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ONE-POT GREEN SYNTHESIS OF BIOCOMPATIBLE SILVER NANOPARTICLES USING LEAF EXTRACT OF *PIPER NIGRUM*

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ABSTRACT

An increase in the commercial demand for nanoparticles, due to their all-embracing applicability in various areas, has led to the formulation of different production processes. The traditional "Wet chemical technique" employs the usage of toxic and flammable reactants for the synthesis of metallic nanoparticles. In the present study, a simple, environment benign and cost-effective technique for green synthesis of silver nanoparticles (AgNPs) using the leaf extract of Piper nigrum (PNLE), as reducing and capping agent, was developed. The studies of characterization: UV–Vis absorption spectroscopy, FTIR, TEM, SAED pattern and SEM were carried out, exhibiting spherical shaped nanoparticles of 14.46–41 nm in size. The antibacterial activity of PNLE-AgNPs was assessed by well diffusion assay against pathogenic strains, thereby, formulating a "green and clean" approach for synthesizing PNLE-AgNPs as potential aspirants for medical settings.

KEY WORDS

Nanotechnology, Green synthesis, Silver nanoparticles, Piper nigrum, Antibacterial activity

INTRODUCTION

The attributes of nanotechnology have emerged as a "hotspot" area of material sciences with an increase in search for novel nanomaterials of diverse dimensions and specifications. Metal derived nanoparticles have received extensive mind of interest in recent years, due to their tunable material properties and resourceful applications in broad quarters such as pharmaceutical, catalysis, chemical sciences, biosensors and electronics (Jacob *et al*., 2012).

The nano silver, (Ag) acquires an imperative spot, among metallic nanoparticles, in terms of its toxicity against various microbial species, with respect to its bulk counterparts. This utility has proven to be multitude of uses ranging in more than 200 commercial consumer goods, from complex nanocomplexes to nano-derived microchips. Albeit ushering remarkable benefits, the constant hysterical release of engineered NPs into the environmental domains, stays as a serious case of concern (Rani and Rajasekhar reddy, 2011; Bhatt and Tripathi, 2011). The synthetic protocols (both physical and chemical) are rapid and produce large amounts of NPs but requires the usage of toxic chemicals as reducing and capping agents (Saifuddin *et al*., 2009; Song *et al*., 2008). Green technology, as an alternative, has materialized the constraints of conventional procedures of nano generation with the application of cost-effective, ecofriendly and efficient catalysis. The "green" principles cover the elements of judicious utilization of various biological entities such as plants and microbial cells with the mechanisms of nano-green chemistry and minimization of non-renewable or toxic reactants (Cherian *et al*., 2018).

Biosynthesis of silver nanoparticles mediated by microbial cells such as bacteria, fungi, yeast, algae, etc. have been vastly exploited. Nevertheless, the exploratory analysis of plant systems as the "prospective nanounits" has heightened the curiosity

towards the biosynthesis of nanoparticles. Synthesis of silver nanoparticles using various plants extracts like *Aloe vera* (Chandran *et al*., 2006), *Azadirachta indica* (Tripathy *et al*., 2010), *Capsicum annuum* (Li *et al*., 2007), *Rhizophora apiculata* (Cherian *et al*., 2018), *Stevia rebaudiana* (Varshney *et al*., 2010), etc. has been reported.

Piper nigrum L., of family Piperaceae, is an aromatic, shade-loving climber cultivated in India and other tropical Southeast Asian nations. It is a perennial woody vine growing upto 13 ft and contains an array of phytochemicals such as amides, piperidines, pyrrolidines and trace amounts of safrole (Dawid *et al*., 2012). Jirovetz *et al*. (2002) reported aroma-contributing terpenes, including germacrene, limonene, pinene, alpha-phellandrene and β-caryophyllene from essential oils of *Piper nigrum* and *Piper guineense*, which confer pharmacological benefits, such as antioxidant, antimicrobial, and anti-inflammatory properties.

The present study was aimed at rapid one-step synthesis of silver nanoparticles using aqueous leaf extract of *Piper nigrum*, its characterization and evaluation of antibacterial activity against pathogenic strains.

