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Research Article

IMPROVED YIELD OF GREEN SYNTHESIZED CRYSTALLINE SILVER NANOPARTICLES WITH POTENTIAL ANTIOXIDANT ACTIVITY

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ABSTRACT

Biogenic nanoparticles are evolving as an important branch of nanomedicine which is cost effective and ecofriendly. The current study deals with the synthesis of silver nanoparticles using *Ocimum sanctum* leaf extract as reducing and capping agent. With increase concentration of $AgNO_3$ (1-5mM), there was considerable increase in yield of silver nanoparticles The prepared nanoparticles were characterized using UV-visible spectroscopy, Fourier transform infrared spectroscopy (FT-IR), Transmission electron microscopy (TEM), X-ray diffraction (X-RD) and Dynamic light scattering (DLS).UV-visible spectroscopy showed maximum absorbance at 420 nm due to surface plasmon resonance of silver nanoparticles. FT-IR spectral analysis indicates the presence of water soluble phenolic compounds as reducing and stabilizing agents in the synthesis of silver nanoparticles. TEM analysis showed the presence of nearly spherical particles of size range 3-15 nm. Additionally, X-RD analysis revealed that the synthesized silver nanoparticles were anisotropic face-centered, cubic crystalline, having a size of 17.082 ± 5.83nm. DLS further support the formation of thermally stable silver nanoparticles, possessing negative surface charge potential. Thus, *Ocimum sanctum* leaf extract promotes rapid bioreduction of optimum concentration of silver nitrate resulting in a significant yield of silver nanoparticles which can be exploited commercially for its antioxidant activity.

Keywords: Silver nanoparticles, Crystalline, Biological synthesis, *Ocimum sanctum.*

INTRODUCTION

Recently, there is a great need of silver nanoparticles because of their antibacterial and anti-inflammatory properties[1,2]. Commercially, SNPs are synthesized by means of chemical methods using reducing agents such as sodium borohydride, hydrazine, N, N-dimethylformamide in the presence of stabilizers like triphenylphosphine, citrate, polyvinylpyrrolidone[3-5]. The prepared SNPs have a high surface area which leads to the adsorption of these potentially toxic chemicals. This might be one of the major reasons for reported toxicity of SNPs[6-8]. The contamination from these toxic chemicals can be avoided by synthesizing SNPs using biological methods like microbes and plants. Many bacteria and fungi have been used for the biosynthesis of SNPs, but the process is relatively slow in comparison to plant extract[9-15]. Moreover, it is very difficult to maintain bacterial and fungal strains for long term use. Plant extract is easily available, generally safe and nontoxic. They consist of a large variety of water-soluble secondary metabolites like terpenoids, flavanoids, organic acids and phenols[16]. These constituents aid in the reduction of silver ions. Thus, the process for the preparation of SNPs is totally aqueous, environment-friendly and economical. Leaf extracts of *Crossandra infundibuliformis, Tephrosia purpurea, Sesuvium portulacastrum, Tamarindus indica, Chenopodium murale, Desmodium gangeticum, Acalypha indicia, Psidium guajava, Murraya koenigii, Azadirachta indica* have been reported for the green synthesis of SNPs[17-26]. The choice of plant extract is very important because it plays a pivotal role in determining the biological activity of prepared SNPs[27]. Leaves of *Ocimum sanctum* were chosen for the present study as this plant is readily available and has enormous therapeutic benefits[28,29]. SNPs prepared using leaves of *Ocimum sanctum* might possess antioxidant properties which can be used for cancer chemotherapy. The aim of the present study is to commercially exploit this biogenic method for large scale synthesis of SNPs. Effect of various silver nitrate concentrations (1-5mM), different temperatures (24 ± 5°C, 50 ± 5°C) at pH 9.20 ± 0.23 were studied for the time taken to prepare SNPs, percentage yield, particle size, zeta potential analysis, and polydispersity index. Further, the optimized concentration of SNPs i.e. 5mM was characterized for crystallinity, thermal stability and biological activity.

MATERIALS AND METHODS

All analytical reagents used in the study were of analytical grade and were purchased from Loba Chemie, Mumbai, India. Fresh leaves of *Ocimum sanctum* were collected from the botanical garden, Punjabi University, Patiala, Punjab. The leaves were identified by Dr. V.K. Singhal, Professor, Department of Botany, Punjabi University, Patiala. The authentication no. was 59627.

