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Silver Nanoparticles Synthesis of *Mentha arvensis* Extracts and Evaluation of Antioxidant Properties

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Abstract

Silver nanoparticle synthesis of selected plant extract were confirmed by Ultra violet visible and Fourier transform infrared spectroscopy The *Mentha arvensis* leaf extract mediated nanoparticles showed absorbance peaks at 340 nm region in the spectral analysis. Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at1650.95 cm^{-1}. The total antioxidant of $AgNO_3$) shows a maximum activity of 40% was observed at 600µg/ml. 1-Dibhenyl-2-Picrylhydrazlradical in *Mentha arvensis* mediated silver nanoparticles showed a maximum activity of 25% was observed at 600µg/ml. Hydrogen peroxide scavenging assay in *Mentha arvensis* mediated silver nanoparticles showed a maximum activity of 10% was observed at 600µg/ml. Reducing power of *Mentha arvensis* silver nanoparticles exhibited a higher activity of 19% in 600µg/ml. The selected plant exhibits better antioxidant properties.

Keywords

Mentha arvensis UV, FTIR, Antioxidant

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1. Introduction

Numerous methods for the synthesis of silver nanoparticles have been reported which includes biological reduction (Lee and Meisel, 1982), warm air decomposition (Yang *et al*., 2007), laser ablation (Simakin *et al*., 2004), and Sonochemical synthesis (Salkar *et al*., 1999). Among these, chemical reduction method and laser ablation method are the most commonly employed synthetic routes. The natural reduction method involves the reduction of metal salt like silver nitrate in an appropriate medium using various reducing agents like citrate, borohydride, etc. to produce colloidal suspensions integrated by nanoparticles (Evanoffand Chumanov, 2005).In recent years there has been growing interest in the preparation and study of silver nanoparticles (AgNPs), because those nanoparticles have been found to exhibit interesting antibacterial activities. (Shahverdi *et al*., 2007) Production of nanosized metallic silver particles with different morphologies and sizes using different routes has been reported (Patel *et al*., 2007). Along with methods, the simple process involving a reduction of silver salts has already been well developed (Chen *et al*., 2007).

The exact antibacterial action of AgNPs is not completely understood. There are reports in the literature that show that electrostatic attraction between negatively charged bacterial cellsand positively charged nanoparticles is crucial for the activity of nanoparticles as bactericidal materials.(Sondi *et al*., 2004).AgNPs were effective against these pathogens. The anti-bacterial activity of the ethanolic extracts of leap of *M. arvensis* L. was studied against Gram-positive & Gram-negative bacteria .The ethanolic extract of *M. arvensis* exhibited a significant anti-bacterial activity. The anti-bacterial activity of *Staphylococcus aureus* was higher than the other bacteria. The inhibition zone diameter of *S. aureus* was 20 mm at 10 % concentration and it was 7 mm at 0.3 % concentration.

The antibacterial activity of *P. aeruginosa* was the lowest and

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the inhibition zone ranging from 7 mm to 12 mm at 0.6 % to 10 % of concentration. The inhibition zone of *E. coli, K. pneumoniae* and *S. flexineri* was ranging from 7 mm to 14 mm at 0.3 % to 10 % of concentration. The plant extract was found to have a moderate antibacterial activity against these three bacteria. *M. arvensi* Leaf extract was classified as very active against *Staphylococcus aureus*, against *E. coli, K. pneumonia and Shigella flexineri,* and partially active against *P. aeruginosa*. (Rachel Madhuri Sugandhi and Meera Bai, 2011) Ag NPs release silver ions, which make an additional contribution to the bactericidal effect.(Feng *et al*., 2000) In fact, showed that Ag NPs (where silver is present in the Ag0 form) also contain micromolar concentrations of Ag+, and they have shown that Ag+ and Ag0 both contribute to the antibacterial activity. The mechanism of inhibition by silver ions on microorganisms is partially known. It is believed that DNA loses its replication ability and cellular proteins become inactivated on silver ion treatment.(Gupta *et al*., 2008) Higher concentrations of Ag+ ions have been shown to interact with cytoplasmic components and nucleic acids.(Lim *et al*., 2006) The antibacterial effect of Ag NPs determined in this study was found to be similar to that described in the earlier reports.(Shrivastava *et al*., 2007) The particle size has an effect on microbes; the effect increased with smaller particle size.

