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ज्ञान-विज्ञान विमुक्तये

GREEN SYNTHESIS OF SILVER NANOPARTICLES AND ANTIMICROBIAL STUDIES IN LEAF AQUEOUS EXTRACT OF *Sophora interrupta Bedd.*

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ABSTRACT

In nanotechnology, the plant mediated synthesis of nanoparticles has terrific application in biomedicine due to its novel properties and its eco-friendly nature. The present study deals with the biosynthesis of stable silver nanoparticles (SNPs) from leaf aqueous extract of Sophora interrupta an Endemic species of Seshachalam hill ranges of Tirumala. The synthesized nanoparticles are characterized by UV–VIS spectroscopy, Zeta potential, FTIR, XRD, with TEM and EDAX. Colour change, Observed from Gray to brown indicates the formation of nanoparticles and UV–VIS surface plasmonresonance spectroscopy observed at 442 nm further confirmed the synthesized nanoparticles as SNPs. FTIR spectroscopic studies confirm that phenols and proteins of leaf extract is mainly responsible for capping and stabilization of synthesized SNPs. The XRD data shows crystalline nature of nanoparticles and EDAX measurements reveal the13.56% percentage presence of Ag metal. Zeta potential at -19.7mV, negitive value indicates the high stability of Nanoparticles. TEM microscopic analysis revealed that the size of synthesized SNPs ranging from 5 to 50 nm with spherical shape. Further, the antimicrobial studies of synthesized SNPs show high activity towards different bacterial isolates.

KEY WORDS

Eco-friendly. Sophora interrupta. Endemic. Plasmonresonance. Silver nanoparticles. Antimicrobial studies.

INTRODUCTION

Nanotechnology is one of the most fascinating research areas in modern material science, Nanoparticles are gaining importance in the fields of biology, medicine and electronics owing to their unique physical and biological properties [1]. Recent studies are focused towards synthesis of nanoparticles using plant materials like, iron, copper, calcium, gold, palladium, zinc and silver. Silver has been recognized of its importance in chemistry, physics and biology due to its unique properties. Over the last few decades, synthesis and characterization of metal nanoparticles gained attention because of their peculiar properties compared to their bulk counterparts, having their high surface to volume ratio [2]. Biosynthesis of nanoparticles using plant materials is easy, efficient, and eco friendly in comparison to chemical mediated or microbe mediated synthesis [3]. Different types of nanoparticles are synthesized using plant materials like Indium oxide nanoparticles from *Aloe vera* [4], Silver has been known to have strong broad spectrum antimicrobial activities even at low concentrations[5].Hence, among the metal nanoparticles, SNPs synthesized from medicinal plants have received, much attention in various biological activities like antibacterial[6a], antifungal[6b].Iron oxide nanoparticles from *Medicago sativa* [7]. Palladium nanoparticles from *Cinnamomum camphora* [8].

Sophora interrupta Bedd. Belongs to the family, Fabaceae (Leguminaceae, Papilionaceae) is commonly called as Adavibillu. There are approximately 219 species in this genus *Sophora*. They were investigated to possess abortifacient, antibacterial, anticholesterolemic, anti-inflammatory, antispasmodic, diuretic, emetic, emollient, febrifuge, hypotensive, purgative, styptic, and tonic properties [9]. *Sophora Interrupta* is one such plant which is traditionally used in the treatment of cancer. Assuming the presence of anticancer compounds its family members are investigated for the antioxidant activity [10]. *Sophora* contains many phyto-constituents like matrine, oxymatrine type of alkaloids [11 12], flavonoids [13 14], anti-cancer activity [15], which possess wide-range pharmacological actions, including antioxidant [16] saponins and polysaccharides [17]. Anti asthamatic, anti-neoplastic, antimicrobial [18].

MATERIAL AND METHODS

Synthesis of SNPs

Sophora interrupta leaves are collected from Seshatheertham area of Tirumala, Chittoor District of Andhra Pradesh, India and identified and herbarium deposited (Voucher No.KP:01) in the Department of Botany, Sri Venkateswara University, Tirupati. 5 gms of powdered leaf was extracted with 100 ml of milli *q* water on boiling water bath for 1 hour. Filter the content with whatman No. 1 filter paper and stored at room temperature for green synthesis of SNPs. 5 ml of plant extract was taken in 250 ml conical flask, titrated with 50 ml of 1mM $Ag(NO3)2$ at 60-80°C with the help of magnetic stirrer. The contents were centrifuged at 10000 rpm for 20 minutes to avoid the presence of any biological impurities. Further, it is used for characterization and antimicrobial studies.

