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Green Synthesis of Silver Nitrate Metallic Nanoparticle Using *Azadirachta Indica* Bark Extract, Characterization and Their Anticancer Activity

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Abstract: The current study describes the synthesis of silver nanoparticles with *Azadirachta indica* bark extract for use against MCF-7 and Hep-2 cells in vitro. Within seven to eight days, silver nanoparticles (AgNPs) were created, and UV-visible spectroscopy provided preliminary confirmation of this. It was further described using HR-TEM and FT-IR. The significant wide peak for Ag nanoparticles at 441 nm was shown by the UV Visible examination. TEM pictures show mostly spherical, biosynthesized AgNPs with a particle size range of 20 nm. *Azadirachta indica* bark extract at doses ranging from 10 to 100µg/ml and silver nanoparticles were used in MTT tests. Compared to Azadirachta indica bark extract, biosynthesized silver nanoparticles had a substantial cytotoxic impact against both MCF-7 and Hep-2 cells at different doses. As a consequence, the findings demonstrate the outstanding uses of *Azadirachta indica* in the green manufacture of silver nanoparticles.

Keywords: *Azadirachta indica* • silver nanoparticles • UV-visible spectroscopy • FTIR • HR-TEM • anticancer activity

Introduction

Cancer refers to a broad variety of cancers brought on by unregulated cell proliferation. Negative consequences, such tissue breakdowns, are likely to occur if normal cells do not control fast growth. It is the second most common cause of cancer-related mortality in industrialized countries, and its prevalence is growing quickly in both developed and developing countries (Gupta *et al*., 2023). Medicinal plants serve as a major source of income for high-altitude inhabitants in the Himalayas, particularly in countries like India, Nepal, and Bhutan (Gupta *et al*., 2023). In the last few decades, synthesis of metallic nanoparticles is one of the emerging fields of nanoscience and nanotechnology (Rawat *et al*., 2023). Nanoparticles were further characterized by UV-visible, XRD, FTIR, SEM and TEM techniques (*Jasrotia et al.*, 2023). Due to their many distinct qualities compared to bulk, metal nanoparticles have drawn a lot of interest from researchers lately. These features have several uses in the areas of medication administration, cancer treatment, diagnostics, cell labeling, and antimicrobial agents (Nel A *et al*., 2006). For several reasons, including their low toxicity and value as an antibacterial agent, silver nanoparticles have garnered significant attention (Seralanthan J *et al*., 2014; Ahmed KB et al., 2014). Plant-mediated synthesis is becoming more economical, environmentally benign, and helpful in scaling up the production of nanoparticles. Because of their dispersion, size, and form, biosynthetic procedures are an efficient technique to create silver nanoparticles utilizing plants or their extract in a limited fashion (Kumar V *et al*., 2009). The possibility of employing advantageous nanoparticles in the detection and management of human malignancies has increased thanks to plant-based nanoparticles (Sankar R *et al*., 2013).

Azadirachta indica commonly known as neem, margosa, or Indian lilac, is a tree in

