



Research article

Synthesis, characterization and antibacterial study of Ag–Au Bi-metallic nanocomposite by bioreduction using piper betle leaf extract



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ABSTRACT

Biological reduction method using plant extract for the synthesis of metal and metal oxides are attracted much to the researchers due to its simplicity, which integrates the chemical technology. The special attention is given to the green synthesis of nanoparticles by easily available plants with eco-friendly system compared to other conventional methods. Silver-gold nanocomposite (Ag–Au NCp's) is synthesized by biological reduction of silver nitrate and gold chloride with biological reduction method. These metal salts are simultaneously reduced by betle leaf extract to form respective silver and gold nanocomposite. The structure and morphology of as prepared Ag–Au NCp's sample was characterized by employing powder X-ray diffraction (XRD) tool and by Scanning Electron Micrograph (SEM) tool respectively. Fourier Transform infrared (FTIR) spectral study was undertaken to know the bonding in the prepared silver sample. Energy dispersive X-ray analysis (EDX) study was undertaken to know the formation Ag–Au NCp's. Antibacterial studies are undertaken for the said nanocomposite to know its activity against bacteria.

1. Introduction

Among various technology, nanotechnology is emerging technology because of its new properties and applications [1, 2]. Next generation technology will be built by tailoring the blocks of many applications. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties they are gaining the interest of scientist for their novel methods of synthesis [3, 4]. Nanomaterials prepared by various plant extraction technique shows enhanced unique properties and applications with proper manipulation compared to its bulk materials [5, 6, 7]. Research on synthesis of metal nanoparticles at by biological route especially by use of plant extract is developing in rapid way because of its simple experimentation. Biological method for the materials synthesis at nanoscale is in accordance to eco-friendly system which avoids the burning with fuel. Currently, there is a growing need to develop environmentally benevolent nanoparticles synthesis processes that do not use toxic chemicals in the synthesis protocol [8, 9, 10]. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing

interest in biological approaches which are free from the use of toxic chemicals as by products. Thus, there is an increasing demand for green nanotechnology [11, 12]. Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants. Synthesis of nanoparticles using plant extracts is the most adopted method of green, eco-friendly production of nanoparticles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites [13, 14]. Silver and gold nanoparticle prepared by biological method has attracted considerable interest due to their extensive applicability in various areas such as drug delivery system, electronics, chemical engineering, energy efficient system and also in the field of medicine [15, 16, 17]. The rapidly developing field of nanotechnology will result inexpensive nanoparticles to humans via several routes (e.g., inhalation, ingestion, skin, etc.). Nanoparticles can translocate from the route of exposure to other vital organs in human body and penetrate cells. Toxicity studies to determine the deleterious effects of nanoparticles on living cells are required. Many techniques of synthesizing silver nanoparticles, such as chemical reduction of silver ions in aqueous solutions with or without stabilizing agents are in literature [18, 19]. Sometimes the synthesis of nanoparticles using

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Fig. 1. (a): Piper Betle leaf. (b): Piper Betle leaf extract.

plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures [20, 21]. Many recent records shows that, the use of various plant leaves for the synthesis of metals in particular AgNCp's and metal oxides with different routes [22]. Researchers concentrated insitu method for the synthesis of nano sized metals and metal oxides integrate the synthetic technology. In addition to this, the prepared method is known for its simplicity and enhanced properties as well as applications [23, 24, 25].

Piper betle leaves are extensively grown in India and are widely used as a post meal mouth freshener. Due to strong pungent aromatic flavour, betel leaves are used as masticators by the Asian people [22, 26]. Production of nanoparticles can be achieved mainly through three methods such as, chemical, physical and biological. Since noble metal such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, a large variety of possible biomedical applications have been examined [27, 28].

The literature survey review the different metals and metal oxide nanoparticle are prepared by biological route successfully, but bimetallic nanocomposite particles by green synthesis route are reported very less. Here is an attempt for the synthesis of Ag–Au NCp's material by reduction of silver nitrate and goldchloride using piper betle leaf extract. Simultaneous in situ bio reduction method was employed for synthesis Ag–Au NCp's material. The prepared sample is well characterized for its structure by X-ray diffraction (XRD), morphology by Scanning Electron Microscope (SEM) and bonding by Fourier Transform Infrared study (FT-IR) techniques. EDX analysis is carried out for the prepared sample to know the formation of Ag–Au NCp's. In addition to this antibacterial activity of the sample was undertaken.

