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RESEARCH ARTICLE

DIFFERENTIAL POTENTIALITIES OF POD BROTH OF *INDIGOFERA LINIFOLIA* (L.F) RETZ. IN FABRICATING NOBLE METAL NANOPARTICLES

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ABSTRACT

This report presents differential potentialities of pod broth of *Indigofera linifolia* in fabricating silver and gold nanoparticles. The biomolecules present in the pod broth induced the reduction of silver and gold ions from silver nitrate and gold chloride respectively, which resulted in the formation of very stable respective nanoparticles. The UV- Visible (UV-Vis) spectroscopic analysis reveals that the Surface Plasmon Resonance (SPR) vibrations have λ max at 455 and 535nm. These λ max values correspond to the formation of silver and gold nanoparticles respectively. Fourier Transform Infrared (FT-IR) spectroscopic analysis explains that biomolecules in the pod broth are responsible as capping agents for the synthesis and stabilization of silver and gold nanoparticles. X-ray diffraction (XRD) analysis shows the particle nature and size. Energy Dispersive X-Ray (EDX) analysis and Scanning Electron Microscopy (SEM) confirm the significant presence of elemental silver and gold nanoparticles in respective reaction media.

INTRODUCTION

The bio-based protocols for synthesis of nanomaterials are both environmentally and economically green as they are based on green chemistry principles, simple and relatively inexpensive (Sharon *et al*., 2012). Synthesis of noble metal nanoparticles (silver, gold and palladium) for the applications such as electronics, environmental and biotechnology are the areas of constant interests for researchers (Virender *et al*., 2009). Research and development in this field is rising rapidly throughout the world. A major significance is the production of materials in nano meter scale regime. These metal nano particles have high specific surface area and a high fraction of surface atoms which results in their unique physicochemical characteristics (Prasad and Elumalai, 2011). Among them, silver and gold nanoparticles play a vital role in to enhance non-linearity of molecular probes used in imaging of the structures and physiology of nanoscale regions in cellular system (Peleg *et al*., 1996; Singh *et al*., 2012). Several studies suggested that the biomolecules present in the plant material such as protein, phenols, flavanoids play a major role in the synthesis and stabilization of metal nanoparticles (Jagadeesh *et al*., 2004; Collera-Zuniga *et al*., 2005; Vedpriya, 2010). In this sense, we have chosen the pods of *Indigofera linifolia*.

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The seeds of *Indigofera linifolia* are rich in K, Ca, Mn and Cu, while albumins and globulins constitute the major proportion of seed proteins in *Indigofera linifolia* (Siddhuraju *et al*., 1995). Seed lipids of the legume contain a large proportion of unsaturated fatty acids with linoleic acid as the predominant one. In addition to this, these contain an adequate levels of all the essential amino acids except sulpho-amino acids in total seed proteins.

Antinutritional factors such as total free phenolics, tannins, phytic acid, hydrogen cyanide, trypsin inhibitor and phytohaemagglutinating activities were also reported in *Indigofera linifolia* (Siddhuraju *et al*., 1995). Researchers have more interest in the synthesis of silver nanoparticles than gold nanoparticles due to its unique properties such as good conductivity, chemical stability, catalytic and antibacterial activity (Tessier *et al*., 2000; Cao *et al*., 2002; Rosi and Mirkin, 2005; Ahmad *et al*., 2011). There are differential bioreduction behaviours of the higher plants during biogenic synthesis of silver and gold nanoparticles (Boruah *et al*., 2012; Awwad *et al*. 2013; Forough and Farhadi, 2010; He *et al*., 2013; Rodríguez-León *et al*., 2013; Malathi *et al*., 2013; Banerjee *et al*., 2014). Apart from this the silver nanoparticles are also reported to be non toxic to human and most effective to microorganisms at very low concentration and do not cause any side effects in human beings (Jeong *et al*., 2005). Being kept all these in mind, we have aimed to screen the differential potentialities of pod broth of *Indigofera linifolia* in the fabrication of silver and gold nanoparticles.