the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*. Although it is native to the Indian subcontinent and some regions of Southeast Asia, it has been naturalized and is cultivated in tropical and subtropical regions all over the world. Neem oil is extracted from the plant's fruits and seeds. The margosa tree grows quickly, reaching heights of 15–20 meters (49–66 feet) and, on occasion, 35–40 meters (115–131 feet). According to Gaur (1999), this evergreen sheds a large number of leaves during the arid winter months. The branches stretch out far. The roundish, rather dense crown can have a diameter of 20–25 m (66–82 ft). The leaves are opposite, pinnate, and range in length from 20 to 40 cm (8 to 16 in). The leaflets are medium to dark green, measuring 3 to 8 cm (1+1/4–3+1/4 in) in length. Frequently, the concluding pamphlet is absent. Petioles are not very long. Up to 25 centimetres (10 in) long, white, fragrant blooms are grouped in almost drooping axillary panicles. There are between 250 and 300 blooms on the third-degree branching inflorescences. A single blossom measures 5.6 mm (3/16-1/4 in) in length and 8–11 mm (5/16–7/16 in) in width. On a single tree, protandrous, bisexual blooms coexist with male flowers. The fruit is a smooth (glabrous), olive-like drupe that, when ripe, measures 14–28 mm (1⁄2-1+1⁄8 in) by 10–15 mm (3⁄8–5⁄8 in). Its form ranges from elongate oval to almost roundish. The mesocarp, or bitter-sweet pulp, is very fibrous and yellowish-white in color, whereas the exocarp, or fruit skin, is thin. The thickness of mesocarp is 3–5 mm (1/8–1/4 in). The fruit's one, rarely two, or three elongated seeds (kernels), which have a brown seed coat, are enclosed by the fruit's white, hard inner shell, or endocarp.The margosa tree may be mistaken for its relative, the Melia azedarach, often known as the chinaberry or bakain, due to their similar appearances. The fruit of Melia azedarach resembles it and it also has toothed leaflets. One distinction is that chinaberry leaves are twice- and three-pinnate, whereas margosa leaves are pinnate.It is believed that *Azadirachta indica* is indigenous to the Indian subcontinent, Bangladesh, Cambodia, Laos, Myanmar, Thailand, and Vietnam in Indochina. It has been widely dispersed over tropical and subtropical areas, ranging from Indonesia to South America. Although margosa tree products have been used for generations in Indian traditional medicine to treat rheumatism and skin conditions, there is not enough clinical data to support the advantages of margosa usage in medicine.Adults: There are no known dosages, and while short-term use of margosa seems safe, prolonged usage may damage the kidneys or liver; margosa oil is poisonous and can be fatal in tiny children. Additionally, margosa may result in low blood sugar, infertility, and miscarriages.

Social media posts from March 2020 across Africa and several Southeast Asian nations supported the usage of margosa leaves to cure COVID-19. The Malaysian Ministry of Health debunked claims about utilizing the leaves to cure COVID-19 and alerted people to the hazards of consuming too many leaves for their health. Because of its many therapeutic benefits, neem has been employed widely in the Indian traditional medical system. Numerous structurally and chemically complex phytochemicals, including limonoids, flavonoids, phenols, catechins, gallic acid, polyphenols, and nimbins, are found in neem. In this study, *Azadirachta indica* was used to create the silver nanoparticles, which were then examined using Fourier transform infrared spectroscopy (FTIR), ultraviolet-visible spectroscopy, and high resolution transmission electron microscopy (HR-TEM). Additionally, the anticancer activity against MCF-7 and Hep-2 cell lines was investigated for the first time.

Materials and Methods

Resources and Processes obtaining and confirming botanical specimens. The Herbarium Forest Research Institute confirmed the authenticity of the bark obtained from the Nagdev forest area in Pauri, Uttarakhand, which *is Azadirachta indica*.

Preparation of the *Azadirachta indica hot extract*

Bark, or *Azadirachta indica*, was gathered and verified at the nearby market. A pestle and mortar was used to finely pulverize the bark of *Azadirachta indica*. After dissolving the powder (20 g) in 100 ml of millipore water, the mixture was heated for 10 minutes at 80°C, and a syringe filter (0.45 µ) was used to filter the mixture (Coates J *et al*., 2000;Geethalakshmi R *et al*., 2012)**.**To reduce Ag+ ions, 10 ml of *Azadirachta indica* concoction was added to 90 ml of a 1 mM silver nitrate solution.

Synthesis of silver nanoparticles

To maximize the synthesis, a water bath was used to maintain a range of temperatures, including RT, 40, 60, and 80°C. For ten minutes, the solution was agitated at 1000 rpm (Sulaiman GM *et al.*, 2013). To confirm that silver nanoparticles were forming, the color variation was seen at different temperatures. The bark mixture of *Azadirachta indica* was used to reduce and stabilize 1 milligram of silver nitrate (Devi JS *et al*., 2012; Devi JS et al., 2012).

