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Biocompatible synthesis of silver and gold nanoparticles using leaf extract of *Dalbergia sissoo*

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ABSTRACT

This report presents a rapid, reproducible and a green biogenic approach for the biosynthesis of gold and silver nanoparticles using leaf extract of *Dalbergia sissoo*. The biomolecules present in the plant induced the reduction of Au^{3+} and Ag^{+} ions from $HAuCl_4$ and $AgNO_3$ respectively, which resulted in the formation of Dalbergia conjugated nanoparticles. The growth of nanoparticles was monitored by UV-vis spectrophotometer that demonstrated a peak at 545 and 425 nm corresponding to Plasmon absorbance of gold and silver nanoparticles respectively. The leaf extract was found to direct different shape and sized gold nanoparticles. Gold nanoparticles were 50-80 nm in size and their shape varied from spherical to few triangular and hexagonal polyshaped. While silver nanoparticle synthesized were spherical, in the range of 5-55 nm in size. X-ray diffraction studies corroborated that the biosynthesized nanoparticles were crystalline gold and silver. Fourier transform infra-red spectroscopy analysis revealed that biomolecules were involved in the synthesis and capping of silver nanoparticles and gold nanoparticles. Copyright © 2012 VBRI press.

Keywords: *Dalbergia sissoo*; gold nanoparticles; silver nanoparticles; polyshaped.

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Introduction

Last two decades have witnessed a rapid advancement in various technologies for the fabrication of nanoparticles and among the various class of nanoparticles, metal nanoparticles are witnessing extreme attention due to their application in various fields of science and technology ranging from medicine to optics, biological labeling and imaging [**1**]. Metal nanoparticles such as silver and gold have been used to enhance non-linearity of molecular probes for their use in selective imaging of the structures and physiology of nanometric regions in cellular system [**2**], potential applicability in bioremediation of radioactive wastes [**3**], sensor technology [**4**], opto-electronics recording media and optics. Many chemical based methods are available for synthesis of silver and gold nanoparticles, but there is a growing concern towards use of these chemicals as they are reported to be very toxic for the environment. Apart from the toxicity these chemical based methods are also not cost effective, a disadvantage for synthesis of nanoparticles at the industrial scale. Due to these problems, various eco-friendly approaches for the synthesis of silver and gold nanoparticles are being adopted. Among them, plant mediated synthesis is being widely explored. Numbers of plants have been successfully used for the extracellular synthesis of silver and gold nanoparticles such as sun dried leafs of *Cinnamomum camphora*, can be used to produce silver and gold nanoparticles (55-80 nm) at an ambient temperature having triangular and spherical shapes [**5**]. Leaf extract of amla *(Emblica officinalis*) have been used to synthesize nanoparticles of silver (10-20 nm) and gold (15-25 nm) [**6**]. *Aloe vera* leaf extract has been used for the synthesis of silver and gold nanoparticles resulting in rapid synthesis of gold nanotriangles and spherical nanoparticles [**7**].

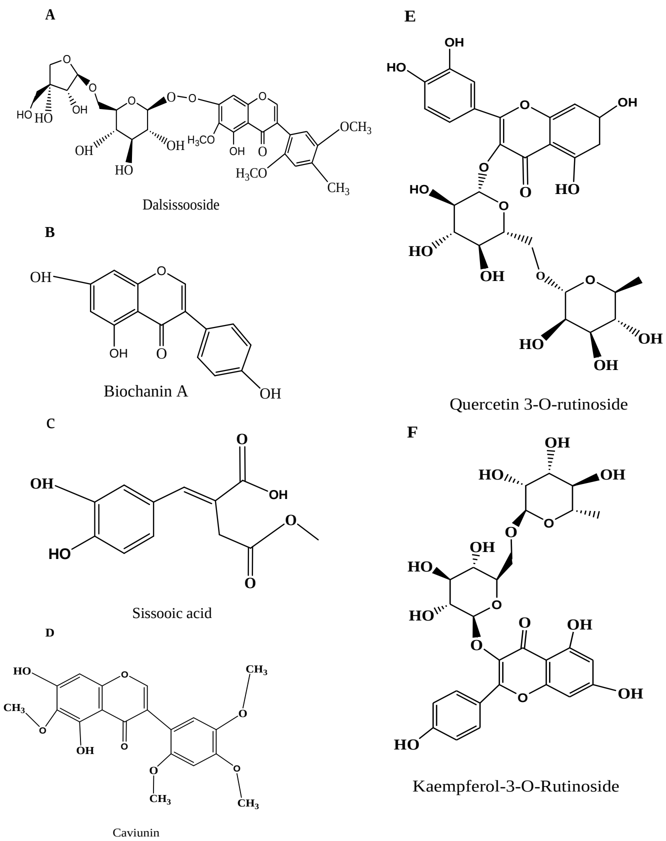


Fig. 1. Chemical structures of important constituents in green leaves of *Dalbergia sissoo*.