MATERIALS AND METHODS

The silver nitrate ($AgNO_3$ 99.0%) and Gold (III) chloride trihydrate ($HAuCl_4$ 3H2O, 99.0%) were purchased from Himedia Laboratories Pvt Ltd (Mumbai, India). *Indigofera linifolia* (L.f) Retz. (Fig. 1) belongs to *Fabaceae* and its pods were collected from the campus of Ayya Nadar Janaki Ammal College, Sivakasi, Tamilnadu, India. It is a prostrate annual herb up to 40cm tall, branchlets are two edged with silvery white oppressed hairs. It is commonly seen in scrub and deciduous forest. Flowering from September to December and fruiting from October to January. In India it is found throughout the plains and hills (Pullaiah and Ramamurthy, 2001).

Fig. 1. *Indigofera linifolia* pod

The collected pod samples were thoroughly washed with tap water followed by distilled water to remove the surface contaminants and dried for 24 hours under the shade. The dried pods were taken and ground to make fine powder using a mixer and sieved using 20 mesh sieve to get uniform size range. 10g sieved pod powder was added to 100ml of distilled water and boiled at 70-80° C for 10 minutes to prepare the pod broth (Sathishkumar *et al.,* 2009; Ganesan *et al*., 2013). In our experience the freshly prepared pod broth was more effective in the synthesis of metal nanoparticles than the stored one. For the reduction of silver nitrate in to silver ions, 10ml freshly prepared pod broth was quickly resuspended in 90ml of an aqueous solution of silver nitrate and this is known as Reaction medium for silver nanoparticles (RMSN). Similarly for the bioreduction of gold ions in to gold nanoparticles, 10ml freshly prepared pod broth was quickly added in 90ml of an aqueous solution of gold chloride (Ghosh *et al*., 2011; Prasad and Elumalai, 2011; Singh *et al*., 2012) and this is known as Reaction medium for gold nanoparticles (RMGN). These media were kept in an Incubator cum shaker (Orbitek-Model) with 150 rpm at 36°C for 24 hours. From each of this reaction media a small aliquot of the sample was used to characterize the presence of respective silver or gold nanoparticles synthesized during the above reaction. The characterization was performed through the following analyses: UV-Vis, FT-IR, XRD, SEM and EDX.

RESULTS AND DISCUSSION

We carried out UV-Vis spectroscopic analysis on a Labomed (Model UV- D3200) UV-Visible spectrophotometer. The colourless solution of silver nitrate and pale yellow coloured pod broth brought the RMSN in to pale yellow colour at zero hour reaction. This pale yellow colour of RMSN gradually changed to brown in colour at various intervals of reaction time, 30min, 1h, 3h, 6h and dark brown at 24h (Fig. 2A). It indicates the formation of silver nanoparticles. However, the addition of pale yellow coloured gold chloride solution and pale yellow coloured pod broth turned the RMGN in to ruby red colour at zero hour of reaction. This ruby red colour remains as such for a while and starts to became dark and after 24h it becomes dark ruby red colour, *ie*., the complete reduction of gold chloride (Fig. 2B). However the reduction of gold chloride was found to be faster than the reduction of silver nitrate by the pod broth of *Indigofera linifolia* (Fig. 3).