Preparation of the extract

Fresh leaves of *Ocimum sanctum* were collected and washed thoroughly with deionized water. The leaves were then kept for sun drying. After drying, the leaves were finely powdered and sieved through mesh 15 (0.19 mm pore size). This fine powder was used for the preparation of leaf extract. The fresh biomass (5 g) was taken and boiled (15 min at 80°C) in the deionized water (100 mL). This extract was filtered through Whatman filter paper no. 1 and stored (4°C) for further use.

Synthesis of SNPs

In a typical reaction procedure, 5 mL leaf extract was added dropwise with continuous stirring to 45 mL $AgNO_3$ solution for the reduction of Ag^+ ions in a 250 mL Erlenmeyer flask. Simultaneously, pH 9.20 ± 0.23 was adjusted with 0.1M NaOH. The solution turned from colorless to reddish-brown indicating the formation of SNPs. The effect of $AgNO_3$ concentration (1–5mM) on the time of formation, percentage yield, particle size, zeta potential was studied at room temperature. In order to study, the effect of temperature on the preparation of SNPs, the study was repeated at 50°C. Percentage yield was calculated according to the formula given below:

$$\% \text{ Yield} = \frac{\text{Weight } of \text{ lyophilized silver nanoparticles}}{\text{Weight of silver nitrate used}} \times 100$$

Characterization of SNPs

The prepared SNPs (5mM) were characterized for their size, shape, and polydispersity[30]. The common techniques used for characterizing nanoparticles are UV–visible spectrophotometry, Transmission electron microscopy (TEM), Dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FT-IR), and powder X-ray diffraction (X-RD).

***In vitro* antioxidant activity**
Determination of Total Phenolic Content

The total phenolic content was determined by the Folin–Ciocalteau method[31]. The ethanolic leaf extract (0.5 mL; 1 mg/mL) and SNPs (0.5 mL;1 mg/mL) were mixed with Folin–Ciocalteau reagent (5 mL, diluted 1:10 with distilled water) for 5 min separately. Na_2CO_3 (4mL, 1M) was then added to the mixture and allowed to incubate for 15 min at room temperature and the phenolic content was determined by the spectrophotometric method at 765nm. The total phenolic content was expressed as Gallic acid (GA) equivalents (mg GA/g dry weight).

DPPH assay

The reported method of Choi et al was carried out for DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. DPPH ethanolic solution (1 mL, 0.1mM) was added in concentrations (5-100 µg/mL) of ethanolic extract and SNPs separately. The reaction mixture was shaken and incubated in the dark for 30 min at room temperature and the absorbance at 517 nm was measured[32]. The antiradical properties of these compounds were evaluated as the IC_{50} DPPH (the concentration of antioxidant which reduces the free radical DPPH about 50%).

Reducing power assay

Fe^{3+} reducing the power of SNPs was conducted based on the method reported by Makari et al.[33]. Different concentrations (10-320 µg/mL) of plant extract and SNPs were prepared and mixed with 2.5 mL phosphate buffer (0.2M, pH 6.6) and 2.5 mL potassium ferricyanide (1%) separately. The mixture was incubated at 50°C for 20 minutes. Further 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, supernatant solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (0.01%) and absorbance were measured at 700 nm in UV-visible spectrophotometer.

Table 1: Time of formation, % yield and particle size of prepared SNPs at room temperature, pH 9.20 ± 0.23

Batch code	Time (minute)	Yield (%)	Particle size (nm)	Zeta potential (mV)	Polydispersiy index
SNPs 1	49.66 ± 0.57	29.37 ± 2.005	78.41 ± 8.67	-7.14 ± 4.52	0.32
SNPs 2	44.34 ± 0.27	32.90 ± 3.06	87.54 ± 3.45	-9.74 ± 5.92	0.24
SNPs 3	20.00 ± 0.23	43.64+2.62	102.65 ± 6.098	-10.6 ± 3.65	0.31
SNPs 4	5.00 ± 0.00	55.26+1.54	127.15 ± 4.78	-15.04 ± 2.76	0.34

*Note: SNPs1-SNPs4 is concentration of $AgNO_3$ (2–5mM)

Table 2: Time of formation, % yield and particle size of prepared SNPs at 50 ± 5°C, pH 9.20 ± 0.23