Dalbergia sissoo (Roxb) known as an Indian rosewood is native to Indian subcontinent. It is mainly considered as a timber tree since woods of this tree is very durable, seasons well and free from wrap or split. Apart from its application in timber industry *Dalbergia sissoo* has been used for treatments of variety of diseases [**8-10**]. Even parts of *Dalbergia sissoo* were traditionally used for treatment of various diseases such as phytochemicals from its leaves were reported for its strong antimicrobial, antipyretic, analgesic and osteogenic activity [**11**] and were also reported to be used for treatment of inflammation and diabetes [**12**]. Due to medicinal value, *Dalbergia sissoo* was investigated for isolation of different flavones, Iso-flavones, flavonols, neoflavonols and coumarins [**13-15**].Among different phytochemicals Isoflavones has been reported for its activity against bone loss and fracture [**16**]. Constituents of *Dalbergia sissoo* leaves includes genstein [**17**], genistein 8-C-β-D-glucopyranoside [**18**], quercetin 3-O-β-D-glucopyranoside [**19**], biochanin A, pratensein [20], caviunin [**21**], quercetin 3-O-rutinoside [**22**], caviunin 7-O-β-D-glucopyranoside [**23**], biochanin 7-O-glucoside, kampferol-3-O-rutinoside [**24**] etc. Recently a new isoflavone, dalsissooside was also reported [**11**] (**Fig. 1**). We hypothesize that, the synergistic reduction potential of different constituents occluded within green leaves of *Dalbergia sissoo* were able to reduce gold and silver salts to corresponding nanoparticles. To best of our knowledge this is the first report on the synthesis of biocompatible gold and silver nanoparticles using green leaves of *Dalbergia sissoo*. The details of this green technological process involving production and stabilization of poly shaped gold and silver nanoparticles are discussed below.

Experimental

Materials

The silver nitrate ($AgNO_3$, 99.8%) and Gold (III) chloride hydrate, ($HAuCl_4.3H_2O$, 99.999%) were purchased from the Fisher scientific (Mumbai, India) and Sigma-Aldrich (USA) have been used for the synthesis of silver and gold nanoparticles respectively. The fresh leaves were taken from the shisham (*Dalbergia sissoo*) located in JUIT campus.

Preparation of leaf extract

For the synthesis of silver and gold nanoparticles 5 g fresh leaves were taken and washed thoroughly to make them free from dust and other impurities. These washed leaves were cut into very fine pieces and immersed 50 ml Millipore water (compared to other plant mediated methods we dispersed shisham leaves in large volume since shisham extract is very viscous) and then boiled for 5 min. The extract was filtered and the residual material was discarded.