2. Experimental

2.1. Materials and methods

The reagents used were of analytical grade obtained from Merck (Mumbai, India). Piper betle leaf extract, silver nitrate and gold chloride solutions were prepared in distilled water. All glass wares are properly rinsed with chromic acid followed by distilled water and dried. Biological reduction of silver nitrate and gold chloride for its nanocomposite by piper betle leaf extract is carried out at room temperature. Agar diffusion method was used for antibacterial activity study.

2.2. Preparation of piper betle leaf extract

Fresh piper betle leaves were collected from the piper betle leaf field (shown in Fig. 1) and washed thoroughly with distilled water. 10 grams of piper betle leaves were cut in to small pieces and put it into conical flask containing 100ml double distilled water. The content is boiled for about 15 min and cooled to room temperature. This solution was filtered through whatmann filter paper no 40 for the aqueous leaf extract.

2.3. Synthesis of Ag–Au NCp's

1 mM aqueous solutions of silver nitrate and gold chloride are prepared in distilled water. These two solutions are equally mixed to form Ag–Au salt solution. 10 ml of above prepared piper betle leaf extract was added to 100 ml of 1mM aqueous salt solution taken in conical flask. This reaction mixture was irradiated with microwaves in microwave oven having 2.45 GHz frequency and power is 800 w for about 15 min and is kept at room temperature for 24 h with suitable closing system.

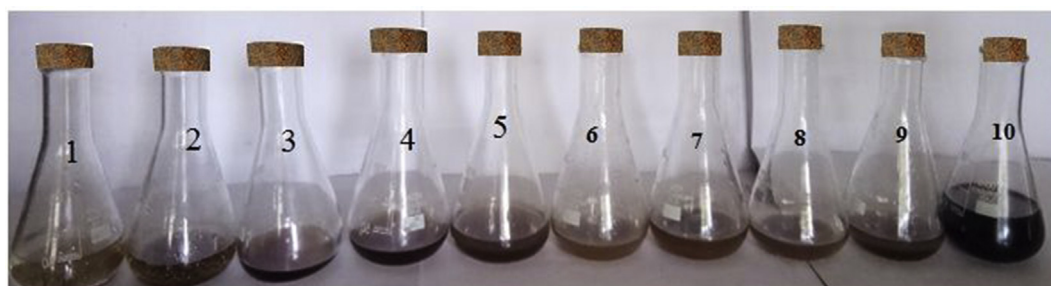


Fig. 2. Sequential colour change from clear solution to brown.

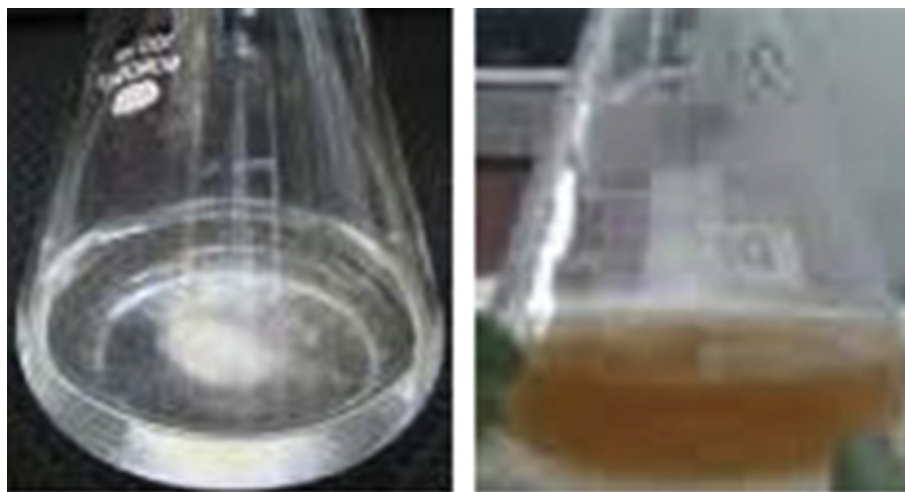


Fig. 3. Colour of Ag–Au salt solution and Ag–Au NCP's.