Characterization of silver nanoparticles

UV-visible spectroscopy was used to characterize silver nanoparticles in an initial manner (Devi JS *et al*., 2012; Renugadevi K *et al*., 2012). Using the nanodrop 2000r, UV–Vis spectroscopic analysis was carried out in the 200–800 nm scanning range. As a blank, millipore water was utilized. Fourier transform infrared spectroscopy (FTIR) was used to examine the interactions between protein-silver nanoparticles in the 4000–400 cm-1 range (Devi JS *et al*., 2012). Using a libra 200 HR-TEM (m/s), TEM images of the biosynthesized AgNPs were acquired in order to determine their size and form.The accelerating voltage was used at 120 and 200 kV (Carl Zeiss, Germany). A drop of the diluted sample was deposited onto the carbon-coated copper grid after the AgNPs were sonicated for five minutes. At room temperature, the liquid portion was allowed to evaporate (Ramteke C *et al*., 2012).

Cell lines and culture

Cell lines for human larynx carcinoma (Hep-2) and breast cancer (MCF-7) were acquired from the King Institute of Preventive Medicine and Research, ICMR, Chennai, India. It was grown in Dulbecco's modified Eagle's medium (DMEM; Hi Media Laboratories, Mumbai, India), which was enhanced with 1% penicillin/streptomycin and 10% fetal bovine serum. The CO2 incubator was kept at 5% CO2 for the cell lines (Prasad TNVKV *et al*., 2011). An inverted microscope was used to assess the confluency of the cultures and ensure that no bacterial or fungal contamination were present (Satyavani K *et al*., 2011).

MTT assay

Azadirachta indica bark extract and silver nanoparticles were tested for cytotoxicity using the MTT reduction assay on cell viability. A 96-well plate was seeded with MCF-7 and Hep-2 cells at a density of 5 × 103 cells/well. The cells were cultured in 200 µl of DMEM containing 10% FBS for 24 hours after being permitted to adhere to the 96-well plate (Krishnaraj C *et al*., 2014).The cells were then cultured for 48 hours after the medium were changed and replaced with a suspension of different concentrations of silver nanoparticles, ranging from 10 to 100 mg/ml (at least 4 wells were seeded with each concentration). Inbathamizh L *et al*., 2014).Following the addition of 10 ml of MTT (5 mg/ml), the cells were incubated for an additional 4 hours at 37°C. Following the removal of the medium, 200 µl of DMSO was applied to each well. Using a multi-well spectrophotometer, the formazan product's

optical density (OD) was measured at 620 nm (Satyavani K *et al*., 2011; Vivek R *et al*., 2012). The average of four separate trials was used to present the results. The following formula was used to sort out the percentage of viability based on the OD value: [(Total initial cell count- Killed cell count)/Total initial cell count] is the percentage of viable cells. x100

Results and Discussion

Figure 1a shows the colorless silver nitrate solution. Figure 1b shows the dark red color of the silver nitrate solution after *Azadirachta indica* bark extract was added. This attests to the reduction of silver nitrate and its transformation into silver nanoparticles.

Figure 1: Visual observation of (a) silver nitrate solution (b) biosynthesized silver nanoparticles

