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Biological Synthesis and Characterization of Silver Nanoparticles of Aconitum Heterophyllum for Antifungal Activity

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KEYWORDS

Ativisha,, silver nanoparticles , SEM, phytoconstituent, Atisine, spectroscopy, antifungal activity.

ABSTRACT:

Green synthesis of metal nanoparticles are plant mediated and these are gaining more attention due tc their easy, rapid and ecofriendly nature. Biomolecules present in the plant extract can be used to reduce metal ions to nanoparticles in a plant extract mediated process. The mono metallic nanoparticles was prepared from the plant extract of Aconitum heterophyllum. The Silver nanoparticles showed that the effective antifungal achievement against C. albicans.

Introduction

Gold and silver nanoparticles have gained considerable attention due to their inherent medicinal properties which make them an ideal candidate on the medical platform. They also play an extensive role in electronic industries, textile coatings, food storage, agriculture industries, biosensor and environmental applications Since ancient times gold nanoparticles with its inherent medicinal properties were used as Swarn bhasm. Swarn jal, Swaran churn etc. Today, gold nanoparticles are widely utilized for drug regulation, cancer therapy, bio-imaging, sensor and treatments. Gold has a marvelous rapport with the human system having low toxicity and adjustable stability. Gold nanoparticles have specific optical properties to absorb infrared light and capability of coating with large surface exhibiting excellent therapeutic potential Silver metal also has specific inherent medicinal activities like anti-inflammatory, wound healing, antimicrobial, antioxidant and anticancer bio-efficacies.[1,2]

Silver is inert in its non-ionized form, however, when dissolved in water or even in the presence of moisture, generate silver ion. The charged silver ions are responsible for antimicrobial activity. Silver ions enter as granules in the cell wall, inhibit the cell division, damage the cellular contents and finally prevent bacterial growth . Silver atoms strongly interact with thiol groups (-SH) and consequently cause the enzymatic deactivation[3,4].

Silver ion-induced catalytic oxidation involves the hydrogen atom of the thiol group of enzymes and oxygen atom from cellular content leading to the formation of disulfide bonds (R- S-S-R). During such structural changes in R-S-S-R bonds, the cellular enzymes get altered and pose an impact on biological functions. Silver ion's interaction with the 30S ribosomal unit results in the disabling of the ribosomal complex and inhibits the translation of proteins . Ag^+ interacts with base pairs (purine and pyrimidine), breaking the hydrogen bonds among the two anti-parallel strands and finally denatures DNA.

However, the exact mechanism is yet to be established. The transport of silver ions across the cell membrane takes place even in the absence of a silver transporter as its putative function is a copper transporter .Silver is non-toxic to the cardiovascular, nervous, immune and reproductive system. It is not carcinogenic . The exposure of a high concentration of silver causes stomach irritation, low blood pressure, dysfunctioning of liver and kidney. Plant bioactives with metallic nanoparticles are much safer than drugs like ativisha. Ativisha (*Aconitum heterophyllum* wall) of family Ranunculaceae is an Ayurvedic herb whichis known for its important medical properties. The root of the plant find use in one form or the other in various Ayurvedic preparations. Ativisha *(Aconitum heterophyllum*) is a popular Ayurvedic herb for treating diarrhoea, fever, and inflammation. Ativisha is a critically endangered species found exclusively in the subalpineHimalayas. Atisine is generally regarded non-poisonous due to its significantly lower toxicity than aconitine and pseudaconitine. Despite the fact that the alkaloid atisine produces hypotension, a full aqueous extract of the root generated considerable hypertension, most likely as a result of an effect on the sympathetic nervous system. Aconitine acts on the central nervous system, cardiovascular system, and respiratory system. As a result, plant extracts may be preferred to microbes for this purpose because to their ease of development, lower toxicity, and more complicated way of cell culture upkeep. It is the optimal platform for nanoparticle synthesis since it is free of toxic substances and contains natural capping agents that enhance the stability of metal nanoparticles[5,6].