The antibacterial properties of silver nanoparticles are associated with its slow oxidation and liberation of Ag+ ions to the environment making it an ideal biocidal agent. Moreover, the small size of these particles facilitates the penetration of these particles through cell membranes to affect intracellular processes from inside. Additionally, the excellent antibacterial properties exhibited by the nanoparticles are due to their well-developed surface which provides maximum contact with the environment (Krutyakov *et al*., 2008). A better understanding of the bactericidal action of silver would require a proper examination of the membrane-bound and intracellular nanoparticles. Silver nanoparticles were found to penetrate into the bacterial cell causing membrane damage and ultimately the death of the organism.

According to the reports of silver nanoparticles exhibited excellent antifungal activity on *Candida albicans* by disrupting the cell membrane and inhibiting the normal budding process (Kim *et al*., 2009). In order to compare the antifungal effects of silver nanoparticles, amphotericin B, an antifungal agent used to treat serious systemic infections was used as a positive control (Hartsel and Bolard, 1996). It showed remarkable antifungal activity against *Trichophyton mentagrophytes* and *Candida species*. Remarkably, these particles exhibited similar activity with amphotericin B, but more potent activity than fluconazole toward all the fungal strains examined. The present investigation was mainly focused on the fabrication of silver nanoparticles synthesis of *Mentha arvensis* plant and its antioxidant properties were evaluated.

2. Materials and Methods

2.1. Collection of Plant Materials

Mentha arvensis are evergreen tree, native to tropical northern South America, southern Caribbean and also India. Its flowers are orange, scarlet and pink in colour and form large bunches. leaf of *Mentha arvensis* were collected from Sorimuthu Ayyanar Koil, Pabanasam, Thirunelveli District, Tamil Nadu, India (8º 39' N and 77 º 20' E) with elevation of 1500 m above sea level, The voucher specimen was identified and deposited in the herbarium of the Ayya Nadar Janaki Ammal College (Autonomous) Sivakasi, Tamil Nadu. The samples were washed, air-dried and powdered.

2.2. Plant Extract Preparation

Mentha arvensis, leafs were broken with help of marton pestle and flowers broken by scissors The fruit pulp (white in color which converts into blue to brown within minutes) was collected for the synthesis of nanoparticles. The plant extracts (broth solutions) were prepared by using 5g of washed and cut leaves flowers petals and fruit pulp in a 250 ml Erlenmeyer flask with 50 ml of sterile distilled water and then boiling the mixture for 5min. The herbal aqueous extract was collected in separate conical flasks by standard filtration method and stored at 4°C in a refrigerator (Gardea-Torresdey *et al*., 2003).

2.3. Preparation of 1mm Aqueous Solution of Silver Nitrate

17mg of Silver nitrate (AgNO3) was added to the 100 ml of distilled water and the solution was stirred well continuously until the silver nitrate is dissolved. This 1mMSilver nitrate solution was stored in brown bottle at 4°C for further use for the synthesis of Silver nanoparticles from Mentha arvensis (Gardea-Torresdey et al., 2003).

2.4. Synthesis of AgNPs

1mM aqueous solution of silver nitrate (Himedia, Mumbai) was prepared for synthesis of silver nanoparticles. For the synthesis of AgNPs, two boiling tubes were taken, one containing 10ml of 1Mm$AgNO_3$ solution as control and the second containing 9ml of 1mM silver nitrate solution and 1ml of plant leaf extract as test solution. These were incubated at room temperature for 1-2 hours. The color change of the leaf extracts from pale yellow to dark brown was checked. The brown color formation indicates that the

silver nanoparticles were synthesized from the plant extracts and they were centrifuged at 5000 rpm for 15 minutes in order to obtain the pellet which is used for further study. Supernatant is discarded and the pellet is dissolved in deionized water. The silver nanoparticles were confirmed by color changes and qualitatively characterized by UV-Visible spectrophotometer.

