, requires a lot of energy, and involves several challenging steps in the purifying process. Since then, scientists have synthesized nanoparticles using environmentally friendly processes. Due to the presence of various phytoconstituents green synthesis techniques use plants as a source for the nanoparticle's synthesis.[3] Using plants, to make nanoparticles is an ingenuous, economical, and biologically friendly process. The 'reduction potential of the plants' which is crucial for nanoparticles synthesis

***Corresponding Author:** Dr. Taranath T C

Address: Environmental Biology and Green Nanotechnology Laboratory, P. G. Department of Studies in Botany, Karnatak University, Dharwad, Karnataka, India.

Email ✉**:** tctaranath@gmail.com

Tel.: +91-9480335735

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ultimately agrees with conclusions concerning reduction, capping and stabilization of nanoparticles by the plant.[4] Generally, silver, gold, platinum, zinc, and copper metals are used to synthesize nanoparticles among which silver nanoparticles were widely synthesized because of their vivid applications in tissue engineering, targeted drug delivery systems, anti-inflammatory, antimicrobial, antioxidant, and anticancer investigations.[5]

The green synthesis protocol involved the preparation of crude plant extract[6] and was carried out using an advanced ultrasonic-assisted extraction (UAE) method, which is a highly efficient, rapid, timesaving, cost-effective and yields a high percentage of crude plant extract.[7]

The hydroacetonic leaf extract of *Moullava spicata* (Dalz) Nicolson (Syn. *Wagatea spicata* Dalz.) (family: Fabaceae) also identified as candy corn plant was used to synthesize the AgNPs. The roots were treated to cure pulmonary tuberculosis and pneumonia and contain rich antimicrobial properties.[8] Bark extracts were applied for skin problems and aqueous leaf extract of *M. spicata* contains different phytoconstituents.[9] As a result of these medicinal properties and no report of green synthesis of nanoparticles on the *M. spicata* (Dalz) Nicolson, the current research was carried out to synthesize AgNPs using ultrasonic-assisted hydroacetonic leaf extract. MsLE-AgNPs were characterized using different spectral methods. As per the earlier reports, the accumulation of different phytoconstituents in plant based AgNPs has been investigated for antioxidant activities.

Furthermore, the most immature, indistinguishable, and malignant type of paediatric cancer develops in the nervous system of children and young progenies known as human bone marrow neuroblastoma (SH-SY5Y) cancer,[10] it increases the risk of low survival time. The treatment strategy mainly involves stage and age-specific surgery and adjuvant chemotherapies, which stand toxic, impacting a high rate of side effects.[11] Thus, the search for safe, less toxic, and potential plant derivatives as biogenic drugs is of prime importance. Hence, the present study extensively analyses the anticancer potentiality of synthesized and capped MsLE-AgNPs against SH-SY5Y cell lines.

Materials And Methods

Collection, Identification and Extraction of the Plant Material

The young leaves of *M. spicata* (Dalz) Nicolson was collected from Handibadaganath Mutt, near Kumbard village, Khanapur taluk, Belagavi district, Karnataka – 591301; 15° 25' 26.2636" N, 74° 34' 23.4361" E; Plant's GPS co-ordinates: 15.39372, 74.58170; Altitude: 478.23m/1569ft; Plus-code: 9HVJ+CM Kudalgaon, Karnataka. The collected leaf samples were taxonomically identified and specimen accession No: Bot/KSCD/28-06-2022/19607 was deposited at the P. G. Department of Botany at Karnataka Science College, Dharwad, Karnataka, India – 580001, for future reference. The collected leaf samples were cleaned with tap water and then Milli-Q water to eliminate all contaminants, shade-dried for ten days, and pulverized to prepare fine powder. UAE was performed using Ultrasonicator Cleaner bath (Model: Athena Multifunctional, ATS-10L), with regulating factors like 40 KHz frequency, 500 W heating power, and 240 W ultrasonic power was maintained and applied direct sonication technique,[7,12] and optimized as per requirements. Extraction was carried out using 50 g of leaf powder with 400 mL of 70% acetone in a 1:8 w/w ratio in a 1 L autoclavable bottle and maintained with 100% sonication power for 75 minutes. at 60°C temperature and then kept for cooling at 25°C for 3 hours. Filtered the extract and air-dried it into pellets. The percentage of total yield can be assessed with the formula:

$$\text{Percentage of yield} = \frac{\text{Weight of the leaf extract}}{\text{Weight of the plant material}} \times 100$$

Moullava spicata Leaf Extract Silver Nanoparticles (MsLE-AgNPs) Synthesis and Characterization

The leaf extract of *M. spicata* (5 g) was solubilized in 50 mL Milli-Q water for preparing the stock solution. For silver ion reduction, 40 mL of 1 mM $AgNO_3$ solution was dissolved in 20 mL of diluted leaf extract and incubated for 2 Hours. at 25°C and adjusted to pH 6 using 1N NaOH with gentle stirring for 5 minutes. The color change was observed for the AgNPs synthesis. Followed by diluted the AgNPs with Milli-Q water (1:5 ratio) and absorption maxima was recorded through the double beam UV-vis spectrophotometer (JASCO NIR V-670) in 300 to 800 nm spectral range. Synthesized AgNPs were purified using REMI Cooling C-24 BL Centrifuge at 5000 rpm for 15 minutes. and washed the reaction mixture thrice using Milli-Q water, discarded the supernatant and collected and air-dried the concentrated slurry for further analyses. MsLE-AgNPs (2 mg) was mixed with dried KBr (200 mg) and thin pellets were prepared and assessed by Thermo-Fischer Scientific Nicolet-6700 analyzer with 400-4000 cm^{-1} wavelength. The crystallinity of synthesized AgNPs was analyzed using RIGAKU Smartlab SE powder X-ray diffractometer equipped with 40 kV, 30 mA current, Cu Kβ (1.3923 Å) radiation, 10.00°/min scanning rate and 5~90° diffraction angle was maintained and the nanocrystallite size was quantified through Debye-Scherrer formula $D = K\lambda/\beta\cos\theta$, In this formula, 'D' denotes the diameter of silver nanoparticles, 'K' represents the Scherrer constant (0.94), diffracted X-rays wavelength 'λ', full-width half maximum (peak width) 'β' and 'θ' shows the Bragg's angle. MsLE-AgNPs were exposed to sonication for 20 minutes, while suspended in deionized water, two drops of silver nanoparticle solution were dropped onto a copper grid, dried under an infrared lamp for 10 minutes., and placed in a vacuum overnight and the shape and size of the AgNPs were measured by Thermo-Fischer, TALOS F200

G2 HR-TEM instrument. For the DLS spectral study, 2 mg of MsLE-AgNPs were added to 10 mL of Milli-Q water, whereby a hydrodynamic solution was formulated. The surface zeta potential, hydrodynamic size, and particle distribution, i.e., polydispersity index (PDI) was analyzed using the HORIBA Scientific SZ-100 instrument.