MATERIALS AND METHODS

The reagents used were of analytical quality, and they were obtained from Merck (Mumbai, India). The chemicals that were employed in the synthesis were of the highest analytical quality. Merck (India) provided silver nitrate ($AgNO_3$),.. All of the solvents were purchased from Sigma Aldrich, Pvt. Ltd. in Mumbai, India. Sabouraud's dextrose broth (SDB) and potato dextrose agar (PDA) were purchased from Hi-Media, India.

Collection of plant

In September (flowering season), the Aconitum Hetreophyllum species was obtained from Razdhan pass (Bandipora), Jammu and Kashmir, India .

Extraction of root of A. Heterophyllum

The 5 g root, was cleaned and cut into tiny pieces, processed into powder with a mechanical grinder. The root powder was then sequentially extracted using a series of solvents, including hexane, ethyl acetate and methanol, over a period of 48 h, with occasional shaking and using intermittent heating over a water bath at their respective boiling temperatures. Filteration and drying of the extracts. [7,8]

Qualitative Detection of Phytochemical Constituents:

Detection of active phytochemical constituents was carried out for all the extracts using the standard procedures.In order to determine the active phytochemical contents in all of the extracts, the usual methodologies were used. [9]

a) Synthesis of silver nanoparticles

An aqueous solution (0.01mM) of silver nitrate ($AgNo_3$) and various concentrations of root extract of Aconitum heterophyllum from 1 to 5 ml were formed independently for the preparation of silver nanoparticles. Each concentration of the root extract was added to a 10 ml solution of 0.01mM $AgNO_3$ that had been previously prepared. It took 20 minutes for the colour of the solution to shift from light yellow to dark brown, indicating the production of $AgNO_3$ in the solution. The resulting colloidal silver solution was subjected to UV – Vis spectroscopy to determine its composition[10,11].

Characterization of biosynthesized nanoparticles

UV–visible spectroscopy

The UV-vis spectra of the biosynthesized Ag nanoparticles in the 200-850-nm wavelength range was investigated. A UV–Visible spectrophotometer was used to observe the reduction of Ag ions in the reaction mixture. [12].

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is used to observe the surface morphologies of Ag, nanoparticles in greater detail. Because the surface of the samples is not sufficiently conductive, charge-up can occur during irradiation with the electron beam when the samples are exposed to it. The surface of the samples is coated with a very thin film sampleprepared using the silver, gold, and silver/gold bimetallic nanoparticles via a method of spin coating (1500 rpm) on a 1x1-cm aluminium foil, after which the samples are allowed to dry for30 minutes at room temperature[13,14].

X-ray diffraction (XRD) technique

PAN analytical X-PERT PRO diffractometer at room temperature was used to determine the crystalline structure and particle size of nanoparticles. It is possible to execute this test procedure by shining an x-ray beam onto a sample andmeasuring the scattered intensity as a function of the outgoing direction. Once the beam has been separated, the scattering pattern, also known as a diffraction pattern, displays the crystalline structure of the material. [15,16].

Fourier transformed infrared spectroscopy (FTIR)

The chemical arrangements of AgNPs was carried out using the FTIR spectrometer (Perkin Elmer Spectrum 100 FTIR Spectrometer) at room temperature. FTIR experiment was carried out to identify the biomolecules present in the extract responsible for the reduction of silver and gold ions. The Infrared spectra were noted in the range of 4000–500 cm 1 [17].

Microscopic (TEM) analyses

A transmission electron microscope (TEM) was used to examine the morphology of the biosynthesized silver nanoparticles. For TEM investigation, we prepared the samples by drop coating the nanoparticle solutions onto carbon-coated copper grids and allowing them tocure at room temperature for several hours. The TEM study was

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carried out using a Tecnai F20model that had been set up with an accelerating voltage. The transmission electron microscope(TEM) images a nanoparticle sample using an electron beam, which provides significantly higher resolution than is feasible with light-based imaging techniques. The transmission electron microscope (TEM) is the method of choice for directly measuring nanoparticle size, grain size, size distribution, and morphology[18,19].