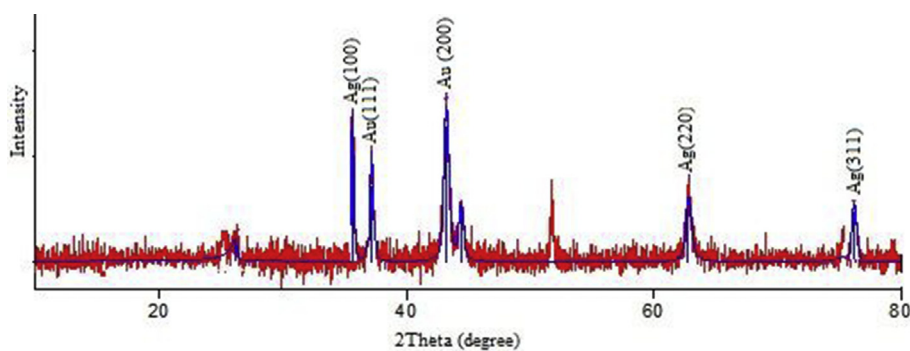


Fig. 4. XRD pattern of Ag–Au nanocomposite.

Sequential colour change from clear solution to dark brown (as shown in Fig. 2) is observed due to start of reduction of metal salts for Ag–Au NCP's. Fig. 3 shows comparison of clear Ag–Au salt solution with completely formed respective composite product. The sample thus formed was centrifuged at 500 rpm for about 10 min followed by re-dispersion in 10 ml of distilled water. For instance, additional experiments were carried out by the addition of 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml plant extract solution in Ag–Au salt solution. The concentration of Ag and Au salt solution is maintaining constant and only variation in extract concentration. Reduction of Ag^+ to Ag^0 , Au^{++} to Au^0 and Ag–Au NCP's was confirmed by the colour change of solution from colourless to brown. Fig. 2 clearly shows the varied brown colour concentration indicating the incomplete reduction in less concentration of

the plant extract solution and complete dark brown colour formation due to the complete reduction reaction. Ag and Au from its salt solution converts in to Ag–Au NCP's at required concentration of the plant extract. As concentration of the plant extract increases, the reaction proceeds towards completion of reduction reaction. Minimum and optimal concentration of the plant extract roles the reaction process.

2.4. Biological studies

2.4.1. Antibacterial study

Agar diffusion method was under taken for antibacterial study of Ag–Au NCP's against the strains *B. Subtilis* and *K. Planticola*. Strains was grown overnight in a single column system in LB broth medium on a rotary shaker at 30 °C. The same is kept for 24 h for incubation in sophisticated incubator. A loop full of bacterial culture was placed on the Muller Hinton broth medium to know the bacterial effect of Ag–Au NCP's. The zones of inhibitions were measured after 24 h at the same conditions [29].

2.5. Characterization

The structures of as prepared Ag–Au NCP's were studied by X–ray diffraction using X' Pert Pro X-ray diffractometer with Cu K α as source of radiation in a θ -2 θ configuration. Morphology and bonding of the above oxide was studied by Phillips XL 30 ESEM and Perkin–Elmer 1600 spectrophotometer in KBr medium tools respectively. The UV visible spectro photo metric measurements were performed on Elico spectrophotometer. JEOL JSM-6380 LA Scanning electron microscope with energy dispersive micro analysis of X-Ray (EDAX) is used to study particle morphology with metal confirmation of the sample.

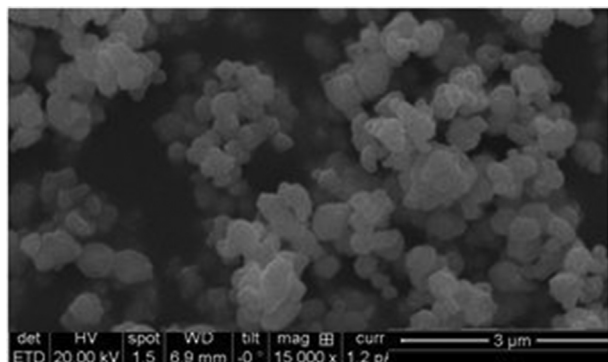


Fig. 5. SEM image of Ag–Au nanocomposite.