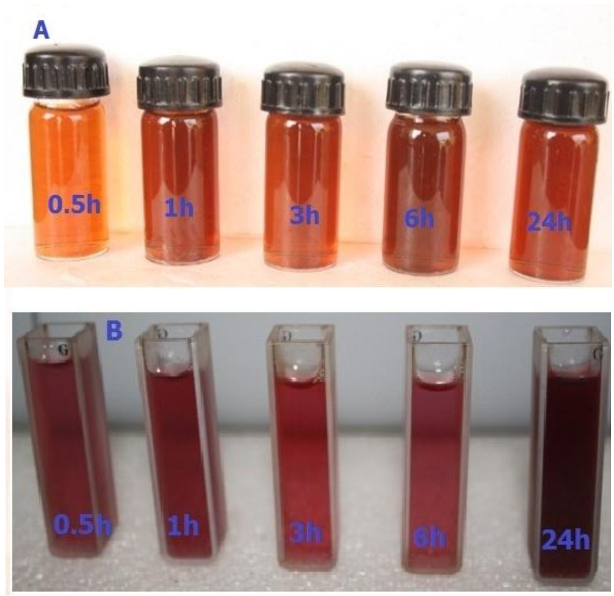

Fig. 2. Colour change of A) RMSN B) RMGN at different time intervals

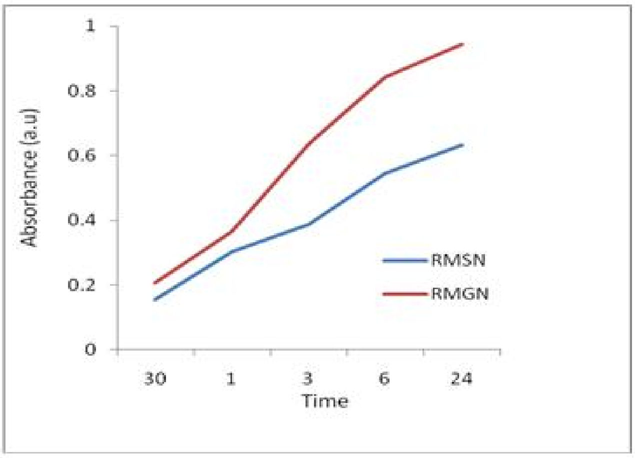

Fig. 3. Spectral plot of absorbance at 455nm (λ max) and 535nm (λ max) of SPR bands shown by RMSN and RMGN respectively as a function of reaction time

The most possible reason for the differential responses in the velocity of reduction in each reaction media could be difference in their redox potential (Singh *et al*., 2012). The pod broth reduced aqueous silver nitrate into silver ions in RMSN and gold chloride into gold ions in RMGN and in turn to silver and gold nanoparticles respectively. The dark brown and ruby red colour of RMSN and RMGN exhibit the vibrations of SPR with λ max 455 and 535nm respectively. These indicate the synthesis of silver and gold nanoparticles through bioreduction of silver nitrate and gold chloride respectively by the pod broth. The excitations of Surface Plasmon Resonanace (SPR) vibrations exhibited by silver and gold nanoparticles were also reported in several cases (Peleg *et al*., 1996; Singh *et al*., 2012). In colloidal state silver nanoparticles are more stable than gold nanoaprticles. In that case silver nanoparticles can withstand to be stable even for three months while gold nanoparticles are found to be stable only for two to three days under room temperature. Gold reaction medium is easily contaminated by pathogenic organisms at room temperature. The possible reason for the long term stability of silver nanoparticles in the reaction medium may be ascribed to the antimicrobial property of silver nanoparticles. Several reports explain the antimicrobial property of silver nanoparticles (Nagaraj *et al*., 2011; Malabadi *et al*., 2012; Umashankari *et al*., 2012).

The isotropic silver nanoparticles could give rise to SPR vibrations with single λ max (Geethalakshmi and Sarada, 2010; Kalyan Kamal *et al*., 2010; Annamalai *et al*., 2011; Kulkarni *et al*., 2011; Sable *et al*., 2012; Nima and Ganesan, 2013; Ganesan *et al*., 2013). The spherical nanoparticles, disks and triangular nanoparticles of silver show one, two and more λ max respectively (Mie, 1908; Mukherjee *et al*., 2002). In the present study, the synthesized silver nanoparticles are of anisotropic (cubical and triangular). Hence the SPR vibrations with two λ max at 325 and 455 nm (Fig. 4) and these may correspond to weak and strong signals. It is clearly justified with the image of SEM (Fig. 5).