Antifungal activity determination of prepared samples

Preparation Potato dextrose Agar media (PDA)

For the fungal growth the easiest and most common culture media is potato dextrose agar media which can be easily prepared in laboratory. Potato infusion and dextrose promote luxuriant fungal growth.[20,22]

Determination of antifungal susceptibility:

Fungal Culture :Fungus in freeze dried condition and revived the culture on agar plate in laboratory.

In vitro antifungal test:

The antifungal activity was performed on petridishes containing potato dextrose agar when temperature of the medium reached at 40°C i.e. slightly above the room temperature. The specific concentration of 100% stock solution nanoparticles (diluted in double distilled water) were added to petriplates of 9 cm diameter containing PDA. The rate of mycelial growth was measured after placing an active mycelial plug of fungi on petridish. Fluconazole was used as positive control. Finally the petriplates were incubated at 28°C. The observation was recorded after 48 hours. [23]:

RESULT:

Phytochemical Characterization of Plant Extract:

Table1: Qualitative phytochemical screening of aconitum heterophyllum

S.no.	Plant constituents	Haxene extract	Ethyl acetate extract	Methanolic extract
1.	Alkaloids	–	+	++
2.	Steroids	+	+	-
3.	Terpenoids	–	+	+
4.	Terpenes	–	–	–
5.	Phenols	+	+	++
6.	Tannins	–	+	+
7.	Saponins	+	+	++
8.	Flavonoids	+	+	++
9.	Glycoside	+	+	-

Note: '++' = Strong presence, '+' moderate presence, and '–' = absent;

Characterization of silver nanoparticles:

UV-vis spectroscopy

The formation of monometallic and bimetallic nanoparticles was monitored using a Microplate reader Epoch 2 (Biotech) to obtain the UV-vis spectra. The nanoparticle solution was scanned between the wavelengths of 300 to 850nm. The peak for silver, gold and bimetallic nanoparticles was found to be at 437nm.

Phyto-fabricated silver nanoparticles:

The phyto-fabrication of $AgNp_s$ was carried out as functions of pH 7.5, 9.5 and 11.5. No perceptible peak was obtained at acidic conditions. As the pH increases (7.5-11.5), the wavelength shifted (447 - 437 nm) towards a lower wavelength. The hypsochromic shift from 447 to 437 nm attributed to the formation of small-sized nanoparticles and having high absorbance intensity. Therefore, pH 11.5 has been considered as optimum pH, having a characteristic peak at λ_{max}: 437 nm. The pH has a significant role in the phyto-fabrication of metal nanoparticles by altering the charge on the molecule, affecting their properties andbehavior. The change in the pH of the solution can alter peak absorption, wavelength and intensity.

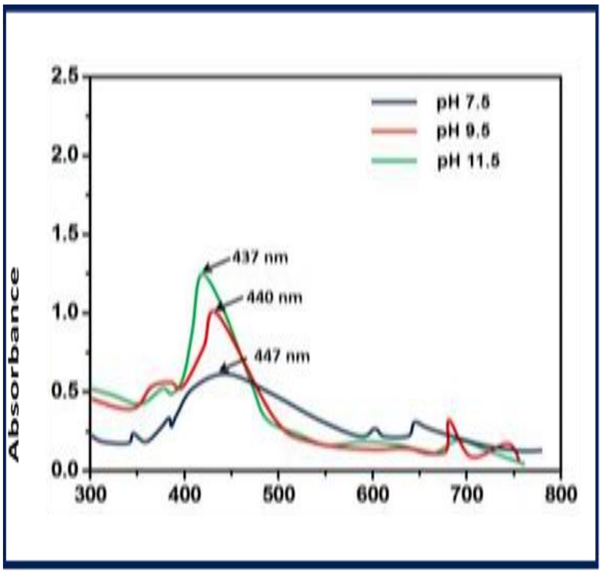

Fig.1 : UV-Vis spectra of $AgNp_s$ at different pH

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Fourier Transform Infrared Spectra

The FTIR spectra can help in study the functional groups present in the synthesized nanoparticles. Since the nanoparticles were synthesized from the *aconitum root* extract, its functional group get attached to the nanoparticles and impart additional characteristics. The region below 1500 cm^{-1} is known as the fingerprint region which is due to the bending vibrations of the molecule. The band noticed between 3371 to 3290 cm^{-1} may be assigned to O-H stretching. The high peak between 1710 and 16 cm^{-1} may indicate a stretching of C=O bonds.