Antioxidant Potentiality of MsLE-AgNPs

Total phenolic content (TPC) of synthesized AgNPs

TPC of synthesized AgNPs was performed using a Folin-Ciocalteu (FC) assay.[13] To maintain optimum concentration, MsLE-AgNPs (1-mg) were dissolved in 1-mg/mL methanol. 1-mL of 50% FC reagent and 1-mL of AgNPs was mixed into 5 mL of Milli-Q water, after three minutes, 3 mL of a 2% saturated Na_2CO_3 solution were introduced. The preparation was placed in the dark for incubation at 25°C for 90 minutes. A blank was prepared from the same procedure. At 720 nm the absorbance was recorded. For calibration, a standard curve of known values (12.5–200 µg/mL) of Gallic acid equivalent was considered as reference.

Total flavonoid content (TFC) of synthesized AgNPs

TFC of MsLE-AgNPs was carried out using an aluminium chloride ($AlCl_3$) assay.[14] 1 mg of AgNPs was mixed into 5 mL of Milli-Q water until fully dissolved, 1-mg/mL of methanol solution and was mixed with 1-mL of 10% $AlCl_3$, 1-mL of 1-M NaOH and the mixture was left to incubate in darkness at 25°C for 30 minutes. Using the same procedure a blank was prepared. At 420 nm absorbance was recorded. For calibration, a standard curve of known values (12.5-200 µg/mL) of quercetin equivalent was considered as reference.

DPPH radical inhibition potential of MsLE-AgNPs

The free radical inhibition potential of MsLE-AgNPs was examined by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.[15] The concentrations (12.5–200 µg/mL) of MsLE-AgNPs were combined with 3 mL of methanol and 5 mL of DPPH (0.1 mM) solution. After proper agitation, solution was placed in the darkness at 25°C for 30 minutes. Ascorbic acid served as the standard, measured the absorbance at 517 nm. The IC_{50} value expressed the potentiality of the experimental drug.

The DPPH radical scavenging activity percentage was determined using the formula:

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

Anticancer Potentiality of MsLE-AgNPs

Cell culture and assay controls

The SH-SY5Y human bone marrow neuroblastoma cancer cell lines were sourced from the National Centre for Cell Sciences (NCCS) in Pune, India. A T25 culture flask (#12556009, Biolite-Thermo) composed of 10% fetal bovine serum (#RM10432, HiMedia) and high-glucose DMEM (HiMedia) was utilized to culture the cell lines and the culture media were incubated in a CO_2 incubator (Heal Force, China) at 5% humidified atmospheric CO_2 and 37 °C temperature. Sub-cultured for every 24 hours. 20,000 cells per well were seeded into a 96-well plate (Corning, USA) using 200 µL of the cell suspension, incubated at 5% CO_2 and 37 °C temperature for 24 hours. Cancer cells treated with camptothecin are treated as a positive control. SH-SY5Y cells in a culture medium without a treatment group served as a negative control and SH-SY5Y cells treated with MsLE-AgNPs in 0.1% dimethyl sulfoxide (DMSO) served as the test group.

Cell viability test

Human bone marrow neuroblastoma cancer (SH-SY5Y) cell lines treated with MsLE-AgNPs and standard camptothecin (#C9911, Sigma-Aldrich). After a 24-hour incubation, the medium was taken off, then MTT reagent (#4060 HiMedia) (0.5 mg/mL) was added and MTT formazan crystals were then dispersed using 100 µL of DMSO (#PHR1309, Sigma-Aldrich). The spectral absorbance was read at 570 nm.[16]

The percentage of cell viability was calculated using the formula:

$$\text{Percentage of cell viability} = \left(\frac{\text{Mean absorbance of treated cells}}{\text{Mean absorbance of untreated cells}}\right) \times 100$$

The IC_{50} value was calculated using the linear regression equation Y=Mx+C.
Where Y = 50, M and C values were taken from the cell viability graph.

Flow cytometric apoptotic assay

A 6-well plates were used to culture the SH-SY5Y cells at a density of 0.5 million cells per 2 mL. MsLE-AgNPs and standard camptothecin were exposed to IC_{50} concentrations for 24 hours. After removal from the medium, the cancer cells were rinsed with PBS, subsequently adding the 200 µL of trypsin-EDTA (#TLC099, HiMedia) at 37°C and the incubation period was 3 to 4 minutes. The cell lines were collected, garnered in 12 x 75 mmipolystyrene tubes. Then centrifuged for 15 minutes at 300 x g with an additional 2 mL of growth medium. The cells were treated with PBS twice for washing, the supernatant was then discarded. 5 µL of annexin V/FITC (#51-65874X, BD-Biosciences) was added, at 25°C and 15 minutes incubation in the dark. Subsequently, PI (Propidium iodide) (#51-66211E, BD-Biosciences) (5 µL) and 1X binding buffer (400 µL) was introduced into every tube and agitated gently using a vortex. [17,18] The flow cytometric, fluorescence-activated cell sorting (FACS) technique was adapted to analyze the cell apoptosis.

Statistical Analysis

The figures and statistical graphs were developed through GraphPad Prism version 8.0.1 software (GraphPad Software, Boston, MA, USA). The tests were repeated three times (n = 3), and the results were stated as the mean ± standard deviation (SD).

RESULTS

Total Yield of UAE Crude Extract

About 50 g of pulverized *M. spicata* leaf powder was ultra-sonicated and produced 14.80 g of hydroacetonic crude extract. The total percentage of yield was 29.6 w/w.