2.5. Characterization of Silver Nanoparticles

A) UV-visible spectroscopy

Synthesis of silver nanoparticles by reducing, the respective metal ion solution with leaves extract may be easily observed by UV- Vis spectroscopy. The absorption spectra of leaves extract quantities and metal concentration was measured using a spectrophotometer in 300-1000 nm range. The formation and completion of silver nanoparticles was characterized by UV-visible spectroscopy using a double beam spectrophotometer

B) FT-IR chemical analysis

Interaction of NPs obtained with PEG and gluconic acid products by reduction of sugar compound were confirmed by FT-IR spectra. (Geethalakshmi and Sarada, 2012).

2.6. Antioxidant Properties

2.6.1. Determination of Total Antioxidant Capacity (TAC)

Briefly, 0.3 ml of sample will be mixed with 3.0 ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture will be incubated at 95ºC for 90 minutes under water bath. Absorbance of all the sample mixtures will be measured at 695 nm after 15 min. Ascorbic acid will be used as standard (Prieto *et al*., 1999)

2.6.2. DPPH Radical Scavenging Assay

The free radical scavenging activity was measured by the 1-1-Diphenyl-2-picryl-hydrazyl (DPPH) following the method by Blois, (1958). DPPH will be used as a reagent which evidently offers a convenient and accurate method for titrating the oxidizable groups of natural (or) synthetic antioxidants. 0.1 mM solution of DPPH in methanol will be prepared and 1ml of this solution will be added to 3ml of seaweed extracts of different concentration (100, 250, 500, 750 and 1000µg). After 10 minutes, absorbance will be measured at 517 nm. The percentage scavenging activity values will be calculated using the following formula

Percentage of Scavenging = $((A_o-A_1) / A_o) \times 100$

Where, A^o is absorbance of control and A_1 is absorbance of sample turbidity factor.

2.6.3. Hydrogen Peroxide Scavenging Assay

The free radical scavenging activity was determined by hydrogen peroxide assay (Gulcin *et al.,* 2004).Hydrogen peroxide (10mM) solution will be prepared in phosphate buffered saline (0.1M, pH 7.4). 1ml of the extract containing samples of different concentration (100, 250, 500, 750 and 1000µg) will be rapidly mixed with 2ml of hydrogen peroxide solution. The absorbance will be measured at 230 nm in the UV spectrophotometer after 10 minutes of incubation at 37°C against a blank (without hydrogen peroxide). The percentage of scavenging of hydrogen peroxide will be calculated using the formula

Percentage scavenging (H_2O_2) = $((A_o-A_1) / A_o) \times 100$

A_o - Absorbance of control; A_1 - Absorbance of sample

2.6.4. Determination of Reducing Power

Reducing power was determined by the following method (Yamaguchi *et al*., 1998). Briefly, 4 ml of reaction mixture, containing samples of different concentration in phosphate buffer (0.2 M, pH 6.6) will be incubated with potassium ferricyanide (1% w/v) at 50°Cfor 20 min. The reaction will be terminated by TCA solution (10% w/v). The solution will be then mixed with distilled water and ferric chloride (0.1% w/v) solution and the absorbance will be measured at 700 nm.