X-ray Diffraction (XRD):

The crystallite size D can be calculated by using Debye-Scherrer formula D=0.9/B (2θ) cos θ, where is the wavelength of the. X-ray, B (2θ) is the full width at the half maximumintensity at the Bragg equation angle.. The result shows that the crystalline habit of the particle ,lattice planes of face centered cubic structure silver nanoparticles .

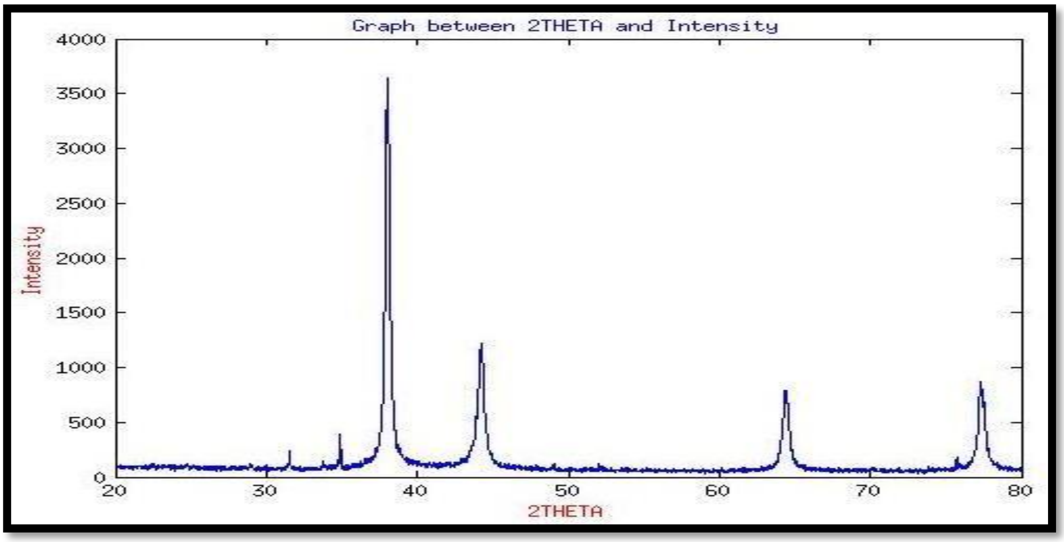

Fig.2 : XRD of monometallic silver nanoparticles

Scanning Electron Microscopy (SEM)

SEM images of all the phyto-fabricated nanoparticles were found in polydispersed spherical shaped surface morphology . The capability of cellular-uptake is influenced by the nanoparticle's shape. The spherical nanoparticles demonstrate the highest uptake among differently shaped nanoparticles. The spherical shaped nanoparticles require a minimum energy barrier with respect to the non-spherical counterparts.