Characterization of MsLE-AgNPs

After 2 hours of incubation the synthesis of MsLE-AgNPs was verified via the colour shift from pale-yellow to brown (Fig. 1). The characteristic surface plasmonic resonance (SPR) vibrational excitation of the synthesized AgNPs at pH 6 represented a sharp UV-Vis absorption spectrum at 405 nm. (Fig. 2). Fig. 3 depicts the phytoconstituents involvement in the capping and reduction of synthesized silver nanoparticles was analyzed by FTIR spectroscopy. The strong absorption peak at 3401.54 cm^{-1} specifies the free alcoholic (O-H) stretching. The weak alcoholic (O-H) spectra were assigned at 2923.90 cm^{-1} and the spectral maxima at 1619.47 cm^{-1} was accompanied by strong peak of cyclic alkenes (C=C group). The absorption peak at 1384.24 cm^{-1} is represented by O-H bending phenolic groups. The vibrational stretch of halogenic compounds was assigned at 562.80 cm^{-1}. The synthesized AgNPs were analyzed using powder XRD, revealing characteristics of a face-centered cubic structure. The XRD patterns of AgNPs exhibited Bragg's reflections at specific 2θ values of 38.18°, 44.29°, 64.50°, 77.43° and 81.52° agreeing with Miller

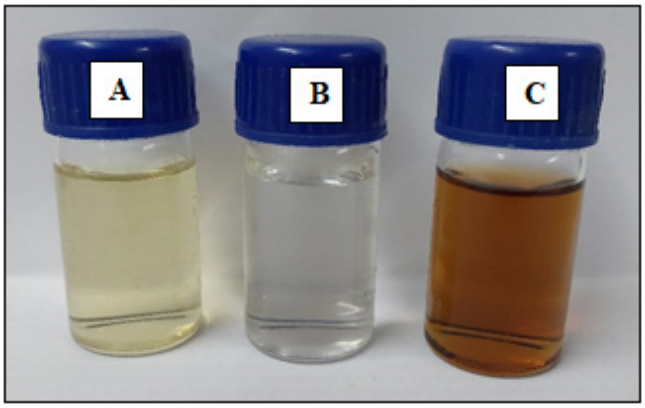


Fig. 1: Synthesis of MsLE-AgNPs (A) Leaf extract (B) 1 mM AgNO3 (C) Reaction mixture after 2 hours. at pH 6

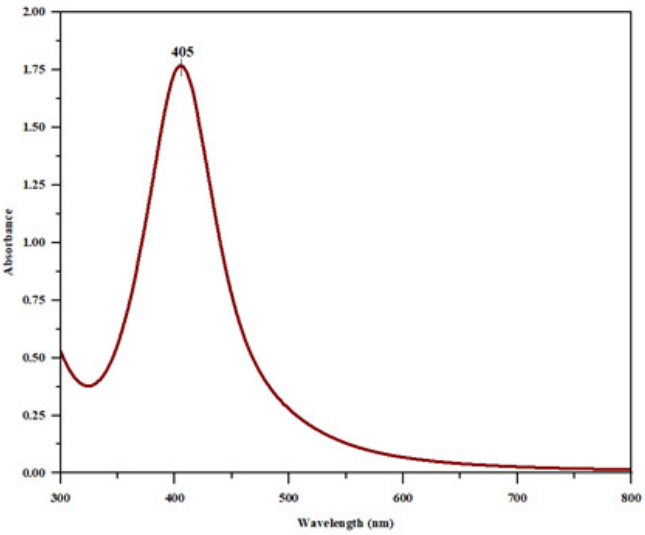


Fig. 2: UV-vis spectrum of MsLE-AgNPs

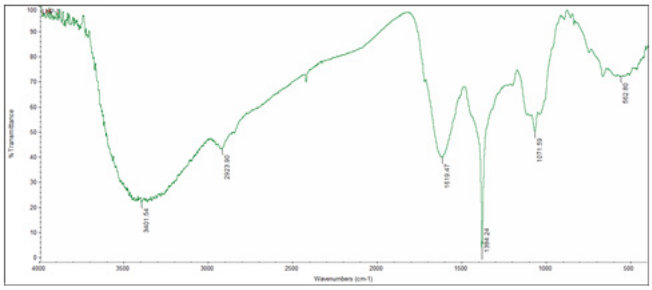


Fig. 3: FTIR spectrum of MsLE-AgNPs

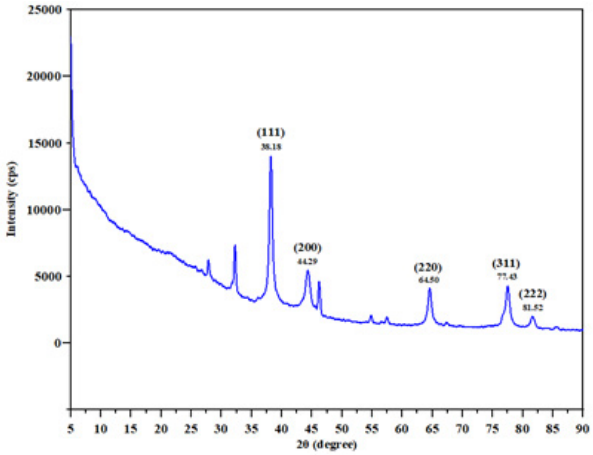


Fig. 4: Powder XRD pattern of MsLE-AgNPs

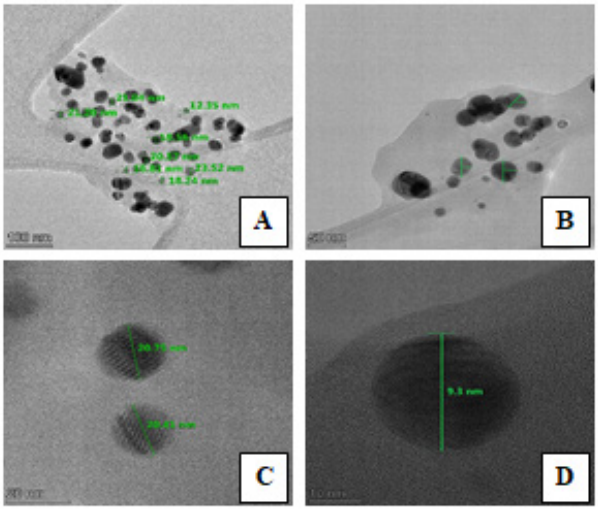


Fig. 5: HR-TEM images of MsLE-AgNPs (9-23 nm in diameter) (A) 100 nm (B) 50 nm (C) 20 nm (D) 10 nm