3. Results

The *Mentha arvensis*flower extract mediated nanoparticles showed absorbance peaks at 370 nm region in the spectral analysis shown in Fig.1. The peaks were stable with time duration also.It indicates that the synthesis of silver nano particles reqires the reduction of α-NADPH to α- $NADP^+$ and the hydroxy quinoline probably acts as electron shuttle tranforming the electron generated during the reduction of nitrate to Ag^+ ions converting them to Ag^0

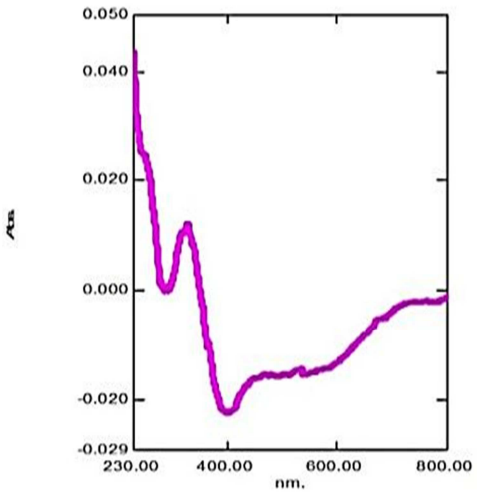

Fig. 1. UV-spectrophotometer for $AgNo_3$ Synthesis of *Mentha arvensis*

Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1650.95 cm^{-1} (Fig.2). The stretching vibration of C=C obtained at 1625.88 cm^{-1} and the single absorbance peak located at 1108.99cm^{-1} is assigned to C-O Polyols, while 3379.05 and 3355.91 cm^{-1} corresponds to O-H and N-H stretching vibration.