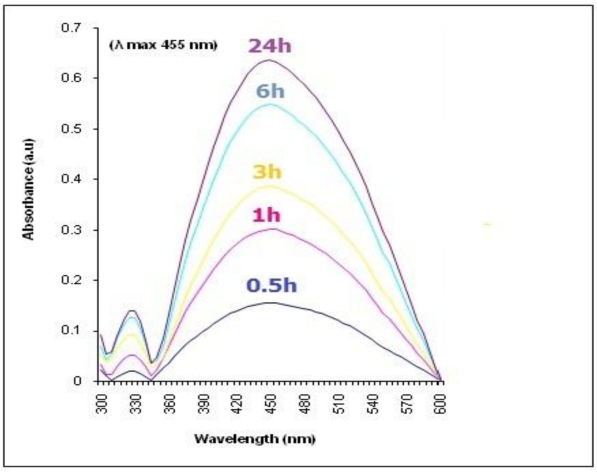

Fig. 4. SPR bands of RMSN at different time intervels

The cubical and triangular structures obtained are supposed to be mixture of full and truncated cubes and triangles of silver nanoparticles similar to the chemical synthesis of gold nanoparticle reported by Shao *et al*., 2004 and Huck *et al*., 2000. Therefore, the SEM analysis clearly indicates highly anisotropic nanostructures of silver due to the presence of two SPR bands in UV-Vis spectrum. The excitation spectrum, as shown in Fig. 4, exhibits SPRs with weak and strong signals of λ max at 325 nm and 455 nm respectively. For the Ag cubes, the induced polarizations lead to two bands that qualitatively match SEM images shown in Fig. 5.

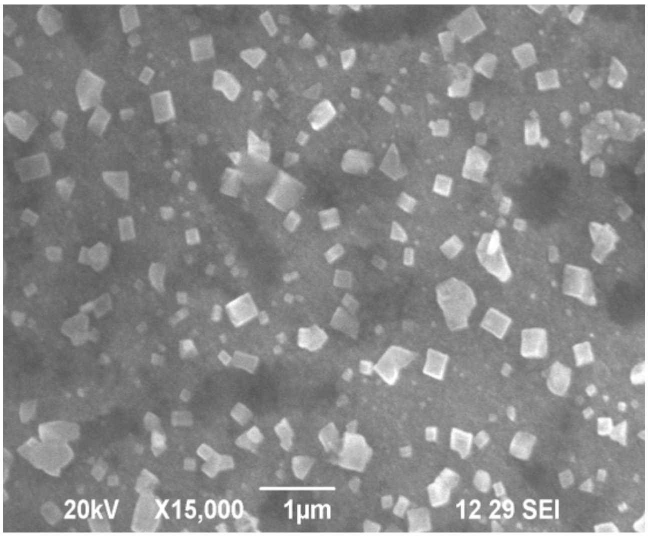

Fig. 5. SEM image of biologically synthesized silver nanoparticle

Analysis of the induced field shows that the 455 nm peak is the in-plane dipole plasmon resonance and the weak 325 nm peak is the out-of-plane dipole resonance. Only the 455 nm peak is found to be very perceptive to the disk thickness and dimensions. These results are almost similar to the report of Hao *et al*., (2004). The gold nanoparticles showed a single SPR vibrations with λ max at 535nm (Fig. 6). It reveals that the gold nanoparticles were almost spherical and isotropic in mophology when compared to anisotropic silver nanoparticles (Fig. 7).