MATERIALS AND METHODS

Plant material and preparation of *Piper nigrum* leaf extract (PNLE)

Fresh leaves of *Piper nigrum* were collected from forest region located in Kottayam district (GIS coordinates: 9°35'29.1876'' N and 76°31'19.8156'' E), Kerala State. Leaves were thoroughly washed with deionized water, air dried and chopped into small pieces. Chopped leaf pieces (50g) were thoroughly grounded and the resulting thick paste was dispersed in 100 ml deionized sterile water. The obtained extract was filtered via Whatman filter paper No.1 and stored until further analysis.

Synthesis of PNLE-AgNPs

PNLE-AgNPs synthesis was carried out by adding 10 ml of *Piper nigrum* leaf extract (PNLE) to 90 ml 1mM $AgNO_3$ solution, pH 8.0 and incubated for 24 h at room temperature under dark conditions (to avoid photochemical reactions). A control setup (flask with same quantity of PNLE) was run under identical conditions. The appearance of greyish colour was an indicative signature of AgNPs formation. The obtained PNLE-AgNPs colloidal solution was purified by repeated centrifugation (Eppendorf Centrifuge 5427 R) at 5000 rpm for 15 min followed by redispersion of pellet in 20 ml double-distilled water. The steps of centrifugation and redispersion were repeated thrice. The obtained pellet was then dried in an oven (Yorco, India) at 40°C for 20-24 h and the resulting dried content was pulverized and stored till further analysis.

Growth conditions and bacterial strains

Four pathogenic strains (*Escherichia coli*, *Vibrio cholerae* MTCC 3906, *Staphylococcus aureus*, *Bacillus subtilis*) maintained in our laboratory, were tested. The pure cultures were cultured on nutrient agar and maintained as slants and glycerol cultures at -20°C.

Characterization of PNLE-AgNPs

The UV–Visible spectra were recorded in UV–Visible double beam spectrophotometer (Perkin Elmer) from 350 to 700 nm, at a resolution of 0.5 nm. HR-TEM analysis was performed on JEOL 100/120kV (JEOL 3010, Tokyo, Japan) operated at 200 kV. For microscopic visualization, samples were prepared by placing 10 µl PNLE-AgNPs on carbon-coated copper grids. TEM was also used for the study of selected area electron diffraction (SAED) patterns. SEM analysis was carried out using powdered PNLE-AgNPs by JSM 6510LV scanning electron microscope (JEOL, Tokyo, Japan) at 15 kV accelerating voltage. FTIR measurements were carried out to identify various biomolecules responsible for AgNPs synthesis. FTIR spectra were recorded using Perkin Elmer FT-IR spectrometer Spectrum Two (Perkin Elmer Life and Analytical Sciences, USA) at a diffused reflectance mode at 4 cm^{-1} resolution in KBr pellets.

Antibacterial activity

The dose dependent PNLE-AgNPs antibacterial activity against pathogenic isolates was determined by well diffusion method as mentioned by Cherian *et al*. (2018). Bacterial culture (0.1 ml, cell density 2×10^8 CFU/ml) was uniformly plated on nutrient agar plates and wells were created by cutting agar with gel puncture. Subsequently, variable concentrations of PNLE-AgNPs (25, 50, 75, 100 µl) solutions, along with PNLE (100 µl), were added to the pre-cut wells in the plates and incubated for 24 h at 37°C. The size of inhibition zones was measured by determining the zone radii.

RESULTS AND DISCUSSION

The chemical reduction of aqueous silver nitrate ($AgNO_3$) solution is one of the widely used methods for

the synthesis of silver nanocolloids. The appearance of greyish colour indicated the formation of silver nanoparticles. The extract (PNLE), after addition of aqueous 1 mM $AgNO_3$, was subjected to optical measurements by UV–vis spectrophotometer, indicating an absorbance peak at 420 nm (Fig. 1), specific for the AgNPs. The change in color was due to the excitation of surface plasmon resonance (SPR) in the metal NPs solution (Mulvaney, 1996). The combined resonance of charged outer valence electrons in a solid lattice excited by incident light is referred to as Surface plasmon resonance phenomena (SPR), which depends upon shape, size, dielectric constant and distribution of charge on metal and its surrounding medium (Eustis *et al*., 2006). Furthermore, it has been reported that absorption spectra of larger metallic colloidal-dispersions can exhibit wide or additional bands in the UV–visible range due to the excitation of plasmon resonances or higher multipole plasmon excitations (Kamat *et al*., 1998). The results are consistent with previous studies reported by Jacob *et al*. (2012); Otunola and Afolayan (2018).