Batch Code	Time (minute)	Yield (%)	Particle Size (nm)	Zeta Potential (mV)	Polydispersiy index
SNPs 5	45.27 ± 0.19	35.67 ± 5.84	40.43 ± 5.04	-12.43 ± 3.72	0.32
SNPs 6	31.02 ± 0.73	48.24+4.56	55.86 ± 7.86	-16.21 ± 4.00	0.27
SNPs 7	15.05 ± 0.87	64.96 ± 2.59	67.23 ± 3.61	-17.83 ± 4.35	0.24
SNPs 8	1.06 ± 0.11	74.73 ± 2.05	83.41 ± 2.23	-21.70 ± 3.06	0.20

*Note: SNPs 5-SNPs 8 is concentration of $AgNO_3$ (2–5 mM)

Table 3: Phenolic compounds present in the plant extract and SNPs

Sample	Total Phenolic Content (mgGA/g sample)
Plant extract	85.00 ± 1.45
SNPs	32.37 ± 1.97

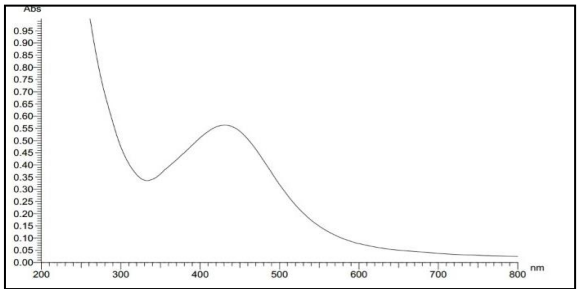

Figure 1: UV–VisIble absorption spectra of colloidal solution synthesized SNPs

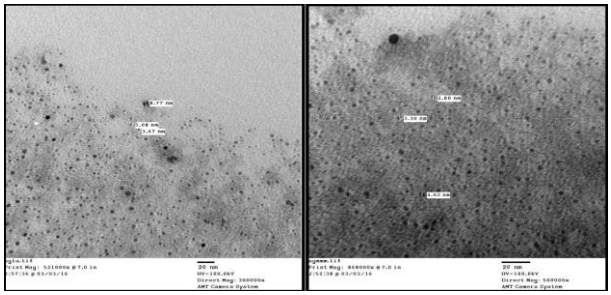

Figure 2: TEM of SNPs at resolution a) 300000x b) 500000x

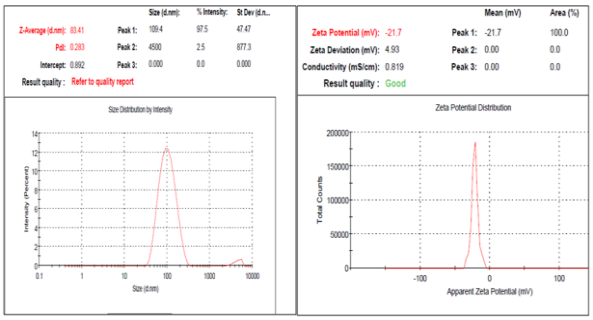

Figure 3: Characterization of SNPs by a) DLS b) Zeta potential

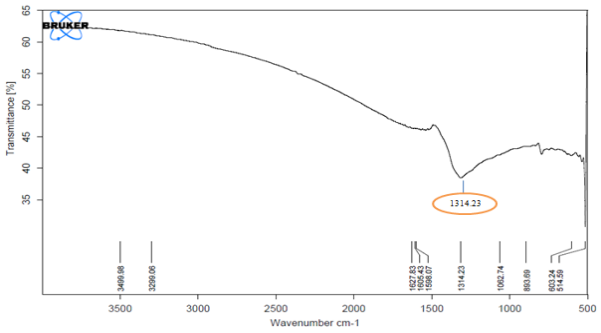

Figure 4: FT-IR spectrum of synthesized SNPs

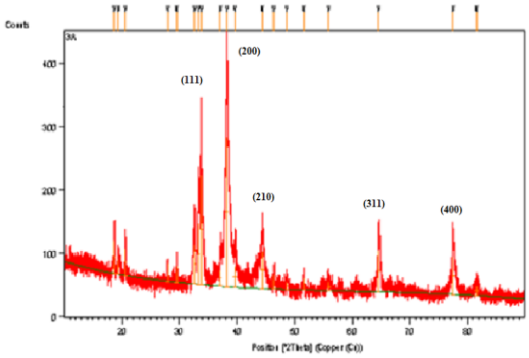

Figure 5: X-ray diffraction pattern of SNPs

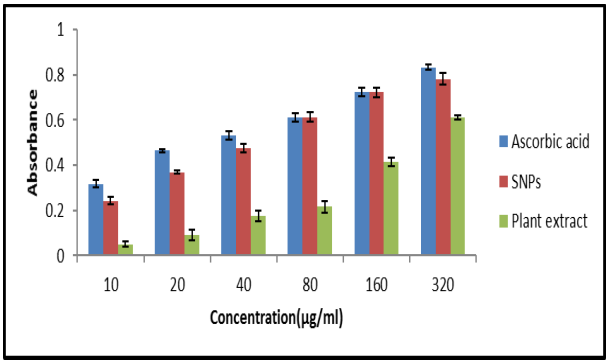

Figure 6: The reducing power assay of the SNPs, plant extract, and ascorbic acid

RESULTS AND DISCUSSION

As shown in Table 1 and 2, all the prepared batches confirm the formation of SNPs due to the color change of *O. sanctum* extract when added to aq. $AgNO_3$ solution (except 1mM). But the time required for color change varies depending upon the operating conditions and $AgNO_3$ concentrations. The formed SNPs in each experiment were analyzed by DLS technique in order to determine their particle size distribution, zeta potential, polydispersity index and % yield. The non-visibility of distinctive color change with $AgNO_3$ (1mM) within 24 hours of study illustrates the absence of SNPs formation. Therefore, from the above data it is concluded that with an increase in $AgNO_3$ concentration (2–5mM), there was a decrease in the time duration of SNPs synthesis and particle size but increase in % yield and stability. Rapid synthesis with maximum yield and stability was obtained with 5mM concentration. Therefore, the batch (SNPs 8) was selected for further studies.

UV spectral analysis

UV–Vis spectroscopy is a powerful tool to observe the formation of metal nanoparticles. Light wavelengths in the range of 200–800 nm are generally used for characterizing various SNPs[34]. The characteristic peak at 420 nm is due to excitation of Surface Plasmon Resonance vibrations (SPR) of synthesized SNPs in reaction medium as shown in Fig. 1. These findings are in consonance with earlier reports by Philip and Unani 2011; Prabhu and Poulose 2012; Nayak et al. 2015.