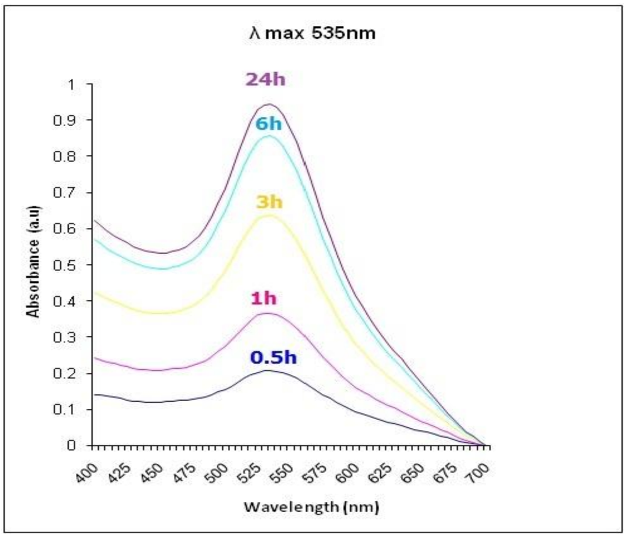

Fig. 6. SPR bands of RMGN at different time intervals

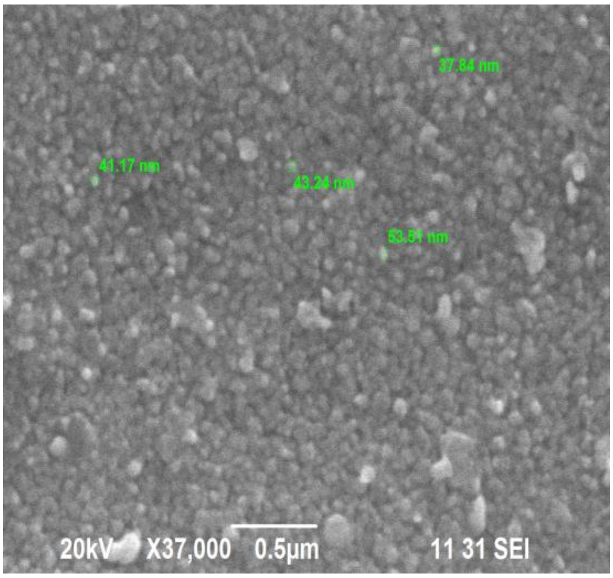

FIg.7. SEM image of biologically synthesized gold nanoparticles

A careful observation of SEM image revealed that the gold nanoparticles had an identical nature with particle size range of about 30-50nm, and there was an aggregated self assembled nanostructures. In the present study we speculate that the biomolecules present in the pod broth may connect the nanoparticles forming an aggregated self assembled nanostructure. This can be justified by various relevant reports by Shankar *et al*. (2004) and Higuchi *et al*. (2007). According to them, the interactions of the gold nanoparticles with uniform sizes may have a lateral driving force thus leads to aggregated or self assembled nano clusters. The aggregation or self assembled nanostructures could be induced by particular biological interactions. The peptides present in the biomaterial bound on the surface of metal nanoparticle could be responsible for connecting the nano particles forming new nanostructures *via* the dipole-dipole interaction pattern. Researchers are focussing an increasing interest in the development of a self-assembled nanostructures by the aggregation of nanoparticles. As in the past reported that these self assembled nanostructures may have new material properties (Shipway *et al*., 2000; Daniel and Astruc, 2004; Ghodake and Lee, 2011).

FT-IR (Shimadzu FTIR spectrophotometer, using KBr pellet method) analysis was performed to identify the biomolecules concentrated on the surface and responsible for the reduction of silver and gold ions into respective nanoparticles. Representative FTIR spectra of the synthesized nanoparticles are shown in Fig. 8 A & B. In case of both silver and gold nanoparticles, spectra show peaks centered at 1670, 1454, 1400, 1336, 1191 and 1122cm^{-1} in the region of 1000-2000 cm^{-1} (Fig 8 A and B). However, the FTIR spectrum of pod broth alone shows peaks centered at 1310, 1406, 1276, 1183 and 1113 cm^{-1} (Fig. 8 C). The absorption peak located at 1670 cm^{-1} can be attributed to the stretching vibrations of C=O, NH_2 bd (Glutamine) (Venyaminov and Kalnin, 1990; Bonwell and Wetzel, 2009; Petla *et al*., 2012). The absorption at 1400cm^{-1} is possibly due to the bending tendency of symmetric CH_3 groups within the acetyl and pyruvyl groups as substituents (Kwon *et al*., 2009). Peaks around 1191and 1122cm^{-1} may be