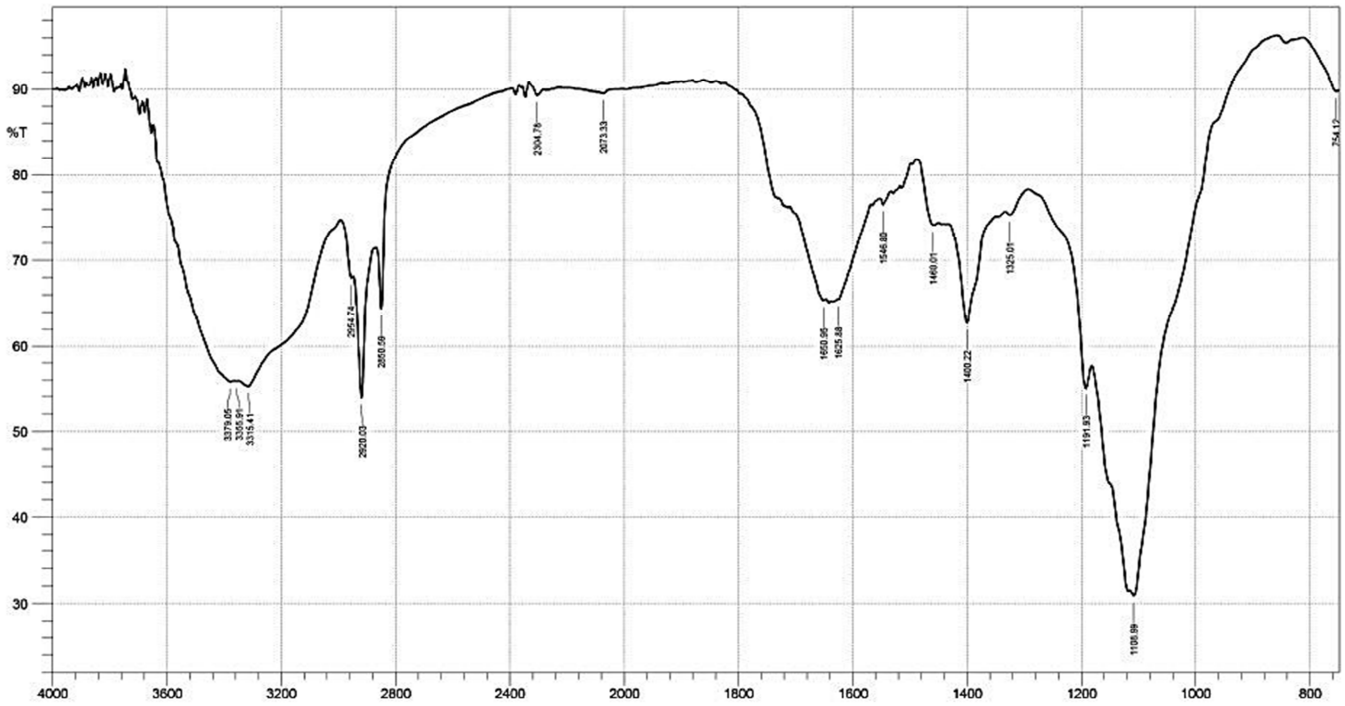

Fig. 2. FTIR analysis for $AgNo_3$ Synthesis of *Mentha arvensis*

3.1. Total Antioxidant Properties

Free radical scavenging activity of the silver nanoparticles was assessed by DPPH solution exhibited a deep purple colour with a maximum absorbance at 517nm. The disappearance of purple colour on adding synthesized silver nanoparticles might due to presence of antioxidant in the medium (Fig.3).

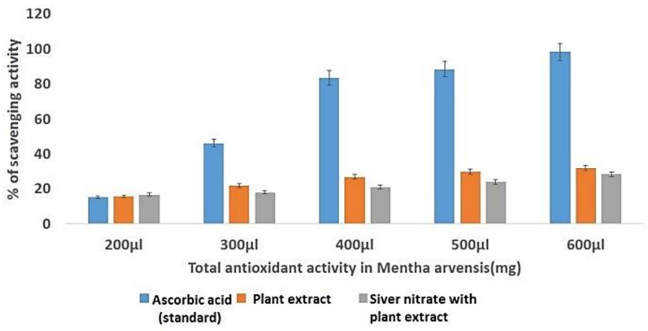

Fig. 3. Total antioxidant activity of *Mentha arvensis*

3.2. Dpph

Free radical scavenging activity of the silver nanoparticles was assessed by DPPH solution exhibited a deep purple colour with a maximum absorbance at 517nm. The disappearance of purple colour on adding synthesized silver nanoparticles might due to presence of antioxidant in the medium. *Mentha arvensis* plant extract of silver nanoparticles exhibit higher activity at 35% in600µg/ml, likewise lower activity observed at 200 µg/ml with 19% followed by the plant extract showing minimum activity at 200µg/ml with 30 % and also maximum activity observed at 600 µg/ml with 80%(Fig.4).

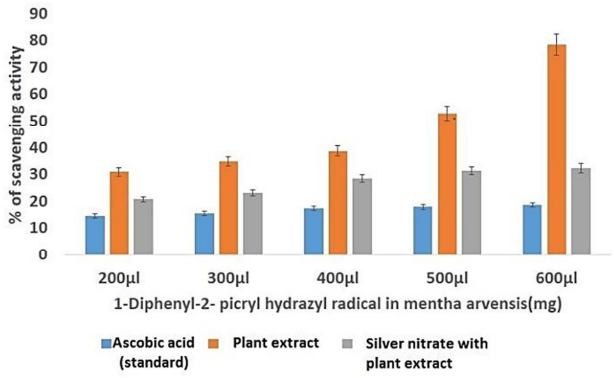

Fig. 4. 1-Diphenyl-2-picrylhydrazyl radicals of *Mentha arvensis*

3.3. Hydrogen Peroxide Scavenging Assay

Mentha arvensis, Hydrogen peroxide scavenging activity of $AgNO_3$showing a minimum activity observed 2% at 200µg/ml and maximum activity observed 10% at 600µg/ml followed by the plant extract showing a minimum activity at 30% at 200µg/ml and maximum activity observed 82% at

600µg/ml (Fig.5).