Characterization of silver nanoparticles

UV–Vis absorption spectrum of SNPs was measured by using Nanodropp 800. Zeta potential analyzed by HORIBA SZ-100, Fourier-Transform Infra Red (FT-IR) spectra of synthesised SNPs were analyzed in the range of 4,000 to 500 cm-1 with an IRAFFINITY-1, IR by ATR method. Crystalline nature of metallic silver nanoparticles was examined using an X-ray diffractometer (XRD) from Bruker, D8 advance, Germany. XRD-6000 equipped with Cu Ka radiation source using Ni as filter at a setting of 40 kV/30 mA. Transmission electron microscopy (TEM) technique was used to visualize the morphology of the AgNps. The 200 kV ultra-high-resolution transmission electron microscope (FEI-TECNAI G2 20 TWIN). TEM Grid were prepared by placing a 5 µL AgNp Solution on Carbon-Coated Copper grids and drying under lamp.

Antimicrobial studies of SNPs

The antimicrobial activity of green synthesized silver nanoparticles from leaf extract was analyzed against two Gram positive bacterial strains like *Bacillus subtilis*, *Staphylococcus aureus* and Two Gram negative bacterial strains like *Escherichia coli*, *Klebsiella pneumoniae*, Disc diffusion method [19] was followed for testing antimicrobial activity against green synthesized SNPs and comparative studies were made with plant leaf extract as a positive control, 1mM $Ag(NO3)2$ as negative control and Streptomycin as the standard. Sterile discs of 7mm size were prepared from whatman No.1 filter paper and 20 µl of each extract was loaded on separate discs with the help of micro pipette and allowed to air dry for one hour in aseptic

conditions. Freshly prepared nutrient agar media for bacterial culture substrate was poured into sterile Petriplates and allowed 30 minutes for solidification. The plates were swabbed with microbial cultures and placed the previously prepared discs; the experiment was carried out in triplicates. The plates were incubated at 37 °C for 24 to 48 h then the zone of inhibition was measured

RESULTS AND DISCUSSION

The formation of silver nanoparticles was monitored by UV-VIS absorption spectra. The colour change from grey to Dark Brown is observed and a typical absorption peak obtained at 442 nm, it is due to surface Plasmon resonance of silver nanoparticles in the reaction Mixture (fig.1). As shown in figure 2, the SNPs obtained posses a negative Zeta potential value. Zeta potential is an essential parameter for the characterization of stability in aqueous nanosuspensions. A minimum of $\pm$ 30 mV Zeta potential values is required for indication of stable nanosuspension [20]. Zeta potential at-19.7mV, negative value indicates the high stability of Nanoparticles. So, this results clearly indicated that the particles are fairly stable due to the electrostatic repulsion. FT-IR spectrum of synthesized SNPs was carried out to know the possible biomolecules responsible for capping and stabilization of nanoparticles. For this the FTIR spectrum was analyzed between the scan ranges from 4000 to 500 cm–1. Here the broad peaks obtained at 3304 cm–1 assigned for O—H (Stretch) bond of phenols, 1635 cm–1 assigned for

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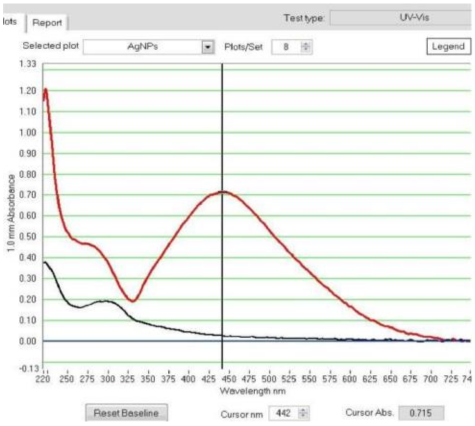
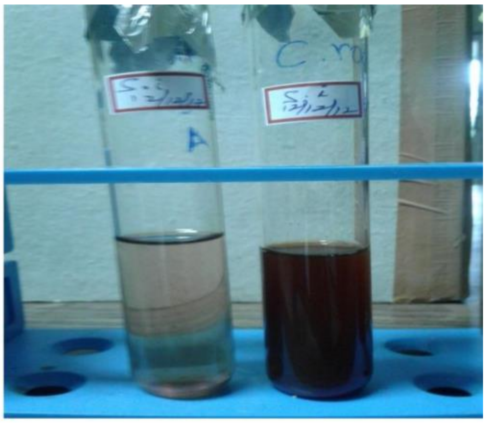
N—H (Bend) bond of primary amines and 514 cm−1 assigned for C-Br (Stretch) of alkyl halides (fig.3). These FTIR studies suggested that the hydroxyl groups of phenols and amide groups of proteins forming a layer to the nanoparticles and acting as capping agents to prevent agglomeration and providing stability to the medium. The nature of the nanoparticles synthesized from leaf extract was analysed by X-ray diffraction analysis. The XRD Shows four plant derived SNPs. An intensive peak at 38.74 44.71 65 and 77.44 of 2θ degrees of X-axis corresponds to 111, 200, 220 and 311 Bragg Reflections of Y-axis (fig4). These Bragg reflections confirm that the nanoparticles are crystalline in nature.