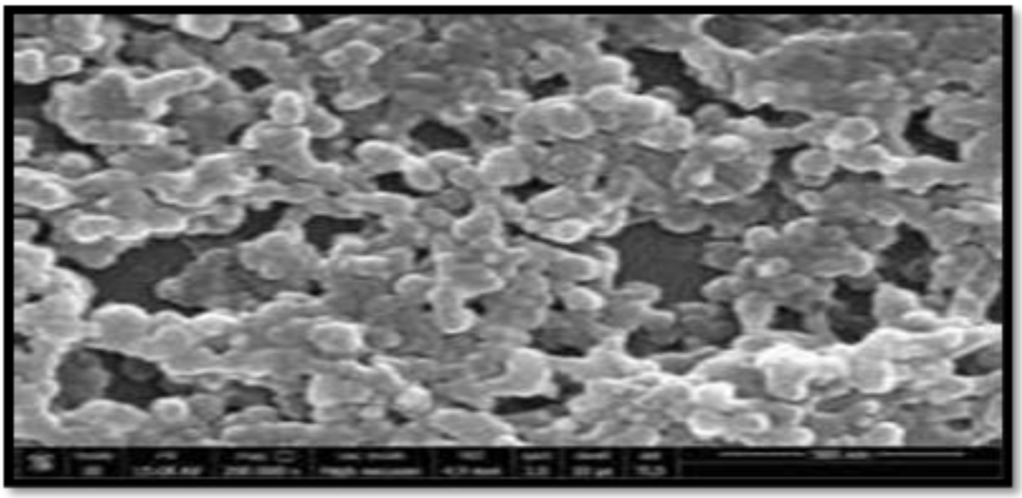

Fig.3 :SEM of AgNps

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Transmission Electron Microscopy (TEM) and Zeta sizer studies

TEM analysis confirmed the spherical shape of all the three phyto-fabricated nanoparticles having the size in the range of 16-30 nm at the magnification of 300,000X. The average hydrodynamic size (Z average) of phyto-fabricated nanoparticles AgNps was 42.32 nm. The phyto-fabricated nanoparticles AgNps, exhibited zeta potentials as -34.5 mV.

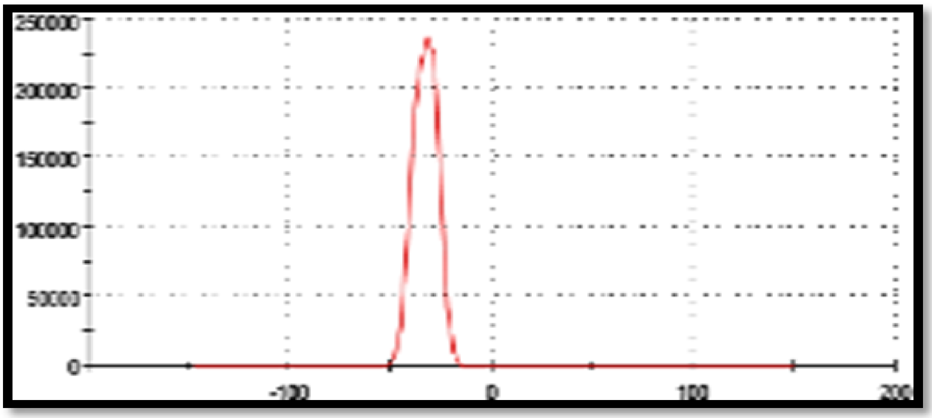

Fig.4 : Zeta potential of silver nanoparticles

Zeta potential values are often used as an indication of the stability of colloidal particles. The absolute values replicate the net electrical charge of the particles of functional groups present on the external surface . The high negative value indicated the stability (repulsive barrier) of the nanoparticles by preventing the agglomeration of nanoparticles. The negative potential, in the present case, might be arising from the loading of negatively charged functional groups (-OH groups of the flavonoids). There was lack of authentic evidence except for the appearance of a fadedlayer around nanoparticles, visible in Transmission Electron Microscopy .