Synthesis of silver and gold nanoparticles

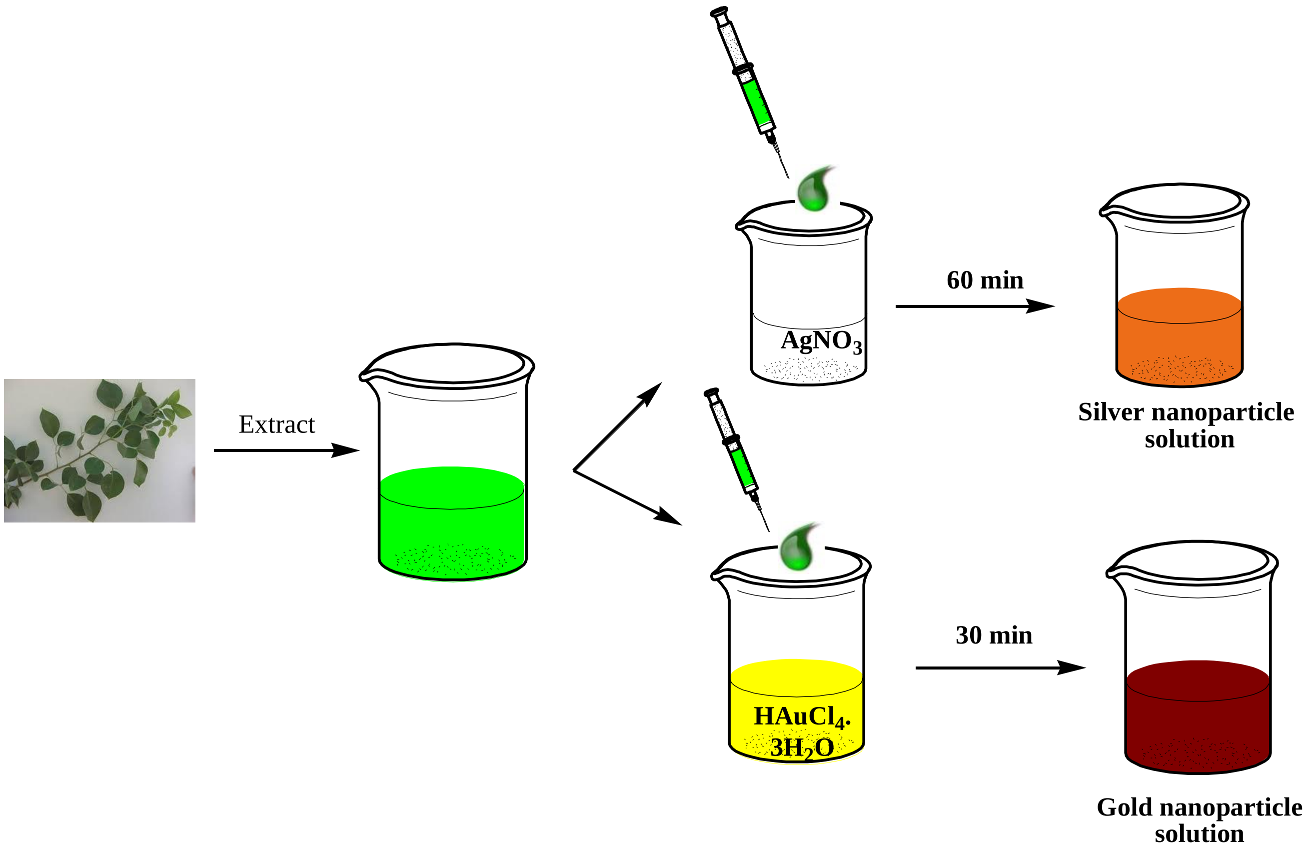
For the bioreduction of Au (III) into the Au (0), a freshly prepared leaf extract (5 ml) was added drop wise using a syringe to 50 ml 10^{-3} M $HAuCl_4$ solution. Similarly for the bioreduction of Ag (I) into the Ag (0), 5ml of leaf extract was added to 50 ml of 10^{-3} M $AgNO_3$ solution. After the addition of leaf extracts both the solutions were kept in the incubator at 37°C (**Scheme 1**).

UV-visible spectroscopy analysis

The reduction of both Ag^+ and $AuCl_4^-$ in the aqueous solution was checked with regular sampling of the 0.3 ml aliquots, diluting it with 3 ml of the Millipore water and measuring the UV-visible spectra of the diluted sample. PERKIN-ELMER spectrophotometer at a resolution of 1 nm was used for the analysis of UV-visible spectrum.

X-ray diffraction analysis

After the complete reduction of $AgNO_3$ and $HAuCl_4$ solution in Ag (0) and Au (0) respectively, solution was maintained at -80°C for 5 hours and then lyophilized for 24 hours. The lyophilized powder was further used for



Scheme 1. Schematic representation for synthesis of silver and gold nanoparticles.

XRD analysis. The XRD analysis was done using an X'Pert Pro X-ray diffractometer operating at 40 mA current and 45 kV voltages with CuKα radiation to confirm the crystalline form of silver and gold nanoparticles.

Fourier transform infrared spectroscopic analysis

For FTIR analysis, 10 ml solution of silver and gold nanoparticles were taken separately and centrifuged at 4000 pm for 10 min. The resulting suspension was re-dispersed into 20 ml of sterile water and centrifuged again. The process of centrifugation and re-dispersion was repeated three times to make the solution free from any biomass which is not present as the capping agent in the solution.

