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GREEN SYNTHESIS OF GOLD NANOPARTICLES USING *CASSIA ALATA* LEAF EXTRACT AND EVALUATION OF ANTIMICROBIAL ACTIVITIES

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ABSTRACT

In present study gold nanoparticles with average particle size of 12.3 nm was synthesized by an easy, simple and ecofriendly method using leaf extract of *Cassia alata*. The synthesis of gold nanoparticles was primarily confirmed by visible colour change of reaction mixture from yellowish to purple /wine red colour and intense peak at λ-max 539 nm due to surface plasmon resonance arising by the collective oscillation of conduction electron on the particle surface. Further the synthesized particles were characterized for structure, size, and possible biomolecules responsible for synthesis, elemental composition and presence of elemental gold by XRD, SEM, FTIR and EDAX respectively. Synthesized gold nanoparticles were proved to be effective against pathogenic bacteria and phyto-pathogenic fungi. *Bacillus subtilis* and *Escherichia coli* both found to be more susceptible to gold nanoparticles with 13.33 mm of inhibition. Phytopathogenic *Aspergillus flavus* was found to be more susceptible with 53.11% inhibition followed by *Didymella bryoniae* (50.07 % inhibition) and *Fusarium oxysporum* (41.92 % inhibition) respectively.

KEYWORDS: Green synthesis, gold, nanoparticles, *Cassia alata*, antimicrobial activity.

INTRODUCTION

The extensive application of nanoparticles revitalize the need for synthesizing the metal nanoparticles, the conventional and long-established chemical and physical methods are usually hazardous and energy consuming[1] (Mishra & Sharma, 2015) which resulted the need

to the develop green process for the synthesis of nanoparticles[2] (Raveendran *et al*., 2006) called green synthesis, evolving into an important branch of nanotechnology.

The field of nanobiotechnology combines the biological principles with physical and chemical procedure to generate nano-sized particle with specific functions is emerging as a rapidly growing field with its application in science and technology. The use of biological materials like bacteria, fungi and plant extracts for the synthesis of metal nanoparticles provides numerous benefits of eco-friendliness and compatibility for biomedical and pharmaceutical applications as this green chemistry approach does not use toxic chemicals in the synthesis protocol[3] (Singh *et al,* 2012). The water soluble plant metabolites like alkaloids, phenolic compounds, terpenoids and co-enzymes present in the biological materials may acts as reducing as well as capping agents during the synthesis of nanoparticles[4] (Mittal *et al*., 2013).

Nanomaterials, especially gold nanoparticles (AuNPs) having unique physico-chemical properties like small size, surface area to mass ratio, and high surface reactivity, presence of surface plasmon resonance (SPR) bands, biocompatibility[5] (Patra *et al.,* 2010) and antimicrobial potentiality, found application in drug and gene delivery[6][7] (Ghosh et al., 2008 & Brown *et al.,* 2010) cancer diagnosis, photothermal therapy and as biosensors in living cells[8] (Huang & El-Sayed, 2010). *Cassia alata* Linn. is a well known plant for its diverse medicinal uses in Indian Systems of Medicine used as vermicide, astringent, purgative, expectorant and to treat skin diseases[9] (Mohideen *et al*., 2005) and also found to exhibit a greater antifungal activity against some human pathogenic fungi justifies the use of this plant in traditional systems of medicine to manage fungal diseases[10] (Timothy *et al*., 2012).

Since the important application of gold nanoparticles as well as the potential involving of plant extract instead of chemicals as reductive factors to synthesize nanoparticles has gain momentum, the study on green synthesis of gold nanoparticles using *Cassia alata* leaf extract and evaluation of their anti-microbial activities had been done to answer questions, if the *Cassia alata* leaf extract can be used for bio-synthesis of gold nanoparticles, similar to its success with silver[11] (Gaddam *et al.,* 2014) and to study the inhibitory effect of synthesized gold nanoparticles against some plant pathogenic fungi and human pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *C. alata* were collected from the medicinal garden (Chandravana) maintained by Mysore Ayurveda College, Mysuru, Karnataka State, India.

Preparation of Leaf extract

Ten grams of fresh leaves were washed repeatedly with tap water to remove the surface dust particles and then with triple distilled water. Leaves were cut into small pieces and boiled in 100ml of triple distilled water for 5-10 minutes, cooled, filtered with muslin cloth and then with Whatman No. 1 filter paper. Obtained extract was stored at 4°C for further use.

Synthesis of gold nanoparticles

For the synthesis of nanoparticles, gold chloride (Chloroauric acid) solution of 1 mM concentration was treated with leaf extract in different proportion viz. 1:1, 3:1 and 9:1 ratio to standardize the metal precursor and extract ratio and the reaction mixture was kept in dark condition at room temperature. Change of colour from yellowish to purple/wine colour indicates the formation of gold nanoparticles. After the completion of reaction the solution was centrifuged at 8000 rpm for 20 minutes, the obtained gold nanoparticles were dried and stored for further characterization.

Characterization of synthesized gold nanoparticles

UV-visible spectroscopy: The bio-reduced sample was studied for the formation of gold nanoparticles by UV-visible spectroscopic using UV-Vis spectrophotometer Beckman Coulter DU730. Sample was taken in a quartz cuvette and optical density was observed at the wavelengths ranged between 300-700nm and results were tabulated.