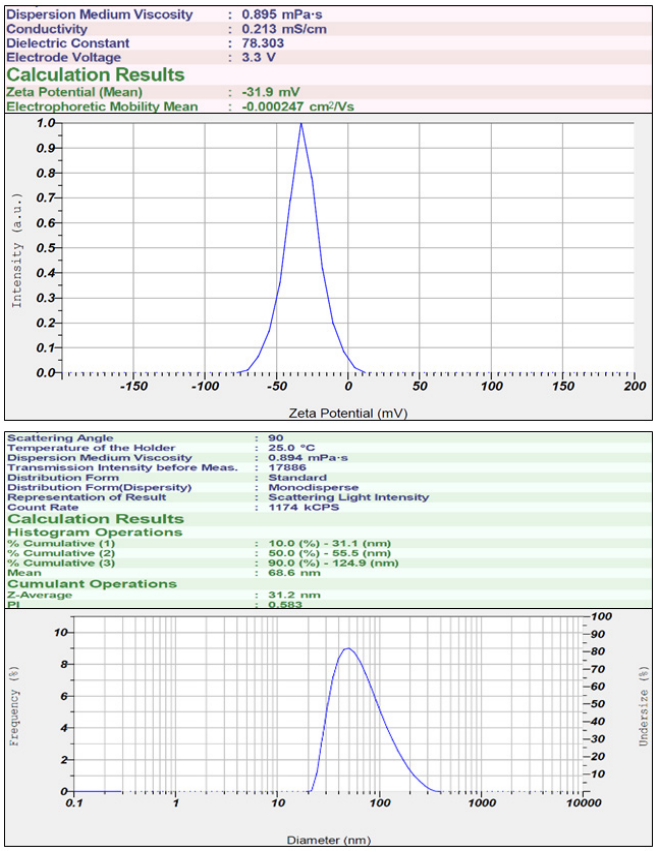


Fig. 6: DLS spectrum of MsLE-AgNPs (A) Zeta potential (B) Particle size distribution

indices at 111, 200, 220, 311 and 222 which ensured the synthesis of elemental silver and MsLE-AgNPs exhibited an average crystallite size of 17.01 nm. The XRD results were compared to International Centre for Diffraction Data (ICDD) (Card No. 00-004-0783) for indexing (Fig. 4). HR-TEM images confirmed the synthesized AgNPs are spherical-shaped, smooth-edged with lattice fringes, polydispersed and different scaled images depict the silver nanoparticles size ranges from 9 to 23 nm in diameter (Fig. 5 A-D). DLS spectrum revealed the surface zeta potential of MsLE-AgNPs was -31.9 mV (Fig. 6A). The hydrodynamic size was 31.2 nm and AgNPs were distributed in a hydrodynamic medium known as polydispersity index (PDI) was determined to be 0.583, which indicates that the synthesized AgNPs were monodisperse distribution (Fig. 6B).

Quantitative Analysis of TPC and TFC assay

The TPC and TFC of MsLE-AgNPs were measured using gallic acid equivalent (GAE) for TPC and quercetin equivalent (QE) for TFC. The TPC of MsLE-AgNPs was 134.763 ± 0.096 mg GAE/100 mL of extract with an R^2 value of 0.9737 (Fig. 7A) and TFC was found to be 32.383 ± 0.121 µg QE/mL of extract with an R^2 value of 0.9738 (Fig. 7B) at a concentration level of 200 µg/mL.

DPPH Radical Inhibition Potential

The synthesized AgNPs efficiently inhibited free radicals in a dose-dependent means. The standard ascorbic acid

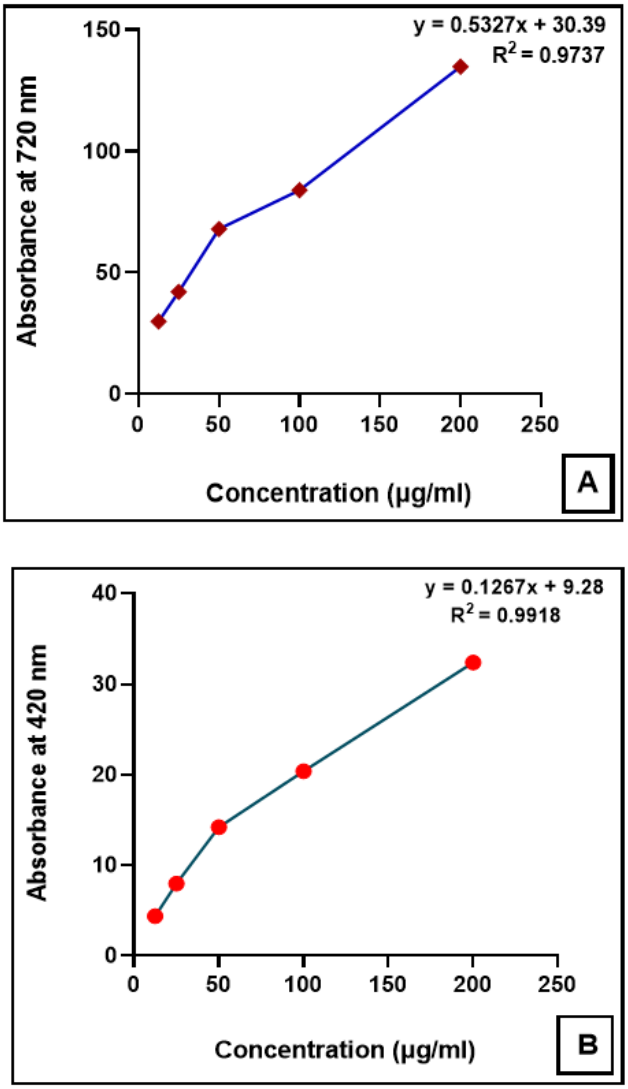


Fig. 7: (A) Total phenolic content and (B) Total flavonoid content of MsLE-AgNPs

Table 1: Percentage of DPPH inhibition between ascorbic acid and MsLE-AgNPs with IC_{50} concentration

Concentration (µg/mL)	*Percentage inhibition (%)*	
	Ascorbic acid	*MsLE-AgNPs*
12.5	37.53 ± 0.026	25.46 ± 0.019
25	48.30 ± 0.057	41.23 ± 0.011
50	59.52 ± 0.073	62.50 ± 0.011
100	69.09 ± 0.018	79.85 ± 0.018
200	93.74 ± 0.067	95.89 ± 0.016
IC50 (µg/mL)	35.39	45.58

Data expressed as mean of triplicates (n = 3) ± Standard deviation (SD)