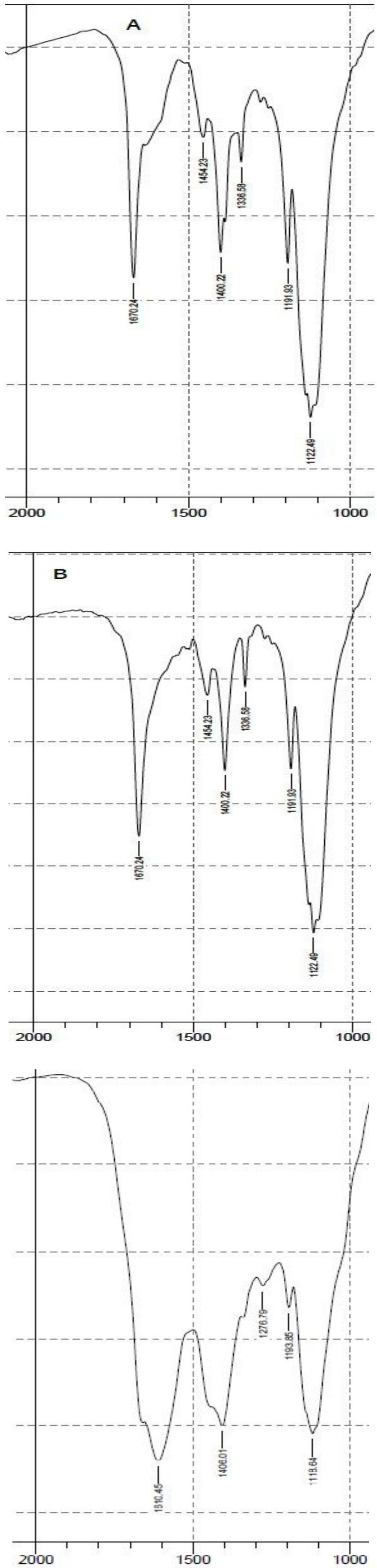

Fig. 8. FTIR spectra of A) RMSN B) RMGN C) Pod Broth alone

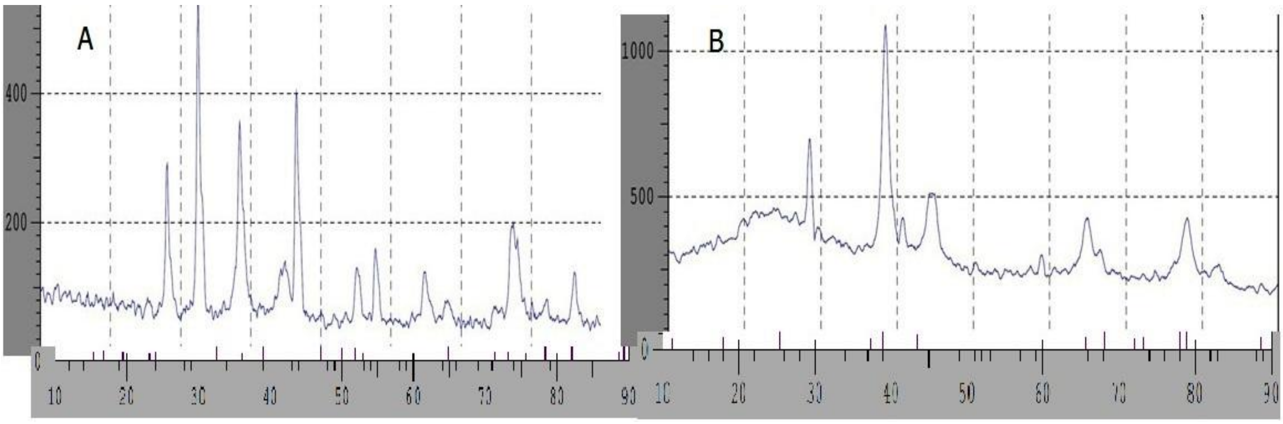

Fig. 9. XRD spectra of A) silver B) gold nanoparticles

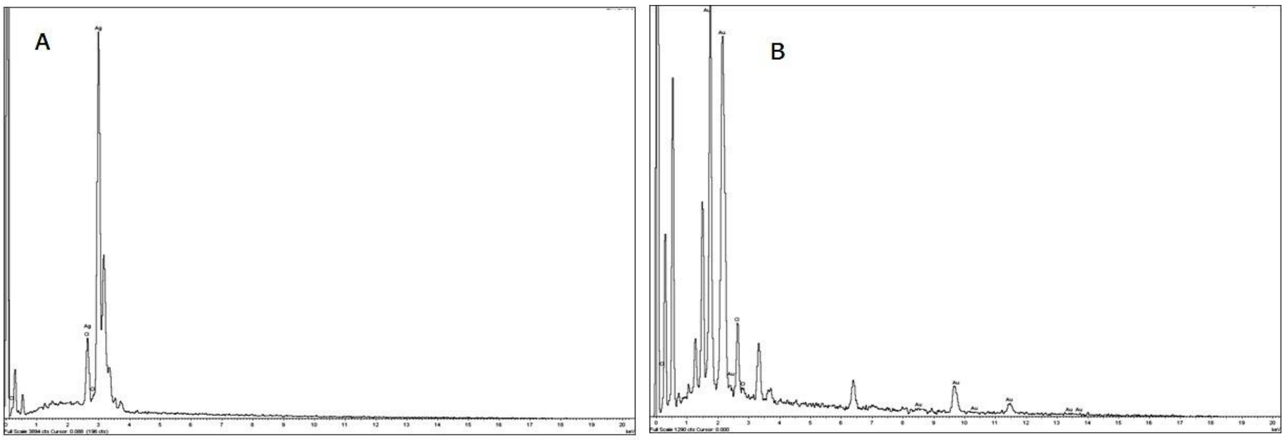

Fig. 10. EDX spectra of A) silver B) gold nanoparticles

due to the C-N stretching vibrations of aliphatic phenols, peak around 1336cm^{-1} is due to C-N stretching for aromatic amines and peak at 1454cm^{-1} may due to germinal methyl functional group (Fazaludeen *et al*., 2012). The appearance and disappearance of the peaks in the spectra may due to the stretching and bending vibrations of carboxyl and methyl groups respectively. Crystalline metallic silver and gold nanoparticles were examined by X-ray diffractometer (Shimadzu XRD 6000)). Fig 9 A & B show the X-ray diffraction spectra of representative silver and gold nanoparticles.

Many Bragg's reflections are visible in the XRD spectrum of silver than the XRD spectrum of gold which are corresponding to the (111), (200), (220) and (311) set of lattice plane. On the basis of these Bragg's reflections, we confirmed the syntheszed silver and gold nanoparticles are Face Centered Cubic (FCC) and crystalline in nature respectively (Ahmad *et al*., 2011; Singh *et al*., 2012). The strong silver and gold peaks in the EDX- spectra (Fig. 10 A & B) confirms the significant presence of elemental silver and gold. Metallic silver nanoparticles generally show typical optical absorption peaks approximately at 3KV due to Surface Plasmon Resonance (Magudapathi *et al.,* 2001). The EDX peak of Ag along with Cl, and O as the mixed components present in the reaction medium. The strong elemental signal along with weak signals that may be originated from the biomolecules bound on the surface of the nanoparticles (Song *et al.,* 2009; Ganesan *et al*., 2013; Nima and Ganesan, 2013).

Conclusion

The differential potentiality of pod broth of *Indigofera linifolia* in the synthesis of silver and gold nanoparticles is established with promising role of carboxyl and methylgroups as reducing and stabilising agent. The present report provides new possibilities for obtaining different sized and shaped silver and gold nanoparticles in an eco friendly manner. In the case of silver nanoparticles we have obtained triangular and cubical shaped particles which make it applicable in the biomedical field for drug loading and sustainable release to the targeted site. Gold nanoparticles were obtained in a fraction of second than silver synthesized. Interestingly we observed that the reduction of gold chloride was found to be faster than the reduction of silver nitrate by the pod broth of *Indigofera linifolia*. The gold nanoparticles were aggregated to form a self assembled nanostructures which may have good material properties for future biomedical applications.

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