Sophora interrupta **(A) Habitat (B) Flowering twig.**





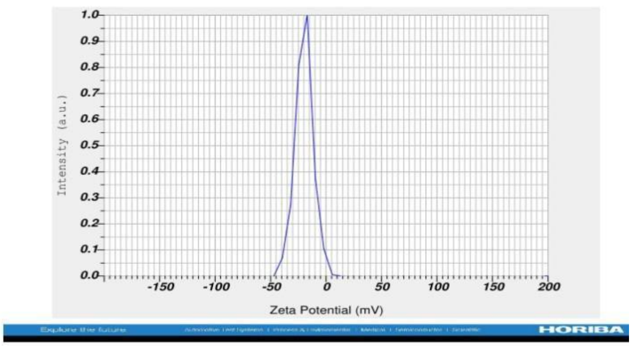
(a) **(b)**

Fig.1 (a) UV-VIS analysis of synthesized SNPs shows peak at 442 nm. (b) Colour change grey to dark brown.

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-19.7 mV	-0.000153 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -19.7 mV
Electrophoretic Mobility Mean : -0.000153 cm2/Vs



Zeta potential = -19mV

(a)Fig.2 (a) Zeta potential of green synthesized SNPs from leaf extract of *Sophora interrupta*

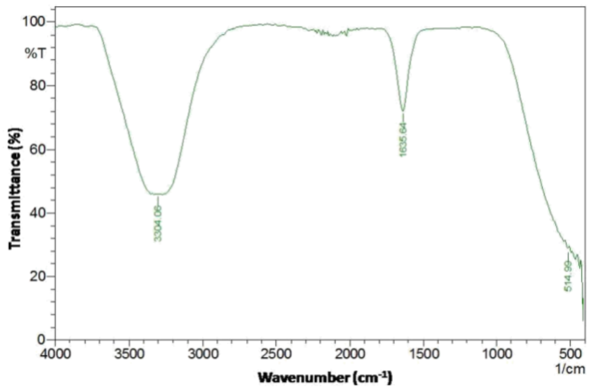


Fig.3 FTIR spectra of green synthesized SNPs from leaf extract of *Sophora interrupta.*

(3304 cm–1 assigned for O—H (Stretch) bond of phenols, 1635 cm–1 assigned for N—H (Bend) bond of primary amines and 514 cm–1 assigned for C-Br (Stretch) of alkyl halides)

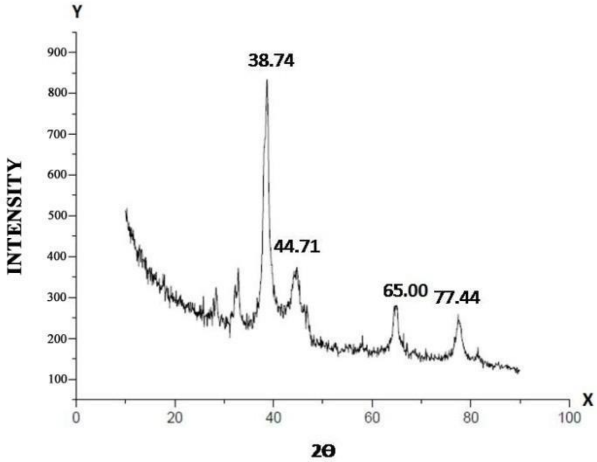
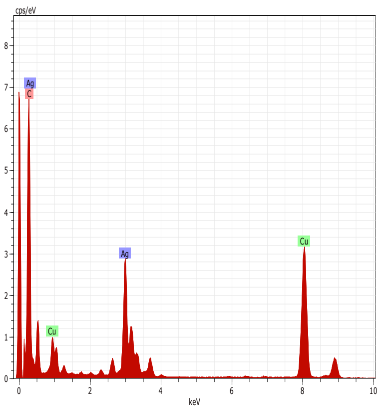