The shape and size of synthesized PNLE-AgNPs were well documented by HR- TEM micrographs (Fig. 2a) exhibiting spherical PNLE-AgNPs in the size range of 14.46-41 nm, with average size found to be 25.12 nm (Fig. 2d) . The SAED (Fig. 2b) pattern revealed that the diffraction rings of synthesized PNLE-AgNPs assigned as (111), (200), (220) and (311) lattice planes of face centred cubic (fcc) lattice, indicated the nano-crystalline nature of biogenic PNLE-NPs. Rani *et al*. (2011) and Rajasekharreddy *et al*. (2010) reported similar SAED patterns of AgNPs synthesized by *Piper betle* L., *Carica papaya* L., *Solanum melongena* L. and *Datura metel* L. leaf extracts. The SEM micrographs revealed variable rod shaped PNLE-capped AgNPs (Fig. 2c). The FTIR spectrum (Fig. 3) of PNLE-AgNPs exhibited distinct peaks: a broad peak at 3427 cm^{-1} may be attributed to the stretching vibrations of hydroxyl (-OH) group. A stretch of C–N vibrations of amino acids was detected at 1637 cm^{-1} while 1097–1397 cm^{-1} correspond to the involvement of C–N vibrations of aliphatic amines, indicating the presence of proteins as ligands, thereby, increasing the stability of synthesized AgNPs. A peak at 2102 cm^{-1} was assigned to C–O group of polyols while alkenes (625–707 cm^{-1}) and aromatic (1037 cm^{-1}) groups, present in the plant extract may act as enhancers in NPs synthesis. Chatterjee *et al*. (1967) isolated two novel alkaloids, piperlongumine and piperlonguminine from *P. longum*, responsible for the reduction of $AgNO_3$ into AgNPs. Shankar *et al*. (2004) reported reductases mediated mechanism of NPs biosynthesis by biological systems. Duran *et al*. (2005) accounted the catalytic reduction of $AgNO_3$ to AgNPs by enzyme nitrate reductase, isolated from *Fusarium oxysporum*, utilizing NADPH as reducing agent. A number of naphthoquinones and anthraquinones with very high redox potential, reported from *F. oxysporum*, could act as an electron shuttle in metal reduction (Newman and Kolter, 2000). Even if, such systems were not reported in plant-mediated synthesis of nanoparticles (NPs), the bioactive constituents can be attributed for the formation of NPs.

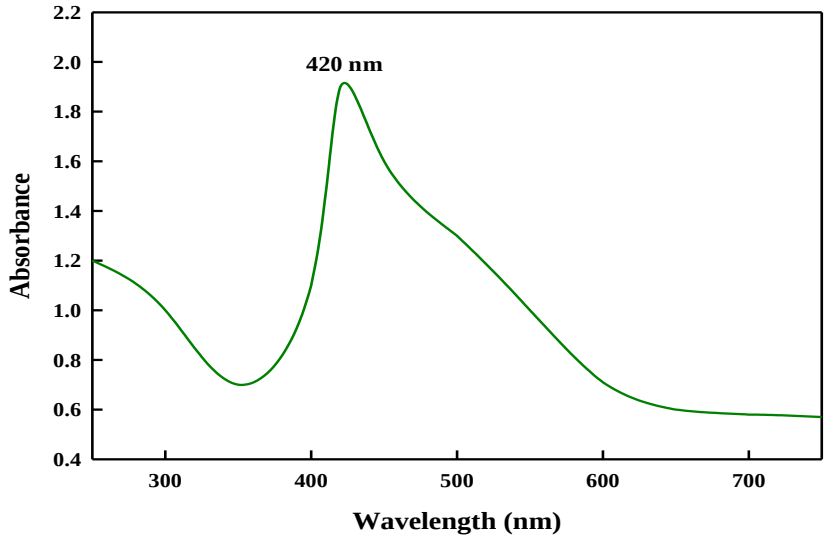

Fig. 1 UV-Visible absorption spectrum of PNLE-AgNPs showing characteristic peak at 420 nm.