TEM analysis

The TEM images of prepared SNPs at 20 nm scale with 3, 00,000x and 5, 00,000x resolution are shown in Fig.2. The particles were found to be spherical with the size range of 3-15 nm. The images revealed narrow particle size distribution. The results are in consonance with earlier reports of Ramteke et al. 2013; Subba Rao et al.2013; Bindhani and Panigrahi 2015[1-3].

Dynamic light scattering and zeta potential

The size distribution of biosynthesized SNPs was measured using dynamic light scattering (DLS) method. Fig.3(a) shows the average particle size of 83.41 ± 2.23 nm with a polydispersity index (PDI) of 0.28. DLS measures the hydrodynamic radius of dispersed nanoparticles, therefore the size of SNPs obtained by this method is slightly bigger than that obtained by TEM. The low value of PDI reveals narrow particle size distribution of SNPs. Fig.3(b) shows the surface zeta potential of -21.70 ± 3.06 mV, which shows that prepared SNPs were stable.

Fourier transformed infrared studies

FT-IR spectroscopic studies were carried out to investigate the plausible mechanism behind the formation of these SNPs and offer information regarding the functional groups. The representative spectra of SNPs are shown in Fig.4. A distinct peak in the region 1314.23 cm^{-1} attributes to the presence of stretching vibrations of alcoholic, carboxylic acids, ethers, and esters functional sites of biomolecules binding with nanoparticles.

X-RD studies

Crystallographic analysis of SNPs using X-RD confirms the crystalline nature of nanoparticles (Fig.5). A number of Bragg reflections with a 2ϕ value of 33.79°, 38.23°, 44.31°, 64.56°, 77.45° corresponds to (111), (200), (210), (311) and (400) set of lattice planes. This may be indexed as the band for face-centered cubic structure (fcc) of silver. The remaining unassigned peaks in Fig.5 could be due to the crystallization of phytochemicals present in the leaf extract on the surface of SNPs.The average crystalline size of prepared SNPs was determined with Debye Scherrer's equation. It was found to be 17.082 ± 5.83 nm, which is fairly in agreement with TEM analysis. In the present study, the peak intensity and peak width were considerably larger than that was reported earlier. It is worth mentioning that the maximum peak broadening at 38.23 indicates the formation of very small size SNPs (13.94 nm).