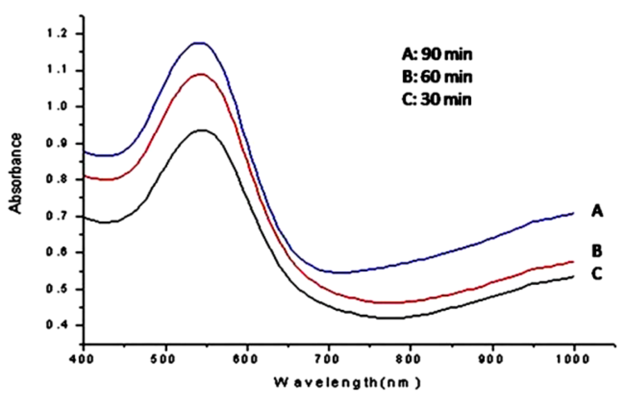


Fig. 2. UV-visible absorption spectra of representative gold nanoparticles synthesized using leaf broth of *Dalbergia sissoo*.

Transmission electron microscopy measurements

After the complete bioreduction of Ag (I) and Au (III) into Ag (0) and Au (0) respectively the solutions were sampled for the TEM observations. TEM samples of aqueous silver and gold nanoparticles were prepared by taking a small drop and putting it on the carbon-coated copper grid and dried at room temperature. The TEM observations were performed on the instrument Morgagni 268(D) (Netherlands), operating at accelerating voltage of 100 kV.

Results and discussion

Qualitative analysis for the formation of silver and gold nanoparticles can easily be followed using spectrophotometer. The excitation of surface plasmon vibrations of silver and gold nanoparticles exhibits yellowish-brown and red wine color, respectively which makes it easy to follow the formation of gold and silver nanoparticles in the aqueous solution [**25**]. In the case of gold nanoparticles the colour of gold solution was pale yellow and after the addition of boiled leaf extract of *Dalbergia sissoo* it transformed into red wine colour within 30 minutes. The reduction continued for 5 hours at 37°C. But, in the case of silver the colorless solution of silver nitrate after the addition of boiled leaf extract of *Dalbergia sissoo* took at least 1 hour to change its colour from colorless to light yellow and further yellow to yellowish–brown but the complete reduction took comparatively more time, i.e., 48 hours at 37°C as compared to that for the complete reduction of gold nanoparticles. The most possible reason could be difference in their redox potential. The maximum absorption for gold nanoparticles was observed at 545 nm (**Fig. 2**). Normally in case of gold nanoparticles, the surface plasmon resonance occurs as a band near about 520 nm. When particles deviate from the spherical geometry, i.e. aggregation of gold nanoparticles in the solution begins to take place, the absorption appears in the long wavelength region as observed in our study. Thus, the formation of anisotropic gold nanoparticles is another explanation for this long wavelength absorption [**26, 27**].

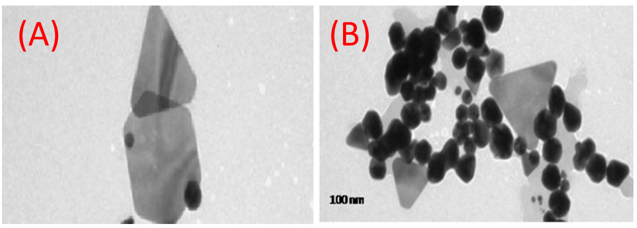


Fig. 3. TEM images (A and B) illustrating the formation of gold nanoparticles biologically synthesized by reduction of $AuCl_4^-$ ions using leaf broth of *Dalbergia sissoo*.

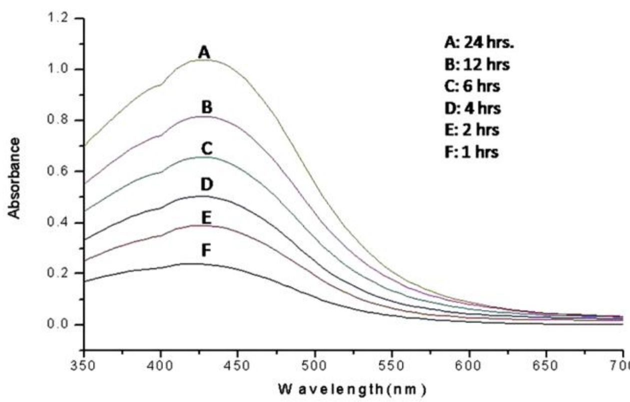


Fig. 4. UV-visible absorption spectra of a representative silver nanoparticles synthesized using leaf broth of *Dalbergia sissoo.*