Fig.4 XRD pattern of green synthesized SNPs from leaf extract of *Sophora interrupta.*

(Intensive peak at 38.74 44.71 65.00 and 77.44 of 2θ degrees of X-axis corresponds to 111, 200, 220 and 311 Bragg Reflections of Y-axis.)

TEM with EDAX analysis provides further insight into the morphology and size of the nanoparticles along with presence of different metal concentrations in the sample. 200 nm resolution studies of green synthesized SNPs show spherical shape with 20-50 nm size of nanoparticles. EDAX analysis was performed to know the percentage of Ag present in the sample. The EDAX spectra shows strong silver (13.56 %) absorption peak along with different elements with their weight percentage like Carbon (73.60 %), Copper (12.84 %) (fig.5) and the results indicated that the reaction product has high purity of SNPs .Presence of C, N and O in the sample analyzed by EDAX indicates proteins as a capping material towards these silver nanoparticles [21]. Higher resolution studies with TEM analysis, to know the size, morphology and agglomeration pattern of nanoparticles. 20 nm resolution studies of nanoparticles on TEM analysis reveals the nanoparticles are 7-11 nm in size owing spherical shape without any agglomeration observed between the particles (fig. 6 and 7). From these microscopic studies with TEM analyses reveals these green synthesized silver nanoparticles from *Sophora interrupta* shows the size range from 5 to 50 nm. Having spherical shape without any agglomeration between the particles.

These green synthesized silver nanoparticles were assessed for antimicrobial activities against two gram positive and Two-gram negative bacteria. Among the bacteria the highest inhibition zones were observed in *Klebsiella pneumonia*. (Fig 8, 9 and Table 1) The SNPs shows less significant effect on Gram positive. Gram-negative bacteria are more susceptible when compared to the Gram-positive bacteria. The Gram-positive bacteria having thick layers of peptidoglycon (together with polypeptide contains proteins) when compared to the Gram-negative bacteria and the penetration of SNPs through cell membrane is easy in case of Gram-negative bacteria. 20-25 nm sized, spherical shaped silver nanoparticles synthesized from Olea europaea leaf extract shows good antibacterial activity Against *S. aureus*, *P. aeruginosa* and *E. coli* [22]. Some of the scientist's state that the SNPs penetrate inside the bacteria and fungi causing damage by interacting with electron phosphorous and sulphur containing Compounds such as DNA and proteins, resulting in cell Death [23].

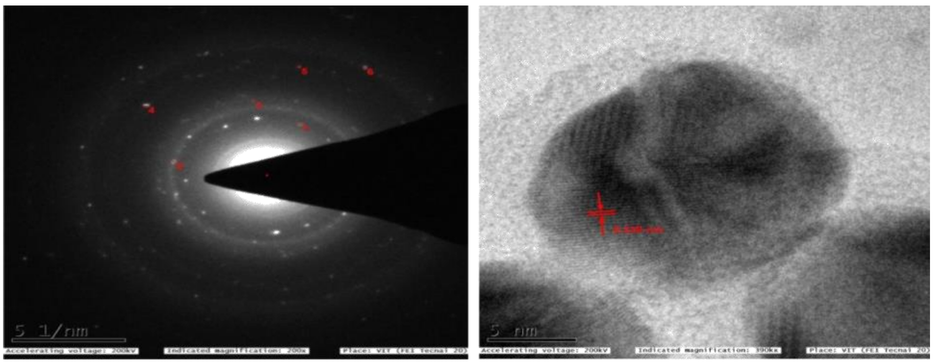


Spectrum: SI

Element	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at. %]	Error (3 Sigma) [wt.%]
Carbon	K-series	36499	73.60	73.60	94.92	6.80
Copper	K-series	49955	12.84	12.84	3.13	1.24
Silver	K-series	4486	13.56	13.56	1.95	1.47
	Total:		100.00	100.00	100.00	