UV-Vis spectral analysis

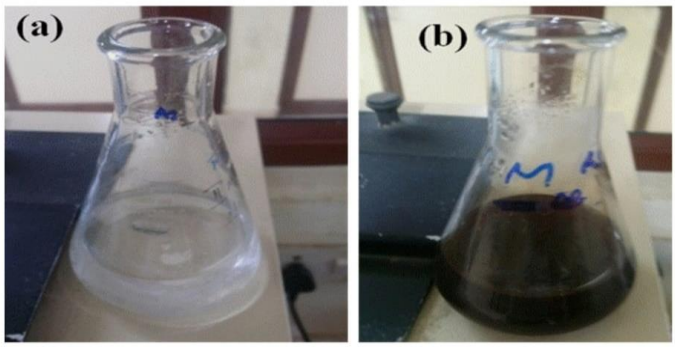

Figure 2 displays the UV-Vis spectra of artificially generated AgNPs from *Azadirachta indica* bark extract at various temperature settings. The color shift indicates that the bark extract from *Azadirachta indica* contains silver nanoparticles. Using *Azadirachta indica* nanoparticle extract, it demonstrates the UV-Vis spectra of silver nanoparticle synthesis at different temperatures: (i) room temperature (RT), (ii) 40°C, (iii) 60°C, and (iv) 80°C in aqueous medium. The region of 420–446 nm was where the colloidal silver surface plasmon resonance bands were found at various temperatures. Compared to other samples, a strong SPR band was seen at a higher temperature of 80°C. The production of silver nanoparticles is confirmed and the spectroscopic data recorded and plotted (Jasrotia S *et al*., 2023) by the strong SPR wide peak seen at 441 nm.

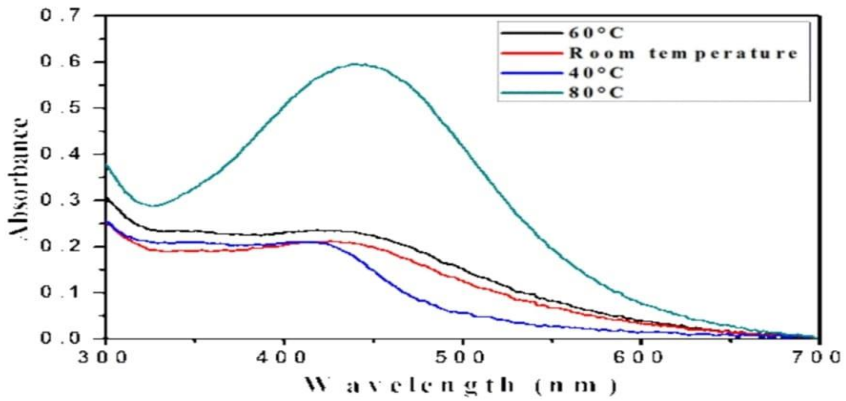

Figure 2: UV-Visible analysis of synthesized AgNPs from *Azadirachta indica* bark extract at different temperature conditions.

FT-IR analysis

Figure 3a shows the FTIR spectrum of. At 3421 cm-1, 2933 cm-1, 2388 cm-1, 1632 cm-1, 1388 cm-1, 1221 cm-1, 1021 cm-1, 565 cm-1, and 521 cm-1, *Azadirachta indica* was discovered. The band located at 3421 cm-1 is indicative of the typical "polymeric" OH stretching mode. The methylene C-H asymmetric and symmetric stretching mode is linked to the peak at 2933 cm-1. The symmetric stretching of alkanes is shown by the peak at 2388 cm-1, whereas the C=O stretching mode of ketones is indicated by the peak at 1632 cm-1. The N=O stretching of the nitro groups in the leaf extract is shown by the peak at 1388 cm-1. The C-C stretch and the C-F stretch of aliphatic fluoro compounds are shown by the 1021 cm-1 peak. The peaks at 521 and 565 cm-1 correspond to the out-of-plane bending of alcohol, OH, aliphatic iodo compounds, and C-I stretch.

The AgNPs *Azadirachta indica* FTIR spectrum is shown in the Figure 3b. Even after adding silver nitrate, the band at 3421 cm-1 remains, corresponding to the "polymeric" OH stretching mode. The C=O stretching mode of ketone is responsible for the strong signal seen at 1645 cm-1 (Figure 3b). The peaks suppressed in comparison to *Azadirachta indica* include those at 1230 cm-1 and 1026 cm-1, which correspond to the amine C–N stretching, the C–C stretching, and the aliphatic fluoro compound C–F stretching, respectively, found in the AgNPs. The addition of silver nitrate results in the total reduction and stability of the silver nanoparticles and entirely suppresses the stretching vibration of the nitro group of the leaf (1388 cm-1), as well as the iodo compounds' OH group out of plane bending (521 cm-1, 565 cm-1). Here, we verify that the bioreduction of silver nitrate to silver nanoparticles is dependent on the regulated transmittance percentage of ketone, fluoro compounds, and amine groups (Coates J *et al*., 2000; Devi JS *et al*., 2012).