Transmission Electron Microscopy

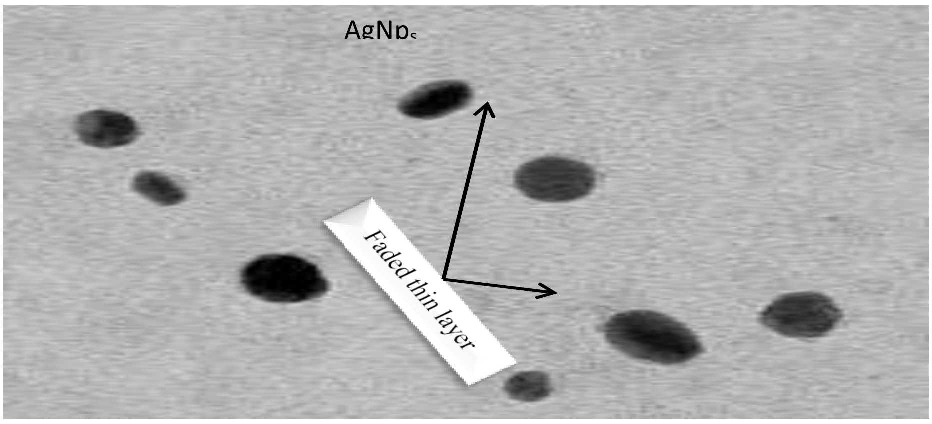

Fig. 5: TEM images of silver nanoparticles exhibiting faded layeraround them

Antifungal activity

Antifungal tested by well diffusion method .Potato dextrose agar plates were inoculated with each fungal culture 10 days old by point inoculation. The filter paper wells 5 mm in diameter impregnated with 20 40 and 60 μL concentrations of the synthesized AgNPS was placed on test organism-seeded plates. Fluconazole (10 μg well) used as positive control After 72 h of incubation at 28°C and inhibition zones were measured in mm.

Table2:Antifungal activity of extract in AgNPs

A.	Nanoparticles (AgNPs)	Zone of inhibition (mm) Concentrations (µg/mL)			(Fluconazole) (mm)
	Pathogenic fungus	**20**	**40**	**60**	
1.	*Candida albicans*	08	12	14	16
2.	*Aspergillusfumicatus*	07	10	12	16

Conclusion

On the basis of complete review of monometallic Nanoparticles of *Aconitum Heterophyllum* it was found that nanoparticles are effective for antifungal activity. Antifungal drugs like *Aconitum heterophyllum* are highly specific against fungus and are widely used in the treatment of various fungal diseases. Now day's eco-friendly methods are gaining importance for synthesis of nanomaterial's. The synthesis process developed in this study could be forwarded for study of various metal combinations to develop most powerful novel antifungal materials.

References

[1] Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A. and Sastry, M. (2006) Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. *Biotechnology Progress*, 22, 577–83. https://doi.org/10.1021/bp0501423

[2] Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X. et al. (2007) Biosynthesis of silver and gold nanoparticles by novel sundriedCinnamomum camphoraleaf.*Nanotechnology*, 18, 105104. https://doi.org/10.1088/0957-4484/18/10/105104

[3] Lin, L., Wang, W., Huang, J., Li, Q., Sun, D., Yang, X. et al. (2010) Nature factory of silver nanowires: *ChemicalEngineeringJournal*,162, 852–8.

[4] Shankar, S.S., Rai, A., Ahmad, A. and Sastry, M. (2004) Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. *Journal of Colloid and Interface Science*, 275, 496–502. https://doi.org/10.1016/j.jcis.2004.03.003

[5] Armendariz, V., Herrera, I., peralta-videa, J.R., Jose-yacaman, M., Troiani, H., Santiago, P. et al. (2004) Size controlled gold nanoparticle formation by Avena sativa biomass: use of plants in nanobiotechnology. *Journal of Nanoparticle Research*, 6, 377–82.

[6] Sangaru, S.S., Rai, A., Ahmad, A. and Sastry, M. (2004) Biosynthesis of silver and gold nanoparticles from extracts of different parts of the geranium plant. *Appl Nanosci*, 1, 69–77.

[7] Shende, S., Ingle, A.P., Gade, A. and Rai, M. (2015) Green synthesis of copper nanoparticles by Citrus medica Linn. (Idilimbu) juice and its antimicrobial activity. *World Journal of Microbiology & Biotechnology*, 31, 865–73.

[8] Gunalan, S., Sivaraj, R. and Venckatesh, R. (2012) Aloe barbadensis Miller mediated green synthesis of mono-disperse copper oxide nanoparticles: Optical properties. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 97, 1140–4.