***In vitro* antioxidant activity**

Phenolic compounds present in the plant extract and SNPs are presented in Table 3. The IC_{50} values of aqueous extract, SNPs, and ascorbic acid were obtained as 39.36 ± 2.37 µg/mL, 10.56 ± 0.65 µg/mL, 5.70 ± 0.29 µg/mL respectively. Fig.6 shows the dose-response bar chart for the reducing power, and was found to be in order: Ascorbic acid > SNPs > extract. The increase in the absorbance of the reaction mixture indicates stronger reducing power. The free radical scavenging activity of SNPs attributes to functional groups of bio-reductant molecules adhered to the surface of nanoparticles[35].

CONCLUSION

The present study demonstrates an eco-friendly, rapid green chemistry approach for the synthesis of SNPs. In this method, *Ocimum sanctum* leaf extract was used as the reducing agent and stabilizing agent. The present study clearly demonstrates that biogenic antioxidant silver nanoparticles can be produced rapidly and efficiently by controlling formulation and processing variables *viz.* concentration of SNPs, temperature, pH.

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ABBREVIATIONS

$AgNO_3$: Silver nitrate
SNPs: Silver nanoparticles
SPR: Surface Plasmon Resonance
UV-vis: UV-Visible Spectroscopy
TEM: Transmission Electron Microscopy
DLS: Dynamic Light Scattering
FT-IR: Fourier Transform Infrared Spectroscopy
X-RD: X-ray Diffraction
PDI: Polydispersity Index

REFERENCES

1. Gurunathan S, Han JW, Kwon D-N, Kim J-H. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. Nanoscale Research Letters 2014;9(1):1-17
2. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, et al. Silver nanoparticles as potential antibacterial agents. Molecules 2015;20(5):8856–74.
3. Zhao X, Xia Y, Li Q, Ma X, Quan F, Geng C, et al. Microwave-assisted synthesis of silver nanoparticles using sodium alginate and their antibacterial activity. Colloids and Surfaces A: Physicochemical and Engineering Aspects 2014;444:180–8.
4. Guzmán MG, Dille J, Godet S. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. 2008;2(3):357-64.
5. Van Dong P, Ha C, Binh L, Kasbohm J. Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles. International Nano Letters 2012;2(1):1-9.
6. Borase HP, Salunke BK, Salunkhe RB, Patil CD, Hallsworth JE, Kim BS, et al. Plant extract: A promising biomatrix for ecofriendly, controlled synthesis of silver nanoparticles. Applied Biochemistry and Biotechnology 2014;173(1):1–29.
7. Arunachalam R, Dhanasingh S, Kalimuthu B, Uthirappan M, Rose C, Mandal AB. Phytosynthesis of silver nanoparticles using Coccinia grandis leaf extract and its application in the photocatalytic degradation. Colloids and Surfaces B: Biointerfaces 2012;94:226–30.
8. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano Letters 2012;2 (1):2-10.
9. Ghorbani HR, Safekordi AA, Attar H, Sorkhabadi SMR. Biological and Non-biological Methods for Silver Nanoparticles Synthesis. Chemical and Biochemical Engineering Quarterly Journal 2011;25(3):317–26.
10. Abbasi T, Anuradha J, Ganaie SU, Abbasi SA, Abdelhamid AA, Al-ghobashy M, et al. Development of biogenic silver nano particle from Pelargonium graveolens leaf extract and their antibacterial activity. Colloids and Surfaces B: Biointerfaces 2013;4(2):194–8.
11. Forough M, Farhadi K. Biological and green synthesis of silver nanoparticles . Turkish Journal of Engineering and Environmental Sciences 2010;34:281–7.
12. Narayanan KB, Sakthivel N. Biological synthesis of metal nanoparticles by microbes.Advances in Colloid and Interface Science 2010;156(1):1–13.
13. Singh R, Shedbalkar UU, Wadhwani SA, Chopade BA. Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. Applied Microbiology and Biotechnology