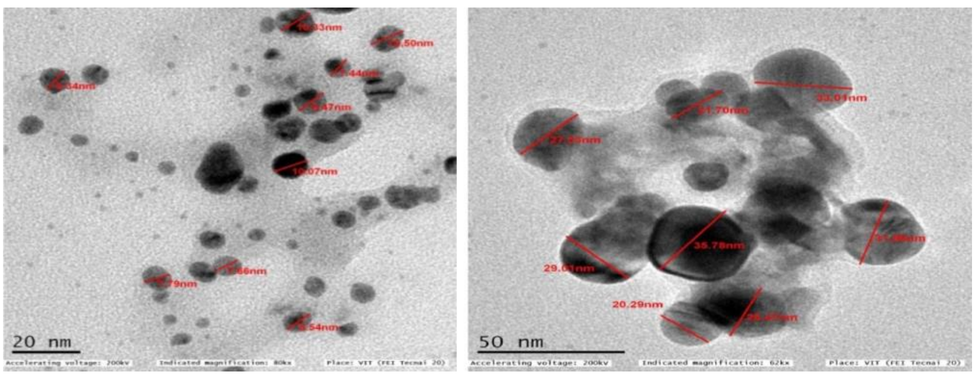
Energy (keV)

Fig 5: EDAX analysis of green Synthesized SNPs shows13.56 weight percentage.



(a) **(b)**

Fig 6 (a) Selected area electron diffraction (SAED) of green synthesized SNPs, (b) 5 nm resolution studies of green synthesized SNPs.



(c) **(d)**

Fig 7 (c) 20 nm resolution studies of green synthesized SNPs show mostly spherical shaped nanoparticles with 7-11 nm size. (d) 50 nm resolution studies of green synthesized SNPs show mostly spherical shaped nanoparticles with 22-36 nm size.

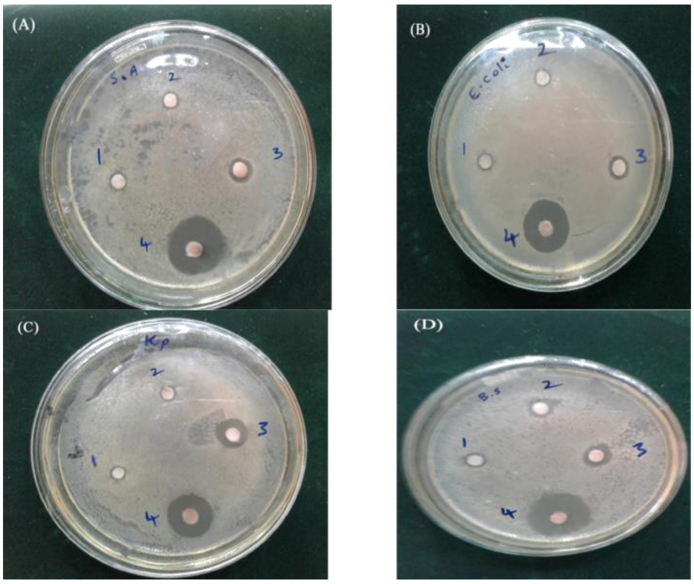


Fig.8 Antimicrobial activities of Synthesized SNPs from leaf extract of *Sophora interrupta* (A)S.aureus, (B) E.coli, (C) K.pneumonia, (D) B.subtilis. (1) Plant extract (2) Ag (NO3)2 (3) SNPs (4) Streptomycin.