[9] Dubey, S.P., Lahtinen, M. and Sillanpää, M. (2010) Tansy fruit mediated greener synthesis of silver and gold nanoparticles. *Process Biochemistry*, 45, 1065–71. https://doi.org/10.1016/j.procbio.2010.03.024

[10] Krishnaraj, C., Muthukumaran, P., Ramachandran, R., Balakumaran, M.D. and Kalaichelvan, P.T. (2014) Acalypha indica Linn: Biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against MDA-MB-231, human breast cancer cells. *Biotechnology Reports*, 4, 42–9. https://doi.org/10.1016/j.btre.2014.08.002

[11] Valodkar, M., Nagar, P., Jadeja, R., Thounaojam, M., Devkar, R. and Thakore, S. (2011) Euphorbiaceae latex induced green synthesis of non-cytotoxic metallic nanoparticle solutions: A rational approach to antimicrobial applications. *Colloids and Surfaces A-Physicochemical and Engineering Aspects - Colloid Surface A*, 384, 337–44. https://doi.org/10.1016/j.colsurfa.2011.04.015

[12] Jiang, Y., Maher, J. and Bassett, D. (1995) Dienone—Phenol Rearrangement Reaction: Design Pathway for Chemically Amplified Photoresists.

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www.jchr.org

JCHR (2025) 15(1), 561-567 | ISSN:2251-6727

Microelectronics Technology, American Chemical Society. p. 228–36. https://doi.org/10.1021/bk-1995-0614.ch015

[13] Ankamwar, B. (2010) Biosynthesis of Gold Nanoparticles (Green-gold) Using Leaf Extract of Terminalia Catappa [Internet]. Journal of Chemistry. https://doi.org/10.1155/2010/745120

[14] Pathak, P.D., Mandavgane, S.A. and Kulkarni, B.D. (2016) Characterizing Fruit and Vegetable Peels as Bioadsorbents. *Current Science*, 110, 2114. https://doi.org/10.18520/cs/v110/i11/2114-2123

[15] MubarakAli, D., Thajuddin, N., Jeganathan, K. and Gunasekaran, M. (2011) Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. 85, 360–5. https://doi.org/10.1016/j.colsurfb.2011.03.009

[16] Prathna, T.C., Chandrasekaran, N., Raichur, A.M. and Mukherjee, A. (2011) Biomimetic synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical prediction of particle size. *Colloids and Surfaces B,Biointerfaces*, 82, 152–9. https://doi.org/10.1016/j.colsurfb.2010.08.036

[17] Sathishkumar, M., Sneha, K., Won, S.W., Cho, C.-W., Kim, S. and Yun, Y.-S. (2009) Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity73, 332–8. https://doi.org/10.1016/j.colsurfb.2009.06.005

[18] Bankar, A., Joshi, B., Kumar, A.R. and Zinjarde, S. (2010) Banana peel extract mediated novel route for the synthesis of silver nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 368, 58–63. https://doi.org/10.1016/j.colsurfa.2010.07.024

[19] Ahmad, N., Sharma, S., Alam, Md.K., Singh, V.N., Shamsi, S.F., Mehta, B.R. et al. (2010) Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. *Colloids and Surfaces B: Biointerfaces*, 81, 81–6. https://doi.org/10.1016/j.colsurfb.2010.06.029

[20] Nabikhan, A., Kandasamy, K., Raj, A. and Alikunhi, N.M. (2010) Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, Sesuvium portulacastrum L. *Colloids and Surfaces B: Biointerfaces*, 79, 488–93. https://doi.org/10.1016/j.colsurfb.2010.05.018

[21] Gopinath, V., MubarakAli, D., Priyadarshini, S., Priyadharsshini, N.M., Thajuddin, N. and Velusamy, P. (2012) Biosynthesis of silver nanoparticles from Tribulus terrestris and its antimicrobial activity: a novel biological approach. *Colloids and Surfaces B, Biointerfaces*, 96, 69–74. https://doi.org/10.1016/j.colsurfb.2012.03.023

[22] Zawawy, N.A.E. (2015) Antioxidant, Antitumor, Antimicrobial Studies and Quantitative Phytochemical Estimation of Ethanolic Extracts of Selected Fruit Peels. *International Journal of Current Microbiology and Applied Sciences*, 4, 298–309.

[23] Annu, Ahmed, S., Kaur, G., Sharma, P., Singh, S. and Ikram, S. (2018) Fruit waste (peel) as bio-reductant to synthesize silver nanoparticles with antimicrobial, antioxidant and cytotoxic activities. *Journal of Applied Biomedicine*, 16, 221–31.