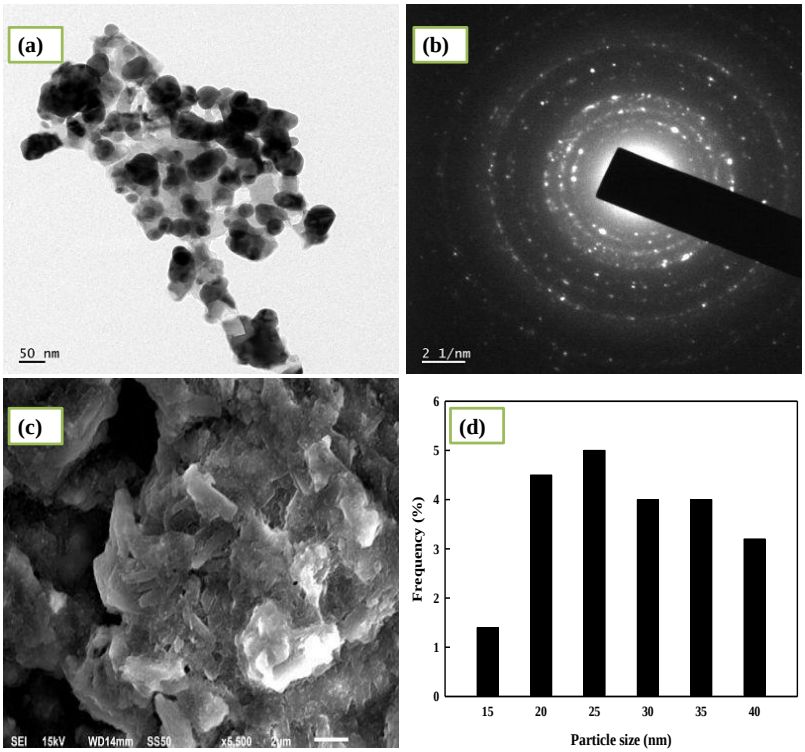

Fig. 2 Electron micrographs of biosynthesized PNLE-AgNPs (a) TEM image (b) SAED pattern (c) SEM image at 5,500X magnification (d) Size distribution histogram.

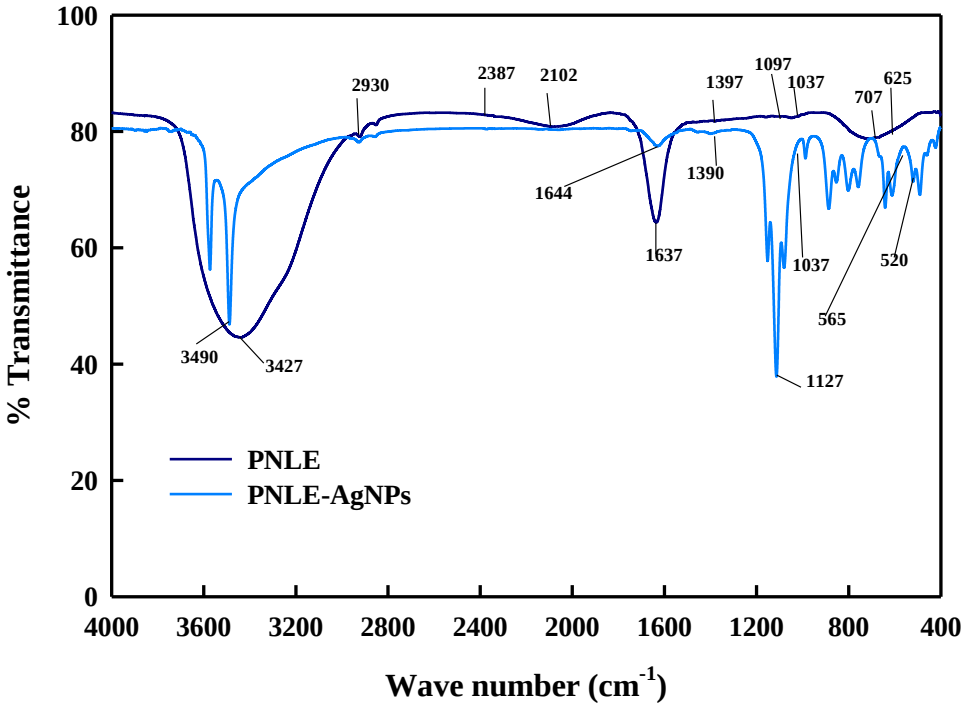

Fig. 3 FT-IR spectra of leaf extract of *Piper nigrum* (PNLE) and biosynthesized silver nanoparticles (PNLE-AgNPs).