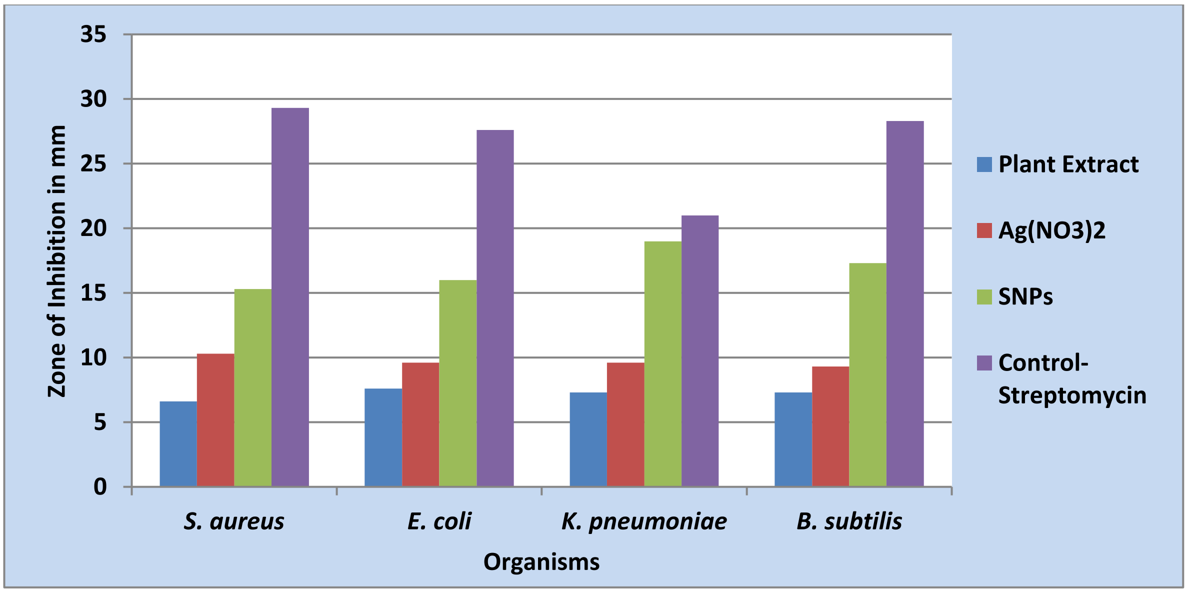


Fig 9 Zone of inhibition of different extracts on clinically isolated bacteria

Table 1: Effect of different extracts and green synthesized silver nanoparticles on clinically isolated bacterial Strains.

Extracts 10mg/Disc	Diameter Zone of Inhibition in mm			
	S. aureus	*E. coli*	*K. pneumoniae*	*B. subtilis*
Plant Extract	6.6±0.32**	7.6±0.32**	7.3±0.32**	7.3±0.32**
Ag(NO3)2	10.3±0.32**	9.6±0.32**	9.6±0.32**	9.3±0.32**
SNPs	15.3±0.32**	16±0.57*	19±0.57*	17.3±0.32**
Control- Streptomycin	29.3±0.32	27.6±0.32	21±0.57	28.3±0.32

All the data are expressed as mean ±S EM: **p<0.01, * p<0.05 as compared to Control group, n=3: (One –way ANOVA followed by Dunnett's test)

CONCLUSIONS

In the present study we develop a fast, eco-friendly and cost-effective method for silver nanoparticles in a greener way. This green method avoids deficiencies of chemical, physical and bacteria mediated approaches instead of plant extracts as reducing agents. Another advantage in this green synthesis approach is nanoparticles are stable for a long time. Further these nanoparticles showed good antimicrobial activity against clinically isolated pathogenic microorganisms. Hence, this type of greener methods for production of silver nanoparticles at lower cost with natural sources is an utmost important to industrial scale due to high importance in various medical fields. High quantity production of nanoparticles with little volume of plant extract is high measurable significance in this medicinal endemic plant.

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REFERENCES

[1] Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ (2005) The bactericidal effect of silver Nanoparticles. Nanotechnology 16:2346–2353. doi:10.1088/ 0957-4484/16/10/059

[2] Thangavel S, Ramaraj R. Polymer membrane stabilized gold nanostructures modified electrode and its application in nitric oxide detection. J Phys Chem C. 2008; 112:19825–19830.

[3] Anamika M, Sanjukta C, Prashant MR, Geeta W (2012) Evidencebased green synthesis of nanoparticles. Adv Mater Lett 3:519–525. doi: 10.5185/amlett.2012.icnano.353

[4] Maensiria S, Laokula P, Klinkaewnaronga J, Phokhaa S, PromarakcV, Seraphin S (2008) Indium oxide (In2O3) nanoparticles using. Aloe vera plant extract: synthesis and optical properties.J Optoelectron Adv Mater 10:161–165

[5] Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB,Ramirez JT, Yacaman MJ (2005) The bactericidal effect of silver. nanoparticles. Nanotechnology 16:2346–2353. doi:10.1088/ 0957-4484/16/10/059

[6a] Savithramma N, Rao ML, Devi PS (2011a) Evaluation of antibacterial efficacy of biologically synthesized silver nanoparticles using stem barks of Boswellia ovalifoliolata Bal. and Henry and Shorea tumbuggaia Roxb. J Biol Sci 11:39–45. doi:10.3923/jbs.2011.39.45