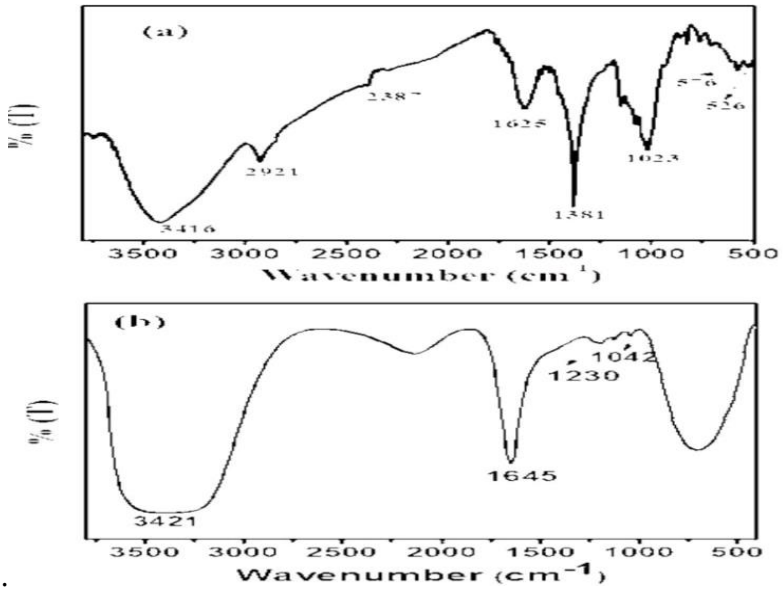

Figure 3: (a) FTIR spectrum of *Azadirachta indica* and (b) FTIR spectrum of biosynthesized silver nanoparticles Figure 3: (a) FTIR spectrum of *Azadirachta indica* and (b) FTIR spectrum of biosynthesized silver nanoparticles

HRTEM analysis

HRTEM is an effective method for analyzing atomic-level material characteristics. AgNPs' HRTEM picture is seen in Figure 4. AgNPs have a particle size that falls between 20 and 40 nanometers. The thick matrix in which the

nanoparticles are lodged might be the organic stabilizing agent found in the extract of *Azadirachta indica*. Silver nanoparticles have a spherical form

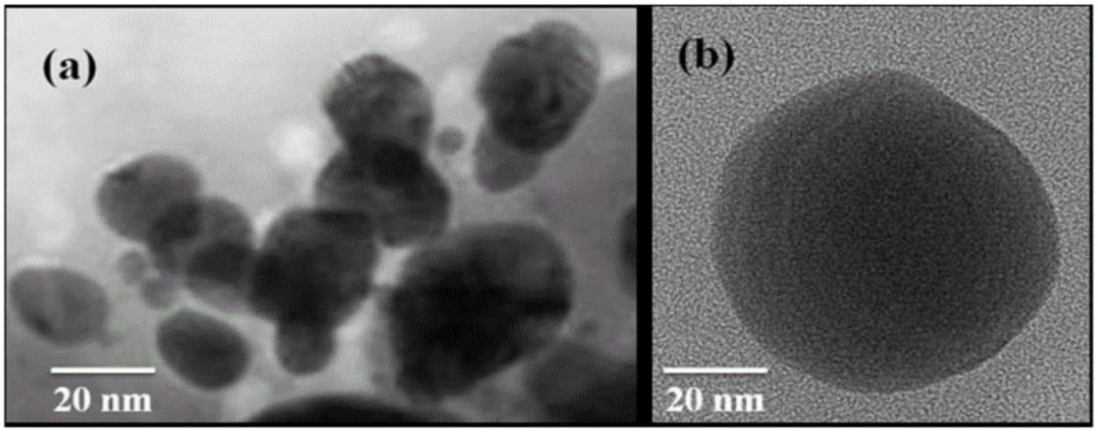

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Figure 4: (a) HRTEM image of AgNPs and (b) 0aJnLfied portion of AgNps.