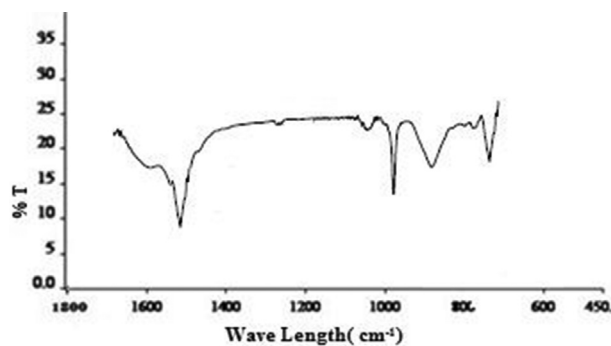


Fig. 6. FT-IR spectra of Ag-Au NCp's.

3. Results and discussion

3.1. X-ray diffraction

Fig. 4 shows indexed XRD pattern of as prepared Ag-Au NCp's sample. The pattern shows the presence of Bragg's reflections due to crystalline nature of the composite. Peaks assigned to the diffraction signals (100), (220 and (311) facets of silver where as signals (111) and (200) facets of gold. d-spacing values of both Ag (JCPDS file 87-0720) and Au (JCPDS file 04-0784) are observed in the pattern. Unit cell parameters were identified by least-square refinement of the XRD data. Presence of both Ag and Au peaks in a single pattern indicates the formation of Ag-Au NCp's and is supported by EDX study explained in the later stage.

3.2. Scanning electron microscopy (SEM)

The surface morphology and size of the particles of the as prepared Ag-Au NCp's was studied by scanning electron micrograph image. Fig. 5 shows SEM image of Ag-Au NCp's sample prepared by piper betle leaf extract. This image shows the clustered crystalline nature of the composite with fine spherical particles. Most of the particles are self-assembled with close compact arrangement [30]. In addition to this, the electrostatic interaction between Ag and Au particle are also observed in the image.

3.3. Infrared study

The bonding nature of the Ag-Au NCp's was studied by Infrared study tool. The centrifuged and dispersed Ag-Au nanocomposite obtained has removed any free residual biomass. Subsequently, the dried powder was obtained by lyophilizing the purified suspension. The resulting lyophilized powder was examined by Infrared tool. Fig. 6 shows FT-IR spectra of Ag-Au nanocomposite. The spectrum shows that, the peak at around 1520 cm^{-1} due to carbonyl stretching and N-H stretch vibrations from amide linkage. Vibrational band at around 1500 cm^{-1} is due to vibrations of aromatic hydrocarbon ring. IR bands at 990 cm^{-1} corresponds to C-O stretching frequency. The shoulders around the band can be specified as overtones and the frequency of the O-H band [31, 32].

3.4. EDX analysis

To know the presence of silver and gold in the synthesized Ag-Au nanocomposite, the analysis of the sample was performed by EDX technique. The Fig. 7 shows EDX spectrum of as synthesized Ag-Au NCp's.

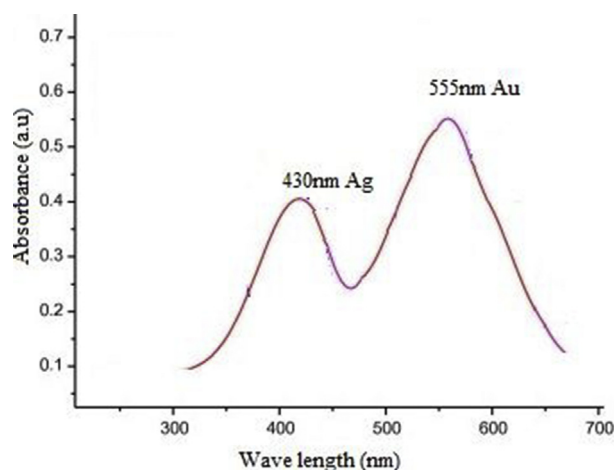


Fig. 8. UV spectrum of Ag-Au NCp's.