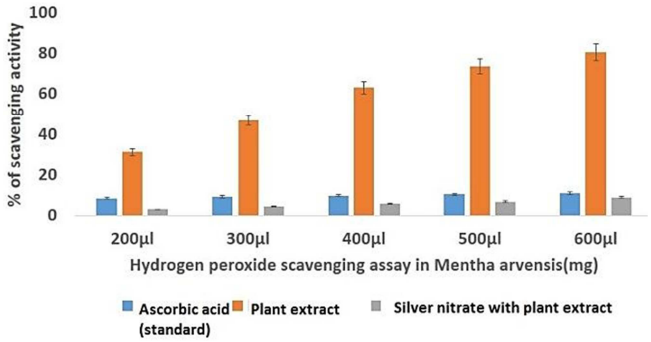

Fig. 5. Hydrogen peroxide scavenging assay in *Mentha arvensis*

3.4. Reducing Sugar

Reducing power of *Mentha arvensis* silver nanoparticles exhibit higher activity at 19% in600µg/ml, minimum activity at200µg/ml with 35 % and also maximum activity observed at 600 µg/ml with 85% (Fig.6).

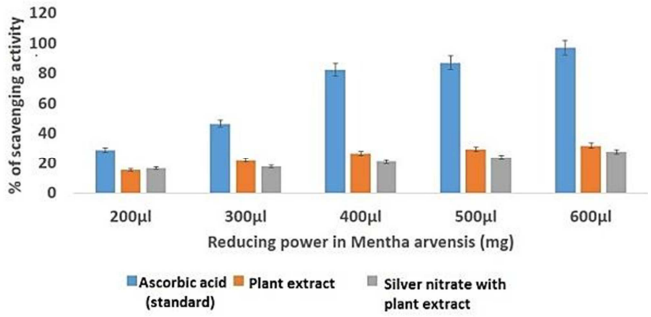

Fig. 6. Reducing power of *Mentha arvensis* silver nanoparticles

4. Discussion

In the present examination, *Mentha arvensis* were studied for antioxidant properties. DPPH is a stable and well characterized synthetic solid radical for evaluation of antioxidant potential of compounds. The DPPH will be reduced by accepting the hydrogen or electron, the DPPH reducing ability of silver nanoparticles was quantified spectrophotometrically by changing the DPPH color from purple to yellow. Inhibition was found to be high in silver nanoparticles, when compared with gold nanoparticles, which may be due to the facts that silver act as a good oxidant can easily lose electrons. The results obtained in the DPPH assay showed effective free radical inhibition by both AgNPs. The average percentage inhibition of synthesized AgNPs was observed in the range from 10%-40 as compared at different concentrations used in this study and the activity increased with increasing concentrations of AgNPs and plant extract also. Similar observations with enhanced DPPH scavenging activity by selenium, platinum, silver nanoparticles (Gao *et al*., 2002; Huang *et al*., 2003; Watanabe *et al*., 2009; Saikia *et al*., 2010) and by torolex and chitosan coated gold nanoparticles (Nie *et al*., 1997; Raghunandan *et al*., 2010) have been reported.

In the present examination, *Mentha arvensis* shows the superoxide scavenging activity of both the plant extract and AgNPs as determined by the PMS-NBT reduction system. Superoxide (O_2 −) radicals easily react with DNA and protein which necessitate their immediate clearance in living systems. The superoxide radical quenching activity of plant extract and AgNPs was found to be increased with increasing concentrations and the average inhibition was about 40%. Similarly, The superoxide radical inhibition has been reported for platinum and selenium nanoparticles (Makari *et al*., 2008).The potential superoxide scavenging activity of gold and silver nanoparticles reported earlier (Ramamurthy *et al*., 2013) support our findings in the present study. In the same way (Huie *et al*., 1993; Pacher *et al*., 2007) explored that the role of nitric oxide radicals in carcinomas and inflammatory process is well established. The toxic effects of nitric oxide will increase when reacts with superoxide radicals that lead to vascular system damage and results in conditions including inflammation, juvenile diabetes and multiple sclerosis Because of the less stability of nitric oxide ions, they accept electrons from silver nanoparticles and form formazan when treated with Griess reagent that can be detected spectrophotometrically.