Antibacterial activity of PNLE-AgNPs

The antibacterial activity of PNLE-AgNPs was assessed against *Escherichia coli*, *Vibrio cholerae* MTCC 3906, *Staphylococcus aureus* and *Bacillus subtilis*. Fig. 4 depicts the results of well diffusion assay of PNLE and PNLE-AgNPs at a dose of 25-100 µl, each against the test strains. A marked increase in zone inhibition size (18–27 mm) with PNLE-AgNPs, was observed while no zone of inhibition reported in case of PNLE. The results demonstrated that growth inhibition induced by PNLE-

AgNPs follows the order *E. coli* > *V. cholerae* > *S. aureus* > *B. subtilis*. A prominent effect was observed at 100 µl PNLE-AgNPs with inhibition zones of 27 and 26 mm for *E. coli* and *V. cholerae*. Furthermore, the Gram-positive cells of *S. aureus* and *B. subtilis* were inhibited with inhibition zone of 24 and 22 mm, respectively. These findings are well in validation with the results of Umashankeri *et al*. (2012) and Cherian *et al*. (2018). Fig. 5 denotes the concentration dependent effect of PNLE-AgNPs (25–100 µl) on the pathogenic test strains with maximum cytotoxic effect observed at 100 µl PNLE-AgNPs.

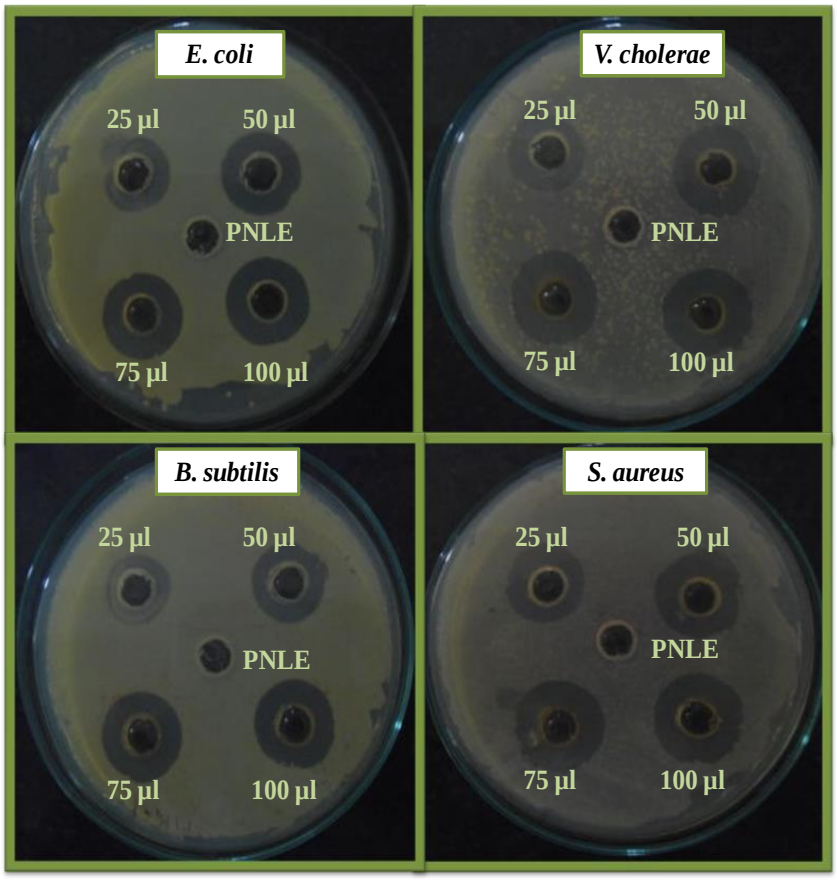

Fig. 4 Antibacterial activity of PNLE and PNLE-AgNPs against pathogenic strains by well diffusion assay.