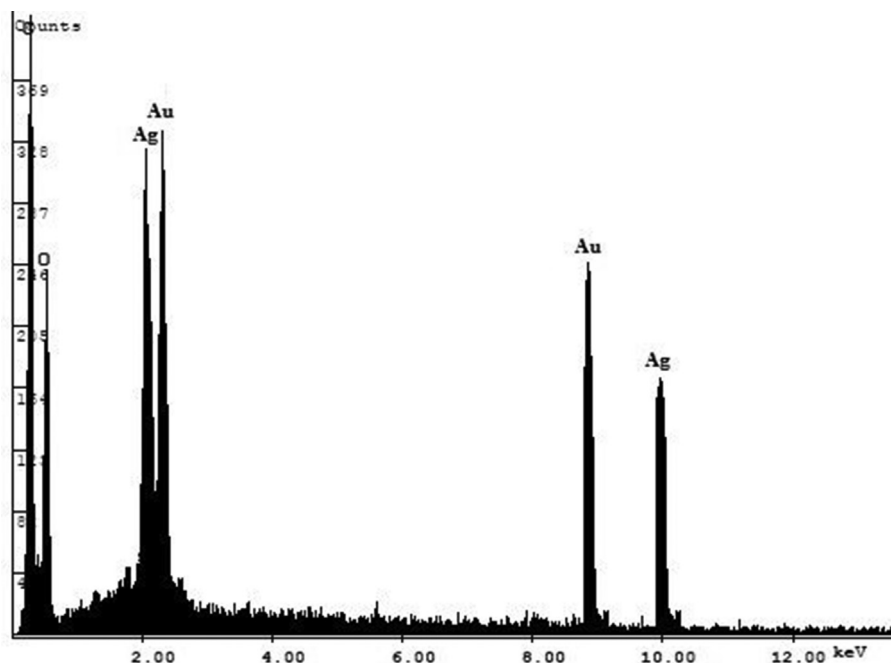


Fig. 7. EDX pattern of as synthesized Ag-Au NCp's.

Table 1

Antibacterial activity of Ag–Au nanocomposite synthesised by piper betle leaf extract.

Sl. No	Concentration of Ag–Au NCP's (μl)	Zone of Inhibition (mm)	
		<i>K. Planticola</i>	<i>B. subtilis</i>
1	10	6	8
2	20	8	9.5
3	30	9	11
4	40	11	13
5	50	13	14

This pattern shows the strong signals of both Ag and Au atom. These characteristic signals at appropriate keV of Ag and Au confirm the formation of Ag–Au NCP's.

3.5. UV-visible study

Fig. 8 shows UV spectrum of Ag–Au NCP's prepared by bioreduction method. The broad surface plasmon resonance band at 430 nm indicates the presence of silver nanoparticles due to excitation of longitudinal plasmon vibration. In addition to this, the figure shows the absorption peak at 555 nm may be assigned due to gold nanoparticle are large and polydispersed. The shifting towards red side indicates the formation of colloidal gel solution containing both Ag and Au. Wavelengths of Ag and Au are distinct and are separated indicating the Ag & Au particles in the solution are formed mostly aggregates and found to be stable in solution as suspension.

3.6. Biological study

The observed zone of inhibition of the green synthesised Ag–Au NCP's for different strains are given in the Table 1. Different concentrations of Ag–Au NCP's like 10, 20, 30, 40 and 50 μl were taken in different cones. The summary of the zone of inhibition of the sample from Table 1, it is clear that the prepared sample show antibacterial drugs activity against the both *Bacillus subtilis* and *Klebsiella Planticola* strains. On comparison of zone of inhibition of both the strains, it is clear that Ag–Au NCP's show higher activity against *Bacillus subtilis* strain.

4. Conclusions

Biosynthesis of Ag–Au NCP's using betle leaf extract is simple, efficient, eco-friendly method and can be used for the synthesis of other metal nanocomposite materials. This method is one of the economic viability methods for synthesis of nanoparticles. Reduction of silver and gold ions takes place simultaneously. Further detailed characterisation, properties and applications of this nanocomposite is future direction of our work.

Declarations

Author contribution statement

Arunkumar Lagashetty: Conceived and designed the experiments; Performed the experiments.

Sangappa K Ganiger: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shashidhar Reddy: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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