The TEM images of gold nanoparticles show that they were hexagonal and triangular in nature and very few of them depicted spherical morphology (**Fig. 3**). The triangular structures obtained can be assumed to be mixture of full and truncated triangles as the formation of truncated like triangles is very common phenomena in case of synthesis of gold nanoparticles and has been reported in various chemical based method [**28, 29**]. A careful observation of few strains of gold nanoparticles in the TEM images is indicated the presence of flat, thin and buckle structures in the sample [**30**]. So, TEM analysis clearly indicates the presence of longer wavelength component of UV-visible-NIR spectra for the biologically synthesized gold nanoparticles on account of the formation of highly anisotropic nanostructures of gold. The absorption in the NIR region is very important from the application point of as was reported in the case of optical coating and hyperthermia of cancer cells, since in these applications nanoparticles need to be selectively excited without exciting the other tissue cells [**31**]. In our study variable dimensions of gold nanoparticles were observed. The average edge length of gold nanotriangles was 80 nm, while the hexagons were 50 nm. The silver showed maximum absorption at the 425 nm and they were very different in morphology as compared to gold nanoparticles (**Fig. 4**). The TEM images of silver nanoparticles clearly show regularity in shape, which was spherical in nature and the average diameter of silver nanoparticles observed was 27 nm, but with variation in diameter, which ranged between 5-55 nm (**Fig. 5**).

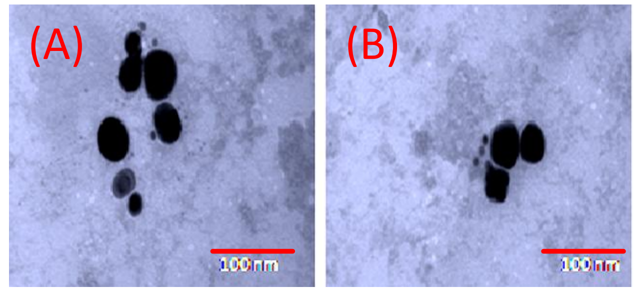


Fig. 5. TEM images (A and B) illustrating the formation of silver nanoparticles biologically synthesized by reduction of Ag^+ ions using leaf broth of *Dalbergia sissoo*.

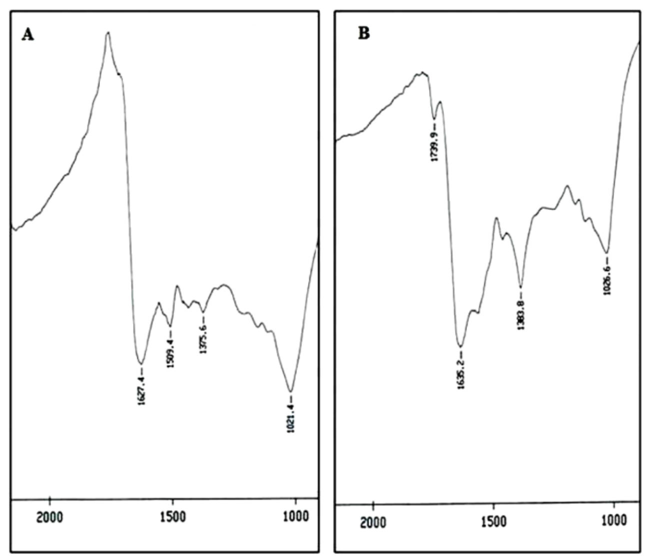


Fig. 6. FTIR absorption spectrum obtained from (A) silver nanoparticles biologically synthesized by reduction of Ag^+ ions and (B) gold nanoparticles biologically synthesized by reduction of $AuCl_4^-$ ions using leaf broth of *Dalbergia sissoo.*