2015;99(11):4579–93.
14. Singh P, Kim YJ, Zhang D, Yang DC. Biological Synthesis of Nanoparticles from Plants and Microorganisms. Trends in Biotechnology 2016;34(7):588–99.
15. Pantidos N, Horsfall LE. Biological Synthesis of Metallic Nanoparticles by Bacteria , Fungi and Plants. Journal of Nanomedicine & Nanotechnology 2014;5(5):1-10.
16. Cheng AX, Lou YG, Mao YB, Lu S, Wang LJ, Chen XY. Plant terpenoids: Biosynthesis and ecological functions. Journal of Integrative Plant Biology 2007;49(2):179–86.
17. Kaviya S, Santhanalakshmi J, Viswanathan B. Biosynthesis of silver nano-flakes by Crossandra infundibuliformis leaf extract. Materials Letters 2012;67(1):64–6.
18. Ajitha B, Ashok Kumar Reddy Y, Reddy PS. Biogenic nano-scale silver particles by Tephrosia purpurea leaf extract and their inborn antimicrobial activity. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 2014;121:164–72.
19. Nabikhan A, Kandasamy K, Raj A, Alikunhi NM. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, Sesuvium portulacastrum L.ColloidsandSurfacesB:Biointerfaces 2010;79(2):488-93.
20. Ankamwar B, Chaudhary M, Sastry M. Gold Nanotriangles Biologically Synthesized using Tamarind Leaf Extract and Potential Application in Vapor Sensing. Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry 2005;35(1):19–26.
21. Abdel-Aziz MS, Shaheen MS, El-Nekeety AA, Abdel-Wahhab MA. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using Chenopodium murale leaf extract. Journal of Saudi Chemical Society 2014;18(4):356–63.
22. Thirunavoukkarasu M, Balaji U, Behera S, Panda PK, Mishra BK. Biosynthesis of silver nanoparticle from leaf extract of Desmodium gangeticum (L.) DC. and its biomedical potential. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 2013;116:424–7.
23. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces 2010;76(1):50–6.
24. Deguchi Y, Miyazaki K. Anti-hyperglycemic and anti-hyperlipidemic effects of guava leaf extract. Nutrition & Metabolism 2010;7:1-9.
25. Suganya A, Murugan K, Kovendan K, Mahesh Kumar P, Hwang JS. Green synthesis of silver nanoparticles using Murraya koenigii leaf extract against Anopheles stephensi and Aedes aegypti. Parasitology Research 2013;112(4):1385–97.
26. Velusamy P, Das J, Pachaiappan R, Vaseeharan B, Pandian K. Greener approach for synthesis of antibacterial silver nanoparticles using aqueous solution of neem gum (Azadirachta indica L.). Industrial Crops and Products 2015;66(1):103–9.
27. Gang DR. The Biological Activity of Phytochemicals (Google eBook). The Biological Activity of Phytochemicals 2011;41:1-210.
28. Mondal S, Mirdha BR, Mahapatra SC. The science behind sacredness of Tulsi (Ocimum sanctum linn.). Indian Journal of Physiology and Pharmacology 2009;53(4):291–306.
29. Jayasinghe C, Gotoh N, Aoki T, Wada S. Phenolics composition and antioxidant activity of sweet basil (Ocimum basilicum L.). Journal of Agricultural and Food Chemistry 2003;51(15):4442–9.
30. Sadeghi B, Gholamhoseinpoor F. A study on the stability and green synthesis of silver nanoparticles using Ziziphora tenuior (Zt) extract at room temperature. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 2015;134:310–5.
31. Robards K, Antolovich M. Analytical Chemistry of Fruit BioflavonoidsA Review. The Analyst 1997;122(2):11R–34R.
32. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, et al. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Science 2002;163(6):1161–8.
33. Makari HK, Haraprasad N, Patil HS, Ravi K. In vitro antioxidant activity of the hexane and methanolic extracts of Cordia wallichii and Celastrus paniculata Internet Journal of Aesthetic and Antiaging MedicineInter.2008;1:1–10.
34. Mulvaney P. Surface Plasmon Spectroscopy of Nanosized Metal Particles. Langmuir 1996;12(3):788–800.
35. Lateef A, Azeez MA, Asafa TB, Yekeen TA, Akinboro A, Oladipo IC, et al. Biogenic synthesis of silver nanoparticles using a pod extract of Cola nitida : Antibacterial and antioxidant activities and application as a paint additive. Integrative Medicine Research 2016;10(4):551–62.

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