[6b] Savithramma N, Rao ML, Basha SKM (2011b) Antifungal efficacy of silver nanoparticles synthesized from the medicinal plants. Der.Pharma Chemica 3:364–372

[7] Herrera-Becerra R, Zorrilla C, Rius JL, Ascencio JA (2008) Electron microscopy characterization of biosynthesized iron oxide. nanoparticles. Appl Phys A Mater Sci Process 9:241–246.doi:10.1007/s00339-008-4420-7

[8] Yang X, Qingbiao L, Wang H, Huang J, Lin L, Wang W, Su Y, OpiyoJB, Hong L (2009) Green synthesis of palladium nanoparticlesusing broth of Cinnamomum camphora leaf. J Nanopart Res12:1589–1598. doi:10.1007/s11051-009-9675-1

[9] Poretz RD, Barth RF. Studies on the interaction of theSophorajaponica lectin and concanavalin A with erythrocytes.and lymphocytes. *R. Immunology,* 1976; 31, 187.

[10] Ren-You Gan, Xiang-Rong Xu, Feng-Lin Song, Lei Kuang and Hua-Bin Li. Antioxidant activity and total phenolic content contentof medicinal plants associated with prevention and treatmentof cardiovascular and cerebrovascular diseases. *Journalof Medicinal Plants Research,* 2010; 4: 2438-44

[11] Liu, M., Liu, X.Y., Cheng, J.F. Advance in the pharmacologicalresearch on matrine. Zhongguo Zhong Yao Za Zhi. 2003;28: 801-4.

[12] Zhang, Y.F Wang, S.Z., Li Y.Y., Xiao, Z.Y., Hu, Z.L. and Zhang,J.P. Sophocarpine and matrine inhibit the production of TNFand IL-6 in murine macrophages and prevent cachexia relatedsymptoms induced by colon 26 adenocarcinoma inmice. International Immunopharmacol. 2008; 8: 1767-2.

[13] Minhaj, N., Khan, H. and Zeman, A. Unanisoflavan, a newisoflavan from *Sophora secondiflora* DC. Tetrahedron Lett.1976; 27: 2391–4.

[14] Minhaj, N., Khan, H. and Zaman, A., Secondifloran, a novelisoflavanone from *Sophora secondiflora* DC. TetrahedronLett. 1977; 13: 1145–8.

[15] Ohyama M, Tanaka T and Iinuma M. Two novel resveratroltrimers, Leachianols A and B, from *Sophora leachiana*. Chem. Pharm. Bull. 1994; 42: 2117-20.

[16] Ding, P.L., Liao, Z.X., Huang, H., Zhou, P., and Chena, D.F. (+)-12*a*-Hydroxysophocarpine, a new quinolizidine alkaloidand related anti-HBV alkaloids from *Sophora flavescens*.Bioorganic & Medicinal Chemistry Letters. 2006; 16: 1231–35.

[17] Tse, W.P., Che, C.T., Liu, K. and Lin, Z.X. Evaluation of theanti-proliferative properties of selected psoriasis-treatingChinese medicines on cultured HaCaT cells. Journal ofEthnopharmacology. 2006; 108: 133–41.

[18] Sato, M., Tsuchiya, H., Takase, I., Kureshiro, H., Tanigaki, S.and Iinuma, M. Antibacterial activity of flavanone isolatedfrom Sophora exigua against methicillin-resistant Staphylococcusaureus and its combination

with antibiotics.Phytotherapy Research.1995; 9: 509–12.

[19] R. Cruickshank. Medical microbiology: a guide todiagnosis and control of infection. Livingston publishers,Edinburghand London, 1986.

[20] Jacobs, C.; Müller, R.H. Production and characterization of a budesonide nanosuspension forpulmonary administration. *Pharm. Res.* 2002, *19*, 189–194.

[21] N. Jain, A. Bhargava, S. Majumdar, et al., Extracellular biosynthesis and characterization of silver nanoparticles using Aspergillus flavus NJP08: A mechanism perspective. *Nanoscale*. 2011, 3: 635- 641.

[22] M.M.H. Khalil, E.H. Ismail, K.Z.E. Baghdady, et al., Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. *Arabian J. Chem*. 2014, 7: 1131-1139.

C. Baker, A. Pradhan, L. Pakstis, et al., Synthesis and antibacterial properties of silver nanoparticles. *J. Nanosci. Nanotechnol*. 2005, 5: 24-29.

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