Cytotoxicity analysis

Utilizing the MTT test, the cytotoxicity of the *Azadirachta indica* bark extract and silver nanoparticle was investigated against the MCF7 (Figures 5a and 5b) and Hep2 cell line (Figures 5c and 5d). Different concentrations of cancer cells (10 µg, 20 µg, 30 µg, 40 µg, 50 µg, 60 µg, 70 µg, 80 µg, 90 µg, and 100 µg) were used to study the cytotoxicity impact. The phytomediated AgNPs were found to have an inhibitory concentration (IC50) value of 54 µg/ml when tested against MCF7 cells and an extract from the *Azadirachta indica*.The bar graph in Figure 6b illustrates the relative effectiveness of biosynthesised AgNps and *Azadirachta indica* against Hep2 cells at varying concentrations. According to Jacob JP *et al*. (2012), silver nanoparticles may really cause harm to cellular components and reactive oxygen species, ultimately resulting in cell death

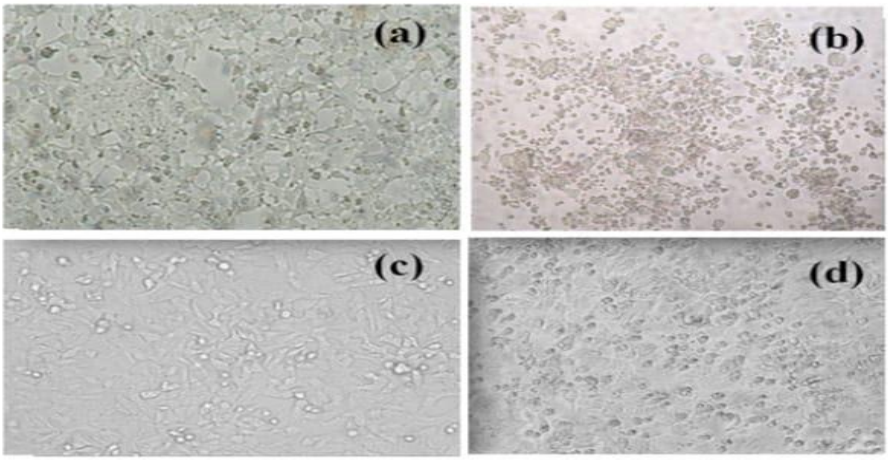

Figure 5: Cytotoxicity of the green synthesized silver nanoparticles against the MCF7. (a) control, (b) cytotoxic and the Hep2 cell line (c) control, (d) cytotoxic.