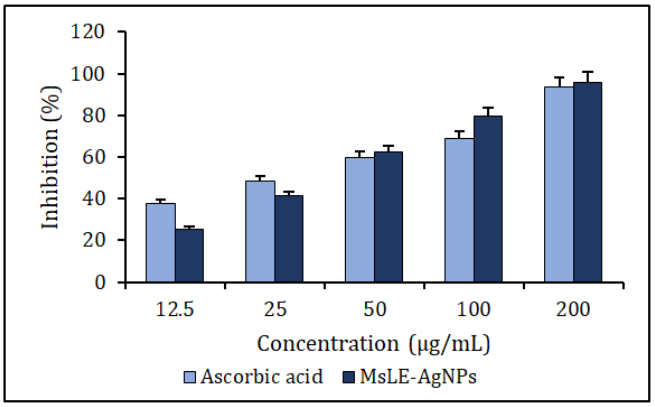


Fig. 8: DPPH free radical scavenging potential of ascorbic acid and MsLE-AgNPs at 517 nm

at high concentrations (200 µg/mL) exhibited 93.74 ± 0.067% inhibition, while MsLE-AgNPs exhibited 95.89 ± 0.016% inhibition at 200 µg/ml concentration. The IC_{50} value for ascorbic acid as a standard was determined to be 35.39 µg/mL. whereas for MsLE-AgNPs it was 45.58 µg/mL. (Table 1 & Fig. 8).

Cell Viability Potential of MsLE-AgNPs against SH-SY5Y Cells

The anticancer potential of MsLE-AgNPs against SH-SY5Y cells was evaluated by the MTT cell viability assay. After 24 hours. of treatment at 12.5 to 200 µg/mL concentrations, the effective cell viability was found to be 49.19 ± 0.022% in standard camptothecin (positive control) and 13.88 ± 0.015% in MsLE-AgNPs (test group) (Fig. 9). The synthesized AgNPs showed potential cytotoxicity against

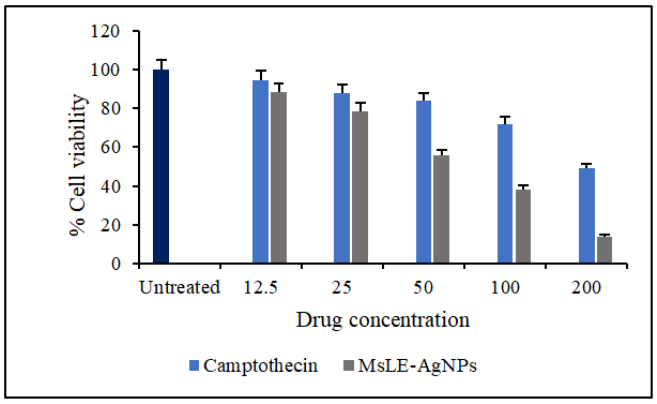


Fig. 9: Cell viability potential of Camptothecin and MsLE-AgNPs against SH-SY5Y cells after 24 hours of treatment

Table 2: IC_{50} concentrations of positive control and test group in µg/mL

Cell line	*Camptothecin*	*MsLE-AgNPs*
SH-SY5Y	195.26	60.05

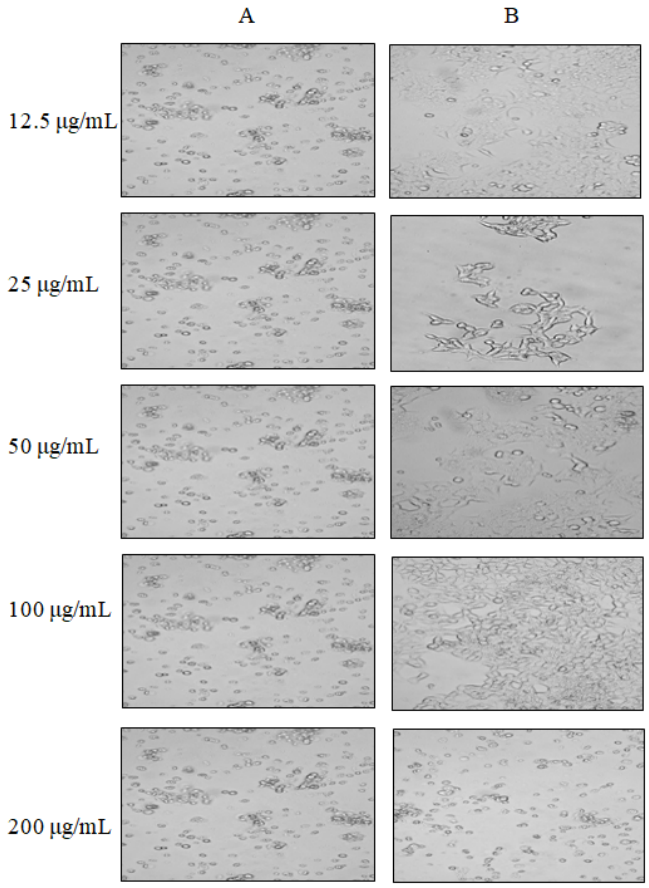


Fig. 10: MTT cell viability potential of MsLE-AgNPs on SH-SY5Y cell lines at 12.5 to 200 µg/mL concentrations (A) Positive control (B) Test group

cancer cell lines. When compared to the standard drug (195.26 µg/mL) the synthesized silver nanoparticles exposed an efficient IC_{50} value of 60.05 µg/mL (Table 2) and the important morphological modifications like shrinkage of cancer cells and cell membrane blabbing were observed in the Fig. 10.

FITC Annexin V/PI Mediated Apoptotic Study in SH-SY5Y cells

Subject to IC_{50} concentrations of positive control and test group for 24 hours. of treatment induced apoptosis in cancer cells. The percentage of apoptosis were controlled by FITC annexin V/PI stains. Figs 11 and 12 determined the rate of induction of apoptosis in SH-SY5Y cell lines was increased from 3.63% (negative control) to 52.83% (positive control) and 74.68% (test group).