Mentha arvensis of H_2O_2 scavenging activity of Plant extract and AgNPs are active in quenching H_2O_2 radicals and the average inhibition was found to be 80 % By the same token, PLAgNPs were as effective as PLFE in quenching H_2O_2 radicals and the average inhibition was found to be 96% as compared to PLFE. In this study, it could be noted that the superoxide radical quenching activity and NO quenching activity of PLAgNPs was 60% and 70% respectively as compared to PLFE which can be explained on the fact that the concentration of phytocompounds responsible for the scavenging activities was higher in the extract than adhered to the nanoparticles. On the other hand, the observed increase in H_2O_2 scavenging activity of PLAgNPs (96%) may be because of the plant condensed tannins present in the extract that are involved in confirmation of nanoparticles (Subramanian *et al.,*2013).

5. Conclusion

Nanoparticles synthesis of *Mentha arvensis* exhibited high antioxidant properties.

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References

[1] Blois MS. (1958). Antioxidant determinations by the use of a stable free radical. Nature.181:119-123Chen Z, and Gao L. (2007). A facile and novel way for the synthesis of nearly monodisperse silver nanoparticles. Mater Res Bull. 42:1657-1661.

[2] Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO.(2000). A mechanistic study of the antibacterial effect of silver ions on E. coli and Staphylococcus aureus. J Biomed Mater Res. 52:662-8.

[3] Gupta P, Bajpai M, Bajpai SK. (2008).Investigation of antibacterial properties of silver nanoparticle-loaded poly (acrylamide-co-itaconic acid)-grafted cotton fabric. J Cotton Sci. 12:280-6.

[4] Gulcin I, Buyukokuroglu ME, and Kufrevioglu OI. (2004). Metal chelating and hydrogen peroxide scavenging effects of melatonin. J Pineal Res. 34(4): 278-81.

[5] Gao X, Zhang J. and Zhang, I. (2002). Hollow sphere selenium nanoparticles: their invitro anti hydroxyl radical effect. Adv. Mat.Sci., 14: 290.

[6] Gardea-Torresdey JL, Gomez E, Peralta-Videa J. (2003).Synthesis of gold nanotriangles and silver nanoparticles using Alfalfa sprouts: A natural source for the synthesis of silver nanoparticles. Langmuir.19:1357–1365.

[7] Geethalakshmi R, and Sarada, DVL, (2012).Gold and silver nanoparticles from Trianthem adecandra: synthesis, characterization, and antimicrobial properties. Int J Nanomedicine. 7: 5375–5384.

[8] Huie RE. and Padmaja, S. (1993).The reaction of NO with superoxide. Free Radic. Res. Commun. 18:195-199.

[9] Hartsel S, and Bolard J. (1996). Amphotericin B: New life for an old drug, Trends Pharmacol. Sci.17: 445–449.

[10] Huang B, Zhang J, Hou J. and Chen, C. (2003). Free radical scavenging efficiency of nano-Se in vitro. Free Radic. Biol. Med. 35:805-812.

[11] Krutyakov YA, Kudrinskiy A, Yu Olenin A, and Lisichkin GV. (2008). Synthesis and properties of silver nanoparticles: advances and prospects, Russ. Chem. Rev. 77(3):233–257.

[12] Kim JS, Yoon T, Yu KN, Kim BG, Park SJ, Kim HW, Lee KH, Park SB, Lee JK, and Cho MH.(2009).Toxicity and tissue distribution of magnetic nanoparticles in mice. Oxford Journals. 89(1):338–347.

[13] Lee PC, and Meisel D. (1982). Adsorption and surface-enhanced Raman of dyes on silver and goldsols, J. Phys. Chem. 86:3391–3395.

[14] Lim PY, Liu RS, She PL, Hung CF, Shih HC. (2006). Synthesis of Ag nanospheres particles in ethyleneglycol by electrochemical-assisted polyol process. Chem Phys Lett. 420:304-8.