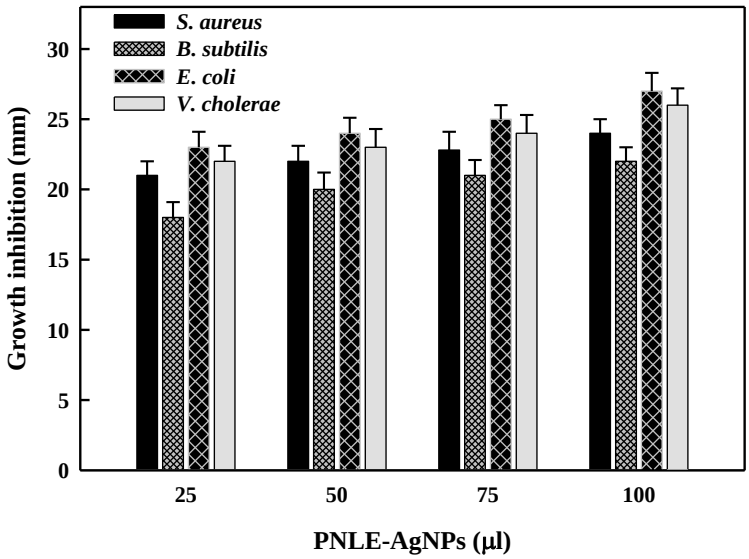

Fig. 5 Comparative antibacterial activity of PNLE-AgNPs (25-100 µl) against tested pathogenic strains.

In the recent times, the rising patterns of multi-antibiotic resistance among microbial forms has become a global menace. Many antibiotics, from solo to combinatorial medications, have been used and are still being used in various settings, but, the efficacy and potency has been severely limited by microbial drug resistance. There is an urgent call to contemplate and devise out novel antimicrobial agents to control and limit the drug resistant forms (Kapil, 2005). Colloidal silver has been used as antibacterial agent since prehistoric times (Hipler and Elsner, 2006). Unlike antibiotic drugs, the bacterial forms are unable to develop resistance as silver targets multiple components in the bacterial cell constitution. According to Sondi and Salopek-Sondi (2004) and Gogoi *et al*. (2006), Gram-negative bacteria are found to be more susceptible to AgNPs due to the interaction of Ag^{+} ions with negatively charged lipopolysaccharide of cell membrane with greater affinity as compared to Gram-positive cells, resulting in the formation of holes in the cell membrane, thereby, leading to leakage of intracytoplasmic contents and eventually to cell death. Moreover, the shape and size of nanoparticles are key determinants of antimicrobial action; as smaller sized spherical shaped AgNPs have been reported to exhibit excellent antibacterial activity (Morones *et al*., 2005; Jain *et al*., 2015). Otunola and Afolayan (2018) reported high antibacterial activity of spherical AgNPs of diameter (6–28 nm) synthesized from the spice blend (garlic, ginger and cayenne pepper) extract.

CONCLUSION

A simple, facile, eco-friendly one-pot synthesis of AgNPs, based on the bioreductive potential of leaf extract of *P. nigrum*, has been developed. The bioactive constituents present in *P. nigrum* act as both reducing and capping agent to protect the NPs surface and prevent particle aggregation, with advantage of wide surface availability and activity. The synthesis illustrated an inexpensive and safe protocol of judicious use of environmentally safer materials (plant origin), compared to physical and chemical procedures, often involving toxic or detrimental substances. The synthesized PNLE-AgNPs exhibited promising antibacterial activity, emphasizing its potential relevance in biomedical field, thus, steering compatibility for remedial and pharmaceutical applications.

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REFERENCES

Bhatt I., Tripathi B.N., Interaction of engineered nanoparticles with various components of the environment and possible strategies for their risk assessment. Chemosphere, 82: 308–317, (2011)

Chandran S.P., Chaudhary M., Pasricha R., Ahmad A. and Sastry M., Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol. Prog., 22: 577–583, (2006)

Chatterjee A., Dutta C.P., Alkaloids of *Piper longum* Linn. I. Structure and synthesis of piperlongumine and piperlonguminine. Tetrahedron, 23 (4): 1769–1781, (1967)

Cherian T., Yalla S.K. and Mohanraju R., Assessment of green synthetic route of silver nanoparticles synthesis by mangrove species *Rhizophora apiculata* of Andaman coast. Int. Journal of Pharm. and Bio. Sci., 8: 819-826, (2018)

Dawid C., Henze A., Frank O., Glabasnia A., Rupp M., Büning K., Orlikowski D., Bader M., Hofmann T., Structural and sensory characterization of key pungent and tingling compounds from black pepper (*Piper nigrum* L.). Journal of Agricultural and Food Chemistry, 60 (11): 2884–2895, (2012)

Duran N., Marcato P.D., Alves O.L., Souza G.I., Esposito E., Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J. Nanobiotechnol., 3 (8) (2005)