Discussion

M. spicata (Dalz) Nicolson hydroacetonic leaf extract served as the bio-reductant for producing silver nanoparticles (AgNPs) through a green synthesis method employing an ultrasonic assisted extraction technique. UAE method with a combination of highly polar solvents like Milli-Q water and 70% acetone (hydroacetone) played a significant

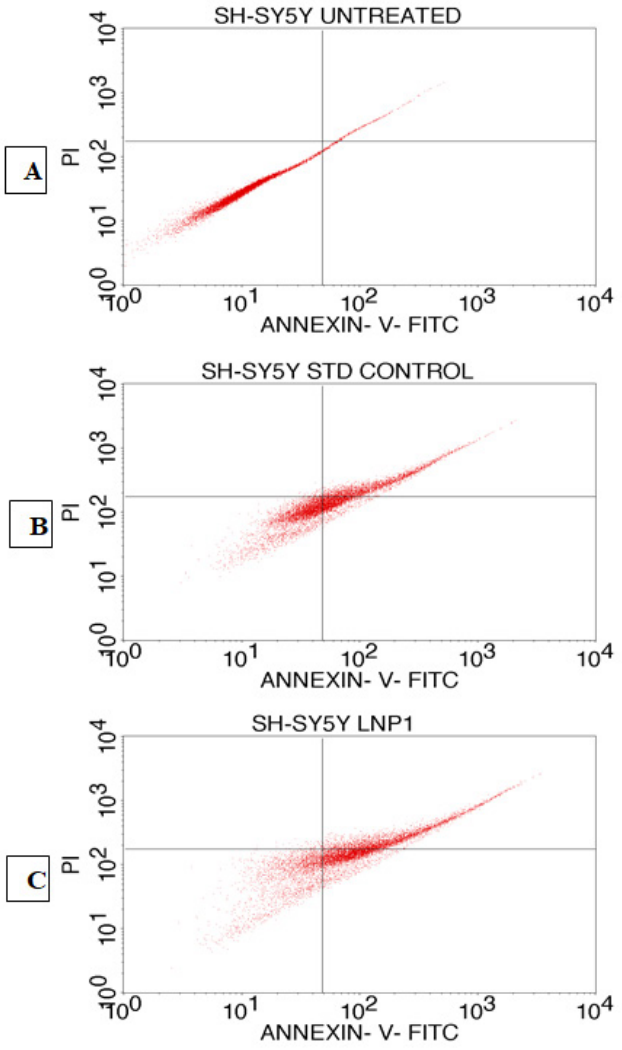
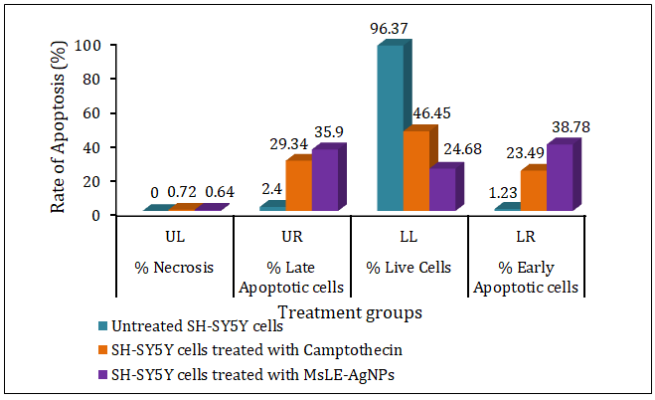


Fig. 11: FITC Annexin V/PI expression in SH-SY5Y cell lines using FACS flow cytometry (A) Negative control (B) Positive control (C) Test group



UL- Upper left, UR- Upper right, LL- Lower left, LR- Lower right

Fig. 12: Percentage of apoptotic induction in SH-SY5Y cell lines treated with different treatment groups

role in the extraction process. UAE technique employed ultrasound which assists in the discharge of intracellular materials through cell disruption. Ultrasonic vibrations accelerate the expansion and implosive collapse of water bubbles and cavitation causes mist-like micro-droplets to develop in the liquid which reduces the reaction time and increases the total percentage of extraction yield.[7,19] The reaction mixture contains several experimental conditions like concentration of reaction mixture, temperature, pH and incubation time for green synthesis. [20] The reaction mixture's colour got shifted to brown from pale-yellow depending on the experimental factors which illustrates the synthesis of MsLE-AgNPs. The articulate oscillation of free electrons at the nanoparticle surface produces the surface plasmonic resonance (SPR) band or waves and the aqueous reaction mixture was induced by these SPR waves which managed the color development and absorption of the substance.[21] The SPR absorption maxima of synthesized AgNPs were validated at 405 nm. FTIR study was utilized to recognize the chemical groups existing on the outer layer of the AgNPs which gives reasonable evidence about the capping, reduction and stabilization of synthesized nanoparticle. The shift in absorption peak from 3401.54 to 2923.90 cm^{-1} indicates the silver ions bind with hydroxyl groups which ensures the presence of polyphenols involved in the stability of AgNPs. The cyclic alkenes absorbed at 1619.47 cm^{-1} might be engaged in the capping of synthesized AgNPs. The phenolic compounds at 1384.24 cm^{-1} and halogenic compounds at 562.80 cm^{-1} contribute to the stabilization and reduction of silver nanoparticles.[22] The powder XRD pattern exposed the presence of FCC structure and crystalline phase of silver nanoparticles. Compared to other crystallographic planes, the 111-plane corresponding to 38.18° showed significant crystallinity due to the existence of higher atom density which regulates the particle size and orienta-tion.[23] Further, the presence of different unassigned peaks indicates the occurrence of bioorganic compounds in the extracts,[24] and the mean nanocrystalline size of MsLE-AgNPs was assessed with the Debye-Scherrer's formula, was confirmed as 17.1 nm which conformed to the HR-TEM results. The dimensions of the nanoparticles that were produced are dependent on the interactions of reactants. The interaction of biomolecules with Ag^{2+} ions during the generation of Ag^{0} nanoparticles greatly affect the size of the synthesized nanoparticles.[25] The direct or indirect concentration of the extract can strongly influence the shape of the AgNPs.[26] As per the previous reports, The present study confirmed the size of AgNPs, which varied from 9 to 23 nm in diameter with spherical-shaped AgNPs capped with different phytoconstituents which prevents agglomeration.[27] In the hydrodynamic solution, the temperature is a key factor in influencing the size and surface zeta value of

AgNPs. The negative zeta potential result indicates that the capping material was effectively exposed to negative charges during the stabilization of AgNPs. The PDI value of AgNPs was less than 1, confirming the monodispersity in distribution. [11]

The reduced AgNPs contain numerous phytoconstituents. These phytoconstituents are the chief source of antioxidant and anticancer activities.[28] The presence of 134.763 ± 0.096 mg GAE/100 mL of total phenols and 32.383 ± 0.121 µg QE/mL of total flavonoids at 200 µg/mL concentration revealed that the synthesized AgNPs comprised of the significant number of polyphenolics deposited on the surface. At a concentration of 200 µg/mL, MsLE-AgNPs exhibited a notable DPPH scavenging potential of 95.89 ± 0.016%, with an IC_{50} value of 45.58 µg/mL. This indicates that the synthesized AgNPs have the potential to act as effective antioxidant agents.[29]