[15] Makari HK, Haraprasad N, Patil HS.and Ravi, K. (2008). In vitro Antioxidant Activity of The Hexane and Methanolic extracts of Cordia wallichii and Celastrus paniculata. Int J. Aesthet. AntiagingMed.1:1-10.

[16] Nie S. and Emory, SR. (1997).Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. Science.275:1102-1106.

[17] Pacher P. and Beckman, SJ. (2007). Lucas Liaudet, Nitric oxide and peroxynitrite in health and disease. Physiol. Rev., 87:315-424.

[18] Patel K, Kapoor S, Dave DP and Ukherjee T. (2007).Synthesis of Pt, Pd, Pt/Ag and Pd/Ag nanoparticles by microwave-polyol method. J Chem Sci.117:311-316

[19] Panacek A, Kvitek L, Prucek R, Kolar M, Vecerova R, and Pizurova N. (2006). Silver colloid nanoparticles: synthesis, characterization and their antibacterial activity. J PhyChem.110:16248-16253.

[20] Prieto PD, Rojas AA, and Jordano J. (1999).Seed-specific expression Patterns and regulation by AB13 of an unusual late embryo-genesis-abundant gene in sunflower. Plant Mol Bio.39:615-627.

[21] Raghunandan D, Bedre MD, Basavaraja S, Sawle B, Manjunath, SY. and Venkataraman A. (2010). Rapid biosynthesis of irregular shaped gold nanoparticles from macerated aqueous extracellular dried clove buds (Syzygiumaro maticum) solution. J. Colloid Surf. 79:235-242.

[22] Ramamurthy CH, Padma M, Samadanam IDM, Mareeswaran R, Suyavaran A, Suresh Kumar M, Premkuar K. and Thirunavukkarasu C. (2013). The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. J. Colloid Surf. 102:808-815.

[23] Rachel MSB. and Meera Bai. G Antimicrobial activity of Mentha arvensis L. Lamiaceae. J Advanced Laboratory Research in Biology. 2011; 2(1): 1-4

[24] Simakin AV, Voronov VV, Kirichenko NA, and Shafeev GA. (2004) Nanoparticles produced by laser ablation of solids in liquid environment. Appl. Phy.79:1127.

[25] Salkar RA, Jeevanandam P, Aruna ST, KoltypinY, and Gedanken AT (1999).Chemical preparation of amorphous silver nanoparticles. J. Mater. Chem.9:1333–1335.

[26] Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D.(2007). Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology. 18: 225103-12.

[27] Shahverdi AR, Fakhimi A, Shahverdi HR, and Minaian S. (2007).Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. Nano Med Nanotechnol Biol Med.3:168-171.

[28] Sondi I, and SalopekSondi B. (2004). Silver nanoparticles as antimicrobial agent: a case studyon E. colias a model for gram-negative bacteria. J Colloid Interface Sci. 275:177-18.

[29] Saikia JP, Paul S. and Samdarshi BK. (2010). Nickel oxide nanoparticles: A novel antioxidant. J. Colloid Surf. 78:146-152.

[30] Subramanian R., Subramanian P. and Raj, V. (2013). Antioxidant activity of the stem bark of Shorearox burghii and its silver reducing power. SpringerPlus.2:28.

[31] Watanabe A, Kajita M, Kim J, Kanayama A, Takahashi K, Mashino T., and Miyamoto Y.(2009). Invitro free radical scavenging activity of platinum nanoparticles, Nanotechnol. 20:455105-455114.

[32] Yang Y, Matsubara S, and Xiong L. (2007).Solvothermal synthesis of multiple shapes of silver nanoparticles and their SERS properties. J. Phys. Chem. 111:9095–9104.

[33] Yamaguchi H, Yamaguchi M, and Adachi M. (1998).Specific-detection of alkaline phosphate activity in individual species of marine phytoplankton. Plankton Benthos Res.14: 214-217.

[34] Yuan YV, Walsh NA. (2006). Antioxidant and anti proliferative activities of extracts from variety of edible seaweed. Food ChemToxicol.44: 1144-1150.