Eustis S., El-Sayed M.A., why gold nanoparticles are more precious than pretty gold: noble metal surface Plasmon resonance and its enhancement of the radiative and non-radiative properties of nanocrystals of different shapes. Chem Soc Rev, 35: 209-217, (2006)

Gogoi S.K., Gopinath P., Paul A., *et al*. Green fluorescent protein- expressing *Escherichia coli* as a model system for investigating the antimicrobial activities of silver nanoparticles. Langmuir, 22(22): 9322–9328, (2006)

Hipler U. and Elsner P., Biofunctional textiles and the skin, in: Haug, S., Roll, A., Schmid-Grendelmeier, P., Johansen, P., Wuthrich, B. (Eds.), Coated textiles in the treatment of atopic dermatitis, Karger Publishers, Switzerland pp 147 (2006)

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

Jain N, Bhargava A, Rathi M, *et al*. Removal of protein capping enhances the antibacterial efficiency of biosynthesized silver nanoparticles. PLoS ONE, 10(7) (2015): e0134337. DOI: 10.1371/journal.pone.0134337

Jirovetz L., Buchbauer G., Ngassoum M. B., Geissler M., Aroma compound analysis of *Piper nigrum* and *Piper guineense* essential oils from Cameroon using solid-phase microextraction-gas chromatography, solid-phase microextraction-gas chromatography-mass spectrometry and olfactometry. Journal of Chromatography A, 976 (1–2): 265–75, (2002)

Kamat P.V., Flumiani M., Hartland G.V., Picosecond dynamics of silver nanoclusters. Photoejection of electrons and fragmentation. J. Phys. Chem. B, 102: 3123–3128, (1998)

Kapil A., the challenge of antibiotic resistance: need to contemplate, Indian J.Med. Res., 121: 83–91, (2005)

Li S., Shen Y., Xie A., Yu X., Qiu L., Zhang L., Zhang Q., Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. Green Chem., 9: 852–858, (2007)

Morones J.R., Elechiguerra J.L., Camacho A., *et al*., The bactericidal effect of silver nanoparticles. Nanotechnol., 16 (10): 2346–2353, (2005)

Mulvaney P., Surface plasmon spectroscopy of nanosized metal particles. Langmuir, 12: 788–800, (1996)

Newman D.K., Kolter R., A role for excreted quinones in extracellular electron transfer. Nature 405(6782): 94–97, (2000)

Otunola G.A., Afolayan A.J., In vitro antibacterial, antioxidant and toxicity profile of silver nanoparticles green-synthesized and characterized from aqueous extract of a spice blend formulation. Biotechnology and Biotechnological Equipment, 1-11, (2018), DOI: 10.1080/13102818.2018.1448301

Rajasekharreddy P., Rani P.U., Sreedhar B., Qualitative assessment of silver and gold nanoparticle synthesis in various plants: a photobiological approach. J. Nanopart. Res., 12: 1711–1721, (2010)

Rani P. U., Rajasekharreddy P., Green synthesis of silver-protein (core–shell) nanoparticles using *Piper betle* L. leaf extract and its ecotoxicological studies on *Daphnia magna*. Colloids and Surfaces A: Physicochem. Eng. Aspects, 389: 188– 194, (2011)

Saifuddin N., Wong C.W., Nur-Yasumira A.A., Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. E-J. Chem., 6: 61–70, (2009)

Shankar S.S., Rai A., Ahmad A., Sastry M., Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J. Colloids Interface Sci., 275(2): 496-502, (2004)

Sondi I., Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. J Colloid Interface Sci., 275(1): 177–182, (2004)

Song J.Y., Kim B.S., Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioproc. Biosyst. Eng., 32: 79–84, (2008)

Tripathy A., Raichur A., Chandrasekaran N., Prathna T., Mukherjee A., Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves. J. Nanopart. Res., 12: 237–246, (2010)

Umashankeri J., Inbakandan D., Ajithkumar T.T., Balasubramanian T., Mangrove plant, *Rhizophora mucronata* (Lamk, 1804) mediated one pot green synthesis of silver nanoparticles and its antibacterial activity against aquatic pathogens. Saline Systems, 8(11) (2012)

Varshney R., Bhadauria S., Gaur M.S., Biogenic synthesis of silver nanocubes and nanorods using sundried *Stevia rebaudiana* leaves. Adv. Mat. Lett., 1 (3): 232–237, (2010)

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