FTIR analysis was performed to identify the biomolecules localized on the surface and responsible for the reduction of silver and gold salts into the respective nanoparticles. Representative FTIR spectra of the synthesized nanoparticles are shown in **Fig. 6**, which reflects many peaks. In case of both silver and gold nanoparticles, spectrum shows peaks centered at the 1739, 1635, 1026, 1383 cm^{-1} in the region of 1000-2000 cm^{-1}. The absorption peak located at 1739 cm^{-1}, can be attributed to the stretching vibrations – C=O [**32**], peaks around 1635 and 1627 cm^{-1} may be due to stretching vibrations of -C=C, peaks 1375 and 1383 cm^{-1} are most probably on account of - N-O functional group. In case of silver nanoparticles, absorption about 1383 cm^{-1} is due to existence of NO_3^- in the residual solution [**33**], two absorption peaks 1026 and 1021 cm^{-1} can be assigned as the absorption peaks of - C-O [**32**]. The presence of various groups like –C=O, -C=C, -C-O etc. can be attributed to heterocyclic water soluble components present in the leaf extract of *Dalbergia sissoo*. This strongly support our hypothesis regarding role of various water soluble heterocyclic compounds such as flavones, Iso-flavones, flavonols, neoflavonols and coumarins present in *Dalbergia sissoo* leaf extract as reducing and capping ligands. In addition to this the presence of oxygen atoms may facilitate the absorption of the heterocyclic components on the surface of the particles in stabilizing the nanoparticles of silver and gold. The change in amplitude of peaks and a small shift was observed in both cases, which may be attributed to the difference in capping species and nature of co-ordination with metal surface for silver and gold nanoparticles.

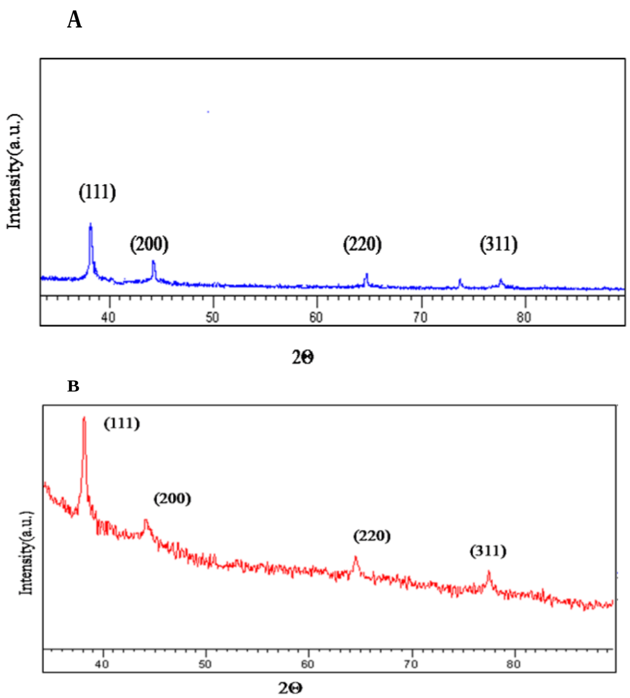


Fig. 7. X-ray diffraction Spectrum of (A) gold and (B) silver nanoparticles synthesized by reduction of $AuCl_4^-$ ions and Ag^+ ions using leaf broth of *Dalbergia sissoo*. Labelled peaks correspond to characteristic diffraction peaks of elemental Au (0) and Ag (0).

The XRD analysis was performed to confirm the crystalline nature of biologically synthesized silver and gold nanoparticles on formation of gold and silver nanoparticles. Various Bragg's reflections are clearly visible in both silver and gold XRD pattern, which are corresponding to the (111), (200), (220) and (311) set of lattice planes (**Fig. 7**). On the basis of these Bragg reflections, we can say that synthesized silver and gold nanoparticles are face centered cubic and essentially crystalline in nature. (200), (220) and (311) set of lattice planes were observed to be very weak and broadened w.r.t. (111) Bragg's reflection, this feature indicates that biologically synthesized nanocrystals are highly anisotropic and nanoparticles are (111) oriented.

Conclusion

Biocompatible and rapid synthesis of silver and gold nanoparticles using the leaf extract of *Dalbergia sissoo* is demonstrated with possible role of different phytochemicals as reducing and stabilizing agent. The present investigation provides a new possibility for synthesis of silver and gold nanoparticles using natural product. In case of gold nanoparticles, special geometrical structures such as triangles and hexagons are obtained having absorption coefficient in the NIR region, which makes it very attractive for its application in photonic devices such as optical sensors and NIR absorbers. Silver nanoparticles were quite different in morphology and size as compared to gold nanoparticles. These rapid time scaled methods for the synthesis of silver and gold nanoparticles using environment friendly natural resources are need to be explored and focused on.

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