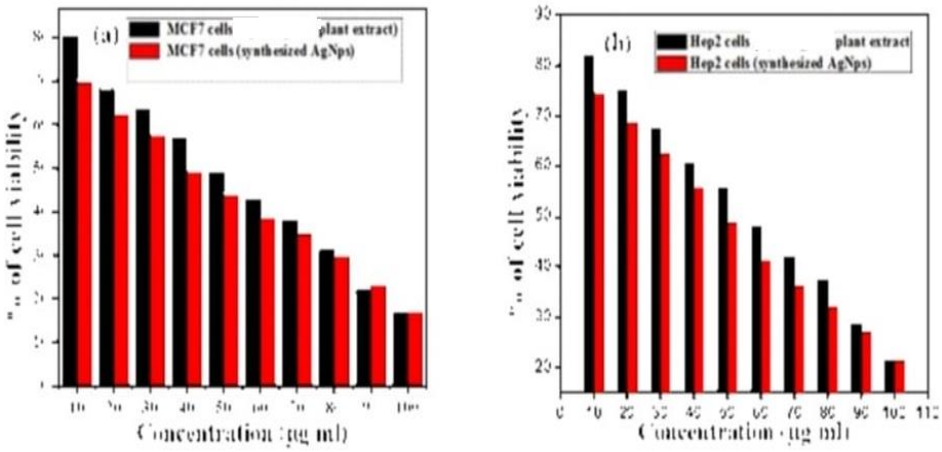

Fig 6: (a) Efficacy of *Azadirachta indica* and biosynthesized AgNps against MCF7 cells at different concentration and (b) Efficacy of *Azadirachta indica* and biosynthesized AgNps against Hep2 cells at different concentration.

Conclusion

We present an easy, cheap, sustainable, and green method for synthesizing silver nanoparticles from *Azadirachta indica* in an aqueous media without the use of synthetic chemicals. The creation of silver nanoparticles has been tentatively verified by FT-IR analysis and UV-Vis spectroscopy. A spherical form with an average particle size of 20–40 nm was seen in the TEM picture Promising anticancer activity was demonstrated by the biosynthesized silver nanoparticles and *Azadirachta indica* bark extract against human pharyngeal cancer cell line (Hep-2) and breast cancer cells (MCF-7). The study's findings indicate that plant-based silver nanoparticles have strong anticancer activity against cell lines, which raises the possibility of these particles' potential medicinal applications.

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Conflict of Interest

The authors state that there are no conflicts of interest associated with the publication of this work.

References

Ahmed K B A, Subramanian S, Sivasubramanian A, Veerappan G and Veerappan A (2014). Preparation of gold nanoparticles using *Salicornia brachiata* plant extract and evaluation of catalytic and antibacterial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *130*, 54-58.

Coates J (2000) Interpretation of Infrared Spectra. John Wiley & Sons Ltd, Chichester.

Devi JS, Bhimba BV (2012) Anticancer activity of silver nanoparticles synthesised by the seaweed Ulva lactuca invitro. *Sci Rep* 1: 242-246.

Devi JS, Bhimba BV (2012) Silver nanoparticles: Antibacterial activity against wound isolates & invitro cytotoxic activity on Human Caucasian colon adenocarcinoma. *Asian Pac J Trop Dis* 2: S87-S93.

Devi JS, Bhimba BV, Ratnam K (2012) Invitro anticancer activity of silver nanoparticles synthesized using the extract of *Gelidiella sp*. *Int J Pharm Pharm Sci* 4: 710-715.

Gaur RD (1999). Flora of the District Garhwal, North West, 1st edition, Transmedia, 459.

Geethalakshmi R, Sarada DVL (2012) Gold and silver nanoparticles from *Trianthema decandra*: synthesis, characterization, and antimicrobial properties. *Int J Nanomed* 7: 5375-5384.

Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281: 1309-1312.

Gupta Stuti, Shivani Jasrotia, and M C Purohit (2023) "Conservation, Cultivation, and Sustainable Use of High Altitude Medicinal and Aromatic Plants for Socio-Economic Development: A Review." *Journal of Mountain Research*, (p ISSN: 0974-3030; e ISSN: 2582-5011), Vol 18 (1), June, 201-209.

Gupta Stuti, Shivani Jasrotia, Anuj Kandwal, Rashmi Rawat and M C Purohit (2023) "Green Synthesis of Silver Nitrate Nanoparticles using L. of Pyracantha crenulata and its Anticancer Activity on Liver Cancer."*Journal of Mountain Research*, (P-ISSN: 0974-3030; E-ISSN: 2582-5011), Vol. 18 (2), 273-283.

Inbathamizh L, Ponnu TM, Mary EJ (2013) In vitro evaluation of antioxidant and anticancer potential of *Morinda pubescens* synthesized silver nanoparticles. *J Pharmacy Research* 6: 32-38.