The MTT assay exposed the therapeutic potential of phytoconstituents located on the surface of AgNPs and exhibited effective anticancer activity against cancer cells.[30] Therefore the synthesized AgNPs showed 13.88 ± 0.015% cell viability against cancer cells and a notable IC_{50} value of 60.05 µg/mL. The potential anticancer activity of silver nanoparticles can be observed in the morphological changes of SH-SY5Y cancer cell lines. The synthesized AgNPs are actively involved in the arresting of cellular division which leads to enhanced apoptosis in the targeted cells. The cytotoxic potential of synthesized AgNP preparations is directly proportional to the silver nanoparticles interaction with cellular compositions.[31] In the apoptotic study, SH-SY5Y cells were categorized into annexin V−/PI+ (necrosis), annexin V+/PI+ (late apoptosis), and annexin V+/PI− (early apoptosis) [32] with different treatment groups at 12.5 to 200 µg/mL concentrations. Compared to the standard camptothecin (52.83%), the synthesized AgNPs were more effectively induced (74.68%) apoptosis in cancer cells and reduced the rate of viable cells. The major contributing variables like AgNP size, increased Ag concentration, and effective capping of phytoconstituents potentially reduced the cell viability. Furthermore, research findings like generation oxidative stress and cell cycle studies can elucidate cellular mechanisms at the molecular level.[33] In the present investigation, the MsLE-AgNPs showed potential antioxidant and anticancer activity against SH-SY5Y cancer cell lines.

Conclusion

The current research investigated the green synthesis of AgNPs mediated by hydroacetonic leaf extract. The extraction was performed using an advanced UAE method. Synthesized AgNPs were characterized using distinctive spectroscopic/analytical techniques which results in the small-sized, spherical-shaped AgNPs. The produced AgNPs were found to be effective in terms of antioxidant and anticancer properties. Therefore, MsLE-AgNPs may be a potential anticancer drug to control human bone marrow neuroblastoma cancer. In the near future, extensive research on these nanoparticles will be carried out for various biological applications.

Acknowledgment

The authors are grateful to; the Chairman, P. G. Department of Studies in Botany at Karnatak University in Dharwad, Karnataka for the laboratory facilities; the USIC, DST-PURSE Program Phase-II, SAIF-Dharwad, Karnatak University, Dharwad, India for the spectral analysis and Soniya Education Trust's College of Pharmacy, Dharwad, Karnataka, India for instrumental facilities.

References

1. Srikar SK, Giri DD, Pal DB, Mishra PK, Upadhyay SN. Green synthesis of silver nanoparticles: a review. Green and Sustainable Chemistry. 2016;6(01):34. Available from: http://dx.doi.org/10.4236/gsc.2016.61004
2. Nair GM, Sajini T, Mathew B. Advanced green approaches for metal and metal oxide nanoparticles synthesis and their environmental applications. Talanta Open. 2022 Aug 1; 5:100080. Available from: https://doi.org/10.1016/j.talo.2021.100080
3. Leela A, Vivekanandan M. Tapping the unexploited plant resources for the synthesis of silver nanoparticles. African journal of biotechnology. 2008;7(17).
4. Dikshit PK, Kumar J, Das AK, Sadhu S, Sharma S, Singh S, Gupta PK, Kim BS. Green synthesis of metallic nanoparticles: Applications and limitations. Catalysts. 2021 Jul 26;11(8):902. Available from: https://doi.org/10.3390/catal11080902
5. Shenoy RU, Rama A, Govindan I, Naha A. The purview of doped nanoparticles: Insights into their biomedical applications. Open Nano. 2022 Nov 1; 8:100070. Available from: https://doi.org/10.1016/j.onano.2022.100070
6. Remya VR, Abitha VK, Rajput PS, Rane AV, Dutta A. Silver nanoparticles green synthesis: a mini review. Chem. Int. 2017;3(2):165-171.
7. Teng H, Jo IH, Choi YH. Optimization of ultrasonic-assisted extraction of phenolic compounds from Chinese quince (Chaenomeles sinensis) by response surface methodology. Journal of the Korean Society for Applied Biological Chemistry. 2010 Oct; 53:618-25. Available from: https://doi.org/10.3839/jksabc.2010.094
8. Surange SR, Deokule SS. Pharmacognistic studies on Wagatea spicata Dalzell. Ancient science of life. 1987 Apr 1;6(4):238-243. PMID: 22557574
9. Dhawal PP, Gharpure M, Joshi MS, Khan RR, Barve SS. Wound healing potential of Vakeri fortified Kampillakadi Taila. Journal of Ayurveda and Integrative Medicine. 2023 May 1;14(3):100721. Available from: https://doi.org/10.1016/j.jaim.2023.100721
10. Monclair T, Brodeur GM, Ambros PF, Brisse HJ, Cecchetto G, Holmes K, Kaneko M, London WB, Matthay KK, Nuchtern JG, Von Schweinitz D. The international neuroblastoma risk group (INRG) staging system: an INRG task force report. Journal of clinical oncology. 2009 Jan 1;27(2):298. Available from: https://doi.org/10.1200/JCO.2008.16.6876
11. Nayak D, Ashe S, Rauta PR, Kumari M, Nayak B. Bark extract mediated green synthesis of silver nanoparticles: evaluation of antimicrobial activity and antiproliferative response against osteosarcoma. Materials Science and Engineering: C. 2016 Jan 1; 58:44-52. Available from: https://doi.org/10.1016/j.msec.2015.08.022
12. Pandhari RM, Taranath TC. In-vitro Antioxidant Activity and Flow Cytometric Analysis of Simarouba glauca DC Bark Extract Induced Apoptosis in Triple Negative Breast Cancer Cells. Asian Pacific