Jacob JP, Finub S, Narayanan A (2012) Synthesis of silver nanoparticles using *Piper longum leaf* extracts and its cytotoxic activity against Hep-2 cell line. *Colloids Surf B Biointerfaces* 91: 212-214

Jasrotia S, Kumar G, Badoni PP, Gupta S. (2023) Removal Of Rose Bengal Dye From The Aqueous Medium Using Biologically Synthesized Zno Nanoparticles *Journal of Mountain Research*, (p ISSN: 0974-3030; e ISSN: 2582-5011), Vol 18 (1), June, 179-187.

Jasrotia, S., Gupta, S., Kumar, G., & Badoni, P. P. (2023). Synthesis And Characterization Of Zinc Oxide Nanoparticles Using Aqueous Extract Of Arisaema Tortuosum Tuber For Dye Removal. *Plant Archives (09725210)*, *23*(2).

Krishnaraj C, Muthukumaran P, Ramachandran R, Balakumaran MD, Kalaichelvan PT (2014) *Acalypha indica Linn*: Biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against *MDA-MB-231*, human breast cancer cells. *Biotechnol Rep* 4: 42-49

Kroemer G, Zamzami N, Susin SA (1997) Mitochondrial control of apoptosis. *Immunol Today* 18: 44-51.

Kumar V, Yadav SK (2009) Plant-mediated synthesis of silver and gold nanoparticles and their applications. *J Chem Technol Biot* 84: 151-157.

Nel A, Xia T, Madler L, Li N (2006) Toxic potential of materials at the nanolevel. *Science* 311: 622-627.

Prasad TNVKV, Elumalai EK (2011) Biofabrication of Ag nanoparticles using *Moringa oleifera leaf* extract and their antimicrobial activity. *Asian Pac J Trop Biomed* 1: 439-442.

Ramteke C, Chakrabarti T, Sarangi BK, Pandey RA (2012) Synthesis of silver

nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. *J Chem* 2013: 1-7.

Rawat, Rashmi, Anuj Kandwal, Reena Purohit, Arun K. Khajuria, Stuti Gupta, and M. C. Purohit. (2023) "Green Synthesis Of Zinc Oxide Nanoparticles Using Sapindus Mukorossi Fruit Extact, Characterization And Their Antioxidant Activity." *Journal of Mountain Research*, (P- ISSN: 0974-3030; E-ISSN: 2582-5011), Vol 18 (2), 25-32.

Renugadevi K, Inbakandan D, Bavanilatha M, Poornima V (2012) Cissus quadrangularis assisted biosynthesis of silver nanoparticles with antimicrobial and anticancer potentials. *Int J Pharm Biol Sci* 3: 437-445.

Sankar R, Karthik A, Prabu A, Karthi S, Shivashangari KS, et al. (2013*) Origanum vulgare* mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity. *Colloids Surf B Biointerfaces* 108: 80-84.

Satyavani K, Gurudeeban S, Ramanathan T, Balasubramanian T (2011) Biomedical potential of silver nanoparticles synthesized from calli cells of *Citrullus colocynthis* (L.) Schrad. *J Nanobiotechnology* 9: 1-8.

Seralathan, J., Stevenson, P., Subramaniam, S., Raghavan, R., Pemaiah, B., Sivasubramanian, A., & Veerappan, A. (2014). Spectroscopy investigation on chemo-catalytic, free radical scavenging and bactericidal properties of biogenic silver nanoparticles synthesized using *Salicornia brachiata aqueous* extract. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *118*, 349-355.

Sulaiman, G. M., Mohammed, W. H., Marzoog, T. R., Al-Amiery, A. A. A., Kadhum, A. A. H., & Mohamad, A. B. (2013). Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using *Eucalyptus chapmaniana* leaves extract. *Asian Pacific journal of tropical biomedicine*, *3*(1), 58-63.

Vivek R, HanJam R, Muthuchelian K, Gunasekaran P, Kaveri K, et al. (2012) Green biosynthesis of silver nanoparticles from *Annona squamosa* leaf extract and its in vitro cytotoxic effect on MCF-7 cells. *Process Biochem* 47: 2405-2410.