Journal of Cancer Prevention. 2024 Jan 1;25(1):201-10. Available from: https://doi.org/10.31557%2FAPJCP.2024.25.1.201
13. Bankalgi SC, Londonkar RL, Madire U, Tukappa NA. Biosynthesis, characterization and antibacterial effect of phenolics-coated silver nanoparticles using Cassia javanica L. Journal of Cluster Science. 2016 Jul; 27:1485-97. Available from: https://doi.org/10.1007/s10876-016-1016-9
14. Chandra H, Kumari P, Bontempi E, Yadav S. Medicinal plants: Treasure trove for green synthesis of metallic nanoparticles and their biomedical applications. Biocatalysis and Agricultural Biotechnology. 2020 Mar 1; 24:101518. Available from: https://doi.org/10.1016/j.bcab.2020.101518
15. Salari S, Bahabadi SE, Samzadeh-Kermani A, Yosefzaei F. In-vitro evaluation of antioxidant and antibacterial potential of green synthesized silver nanoparticles using Prosopis fracta fruit extract. Iranian journal of pharmaceutical research: IJPR. 2019;18(1):430. Available from: PMID: 31089378
16. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983 Dec 16;65(1-2):55-63. Available from: https://doi.org/10.1016/0022-1759(83)90303-4
17. Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, Van Oers MH. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. 1994: 1415-1420. Available from: https://doi.org/10.1182/blood.V84.5.1415.1415
18. Rathikota D, Kattaru S, Potukuchi VG, Yeguvapalli S. Role of Lactiplantibacillus plantarum strain RD1 (Lpb RD1) in mitochondria-mediated apoptosis: an in vitro analysis. Archives of Microbiology. 2022 Sep;204(9):593. Available from: https://doi.org/10.1007/s00203-022-03175-3
19. Saha SK, Chowdhury P, Saini P, Babu SP. Ultrasound assisted green synthesis of poly (vinyl alcohol) capped silver nanoparticles for the study of its antifilarial efficacy. Applied surface science. 2014 Jan 1; 288:625-32. Available from: https://doi.org/10.1016/j.apsusc.2013.10.085
20. Hulkoti NI, Taranath TC. Biosynthesis of nanoparticles using microbes—a review. Colloids and surfaces B: Biointerfaces. 2014 Sep 1; 121:474-83. Available from: https://doi.org/10.1016/j.colsurfb.2014.05.027
21. Bhangale HG, Bachhav SG, Nerkar DM, Sarode KM, Patil DR. Study on optical properties of green synthesized silver nanoparticles for surface plasmon resonance. Journal of Nanoscience and Technology. 2019 May 6:658-61. Available from: https://doi.org/10.30799/jnst.230.19050203
22. Yallappa S, Manjanna J. Biological evaluation of silver nanoparticles obtained from T. arjuna bark extract as both reducing and capping agent. Journal of Cluster Science. 2014 Sep; 25:1449-62. Available from: https://doi.org/10.1007/s10876-014-0722-4
23. Nhung TT, Lee SW. Green synthesis of asymmetrically textured silver meso-flowers (AgMFs) as highly sensitive SERS substrates. ACS applied materials & interfaces. 2014 Dec 10;6(23):21335-45. Available from: https://doi.org/10.1021/am506297n
24. Küünal S, Kutti S, Rauwel P, Guha M, Wragg D, Rauwel E. Biocidal properties study of silver nanoparticles used for application in green housing. International Nano Letters. 2016 Sep; 6:191-7. Available from: https://doi.org/10.1007/s40089-016-0186-7
25. Srividhya B, Subramanian R, Raj V. Green synthesis of gold nanoparticles using black tea extract and their effect on the morphology and their antibacterial activity. Nanofabrication. 2023 Jan 29;8. Available from: https://doi.org/10.37819/nanofab.008.294
26. Kladko DV, Falchevskaya AS, Serov NS, Prilepskii AY. Nanomaterial shape influence on cell behavior. International Journal of Molecular Sciences. 2021 May 17;22(10):5266. Available from: https://doi.org/10.37819/nanofab.008.294
27. Sidhu AK, Verma N, Kaushal P. Role of biogenic capping agents in the synthesis of metallic nanoparticles and evaluation of their therapeutic potential. Frontiers in Nanotechnology. 2022 Jan 31; 3:801620. Available from: https://doi.org/10.3389/fnano.2021.801620
28. Singh S, Sharma B, Kanwar SS, Kumar A. Lead phytochemicals for anticancer drug development. Frontiers in plant science. 2016 Nov 8; 7:224341. Available from: https://doi.org/10.3389/fpls.2016.01667
29. Rajput S, Kumar D, Agrawal V. Green synthesis of silver nanoparticles using Indian Belladonna extract and their potential antioxidant, anti-inflammatory, anticancer and larvicidal activities. Plant cell reports. 2020 Jul; 39:921-39. Available from: https://doi.org/10.1007/s00299-020-02539-7
30. Kalishwaralal K, Deepak V, Pandian SR, Kottaisamy M, BarathManiKanth S, Kartikeyan B, Gurunathan S. Biosynthesis of silver and gold nanoparticles using Brevibacterium casei. Colloids and surfaces B: Biointerfaces. 2010 Jun 1;77(2):257-62. Available from: https://doi.org/10.1016/j.colsurfb.2010.02.007
31. Nayak PS, Pradhan S, Arakha M, Kumar D, Saleem M, Mallick B, Jha S. Silver nanoparticles fabricated using medicinal plant extracts show enhanced antimicrobial and selective cytotoxic propensities. IET nanobiotechnology. 2019 Apr;13(2):193-201. Available from: https://doi.org/10.1049/iet-nbt.2018.5025
32. Kamli MR, Srivastava V, Hajrah NH, Sabir JS, Hakeem KR, Ahmad A, Malik MA. Facile bio-fabrication of Ag-Cu-Co trimetallic nanoparticles and its fungicidal activity against Candida auris. Journal of Fungi. 2021 Jan 18;7(1):62. Available from: https://doi.org/10.3390/jof7010062
33. Ji S, Xiong M, Chen H, Liu Y, Zhou L, Hong Y, Wang M, Wang C, Fu X, Sun X. Cellular rejuvenation: molecular mechanisms and potential therapeutic interventions for diseases. Signal Transduction and Targeted Therapy. 2023 Mar 14;8(1):116. Available from: https://doi.org/10.1038/s41392-023-01343-5

HOW TO CITE THIS ARTICLE: Rangavitala P, Taranath TC. Green Synthesized *Moullava spicata* (Dalz.) Nicolson Leaf Extract Mediated Silver Nanoparticles Potentiate Antioxidant and Anticancer Activity in Human Bone Marrow Neuroblastoma Cancer Cells. Int. J. Pharm. Sci. Drug Res. 2024;16(4):662-670. **DOI:** 10.25004/IJPSDR.2024.160415