XRD analysis: X-Ray Diffraction (XRD) study of synthesized gold nanoparticles was carried out to determine the crystalline nature using Rigaku Destop Miniflex II X-Ray Diffractometer with 2θ angle 10 to 80°. Further, the size of nanoparticles was calculated using Debye-Scherrer formula[12] (Narayanan & Sakthivel, 2010).

$$D=K\lambda/\beta\cos\theta$$

Where; D: Particles size (diameter)

K: Scherrer coustant (K= 0.89)

λ: Wavelength of Xray (0.15406 nm)

β: Full width at half maximum (FWHM) in radians

θ: diffraction angle (Bragg's angle (2θ)

Fourier Transform Infra-red Spectroscopy (FTIR): The Fourier transforms infrared (FTIR) spectroscopy was used for analysis of bio-reducing agent present in the leaf extract as biomolecules responsible for the reduction of the gold ions and capping of the bio-reducer gold nanoparticles synthesis. The FTIR investigations were carried out using PerkinElmer Spectrum Two Spectrophotometer in the range from 600 to 4000 cm^{-1}.

Scanning Electron Microscopy and EDAX analysis: The morphological characteristics of synthesized gold nanoparticles were studied using Hitachi S-3400N Scanning Electron Microscope. Elemental composition and the presence of elemental gold were studied by Energy Dispersive X-ray Spectroscopy (EDAX).

Antibacterial and antifungal activities of synthesized gold nanoparticles

Antibacterial and antifungal activities were carried out by agar well diffusion method.[13,14] Bacterial species *Bacillus subtilis*, *Escherichia coli* and fungal pathogens *Aspergillus flavus*, *Fusarium oxysporum* and *Didymella bryoniae* were procured from Department of Studies in Botany, University of Mysore, Mysuru. For antibacterial studies 100µl of overnight bacterial inoculums were spread over plate containing solidified nutrient agar media and 8mm wells were punched aseptically by sterile cork borer. Then the wells were loaded with 100µl of 1mg/ml gold nanoparticles, plant extract, gold chloride, DMSO and standard antibiotic (Streptomycin 1mg/ml). The standard antibiotic and DMSO served as positive and negative control respectively. The plates were incubated at room temperature for 24 hours and zone of inhibition was measured in mm. Similar method was followed for antifungal activity using PDA media, where standard fungicide (Fenamidone 10% + Mancozeb 50%**)** with commercial name Sectin and DMSO was used as positive and negative control respectively. In this case, plates were incubated at room temperature for 3-4 days and zone of inhibition was measured in mm. All tests were carried out in triplicate.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at $P < 0.05$ level of significance using graphpad prism 5 software.

RESULTS AND DISCUSSION

Visual and UV- spectroscopic analysis

When gold chloride (Chloroauric acid) solution of 1 mM concentration was treated with leaf extract in different proportion namely; 1:1, 3:1 and 9:1, the reaction mixtures changed its colour (Fig.1A & B) within 1 hour of incubation period. Change of colour from yellowish to purple/wine red colour of reaction mixture with 9:1 ratio indicated the formation of gold nanoparticles. Purple colour and maximum absorbance at 539 nm in UV-spectroscopic analysis (Fig. 2A & B) is due to surface Plasmon resonance (SPR) of surface electrons on the gold nanoparticles (Kumar *et al.,* 2016).[15]

X-Ray Diffraction Studies

X-ray diffraction spectra of synthesized gold nanoparticles (Fig. 3) showed two intense peaks at 2θ angles 38.05º and 44.34º which corresponds to (111) and (200) lattice plane and can be indexed as face centered cubic structures of gold which corroborates the earlier results Jayaseelan *et al.,* (2013)[16] and Parida *et al*., (2011).[17] The mean size of synthesized gold nanoparticles was calculated using Debye–Scherrer's equation and found to be 12.3 nm (Narayanan & Sakthivel, 2010).[12]

Fourier Transform Infra-red Spectroscopy (FTIR)

The FTIR analysis result showed the peaks at 3232cm^{-1} corresponds to the OH stretch of hydroxyl groups and 2324cm^{-1} corresponds to P-H stretch of Phosphines (Fig. 4). Peaks at 2114cm^{-1} and 1014 cm^{-1} correspond to the C≡C of terminal alkynes and C-C vibration /C-F stretch of fluoro compound respectively. The peaks observed at 760cm^{-1} and 717cm^{-1} indicates the presence of CH out of plane of benzene and Ar-OH group in phenols. The peaks at 675cm^{-1} and 655cm^{-1} corresponds to the C-O-H bending in alcohols (Coates, 2000; Lambert, 1987).[18,19] The results showed that the different functional groups in water soluble phytochemicals present in the leaf extract may involve in the reduction of the metal precursor and also acts as stabilizing agent in the synthesis of nanoparticles.

Scanning Electron Microscopy and EDX analysis

The Scanning electron microscopic image clearly showed that the particles were monodispersed and spherical in shape with average particle size of 12.3 nm (Fig. 5). Further, the EDX spectrum of synthesized gold nanoparticles showed strong signal of gold particles at approximately 2.3 Kev with 80% of atomic gold component (Fig. 6). Along with gold other signal for carbon and oxygen were also obtained which may be due to the biomolecules and

capping agents attached to the gold nanoparticles which corroborates earlier reports (Milaneze *et al*., 2016 and Isaac *et al.*, 2013).[20,21]

Antibacterial activity

Antibacterial activity was carried using the synthesized gold nanoparticles against *Bacillus subtilis* and *Escherichia coli* in comparison to standard antibiotic streptomycin. Both bacterial pathogens *Bacillus subtilis and Escherichia coli* were found to be more susceptible to synthesized gold nanoparticle with inhibition zone of 13.33mm. However, the antibiotic did not show any inhibition against *Bacillus subtilis* even with repeated experiments, but was effective against *Escherichia coli* with 13.33mm inhibition. Nonetheless, 13.33mm was considered the maximum inhibition of antibiotic against *Escherichia coli* as standard zone of inhibition to calculate percentage inhibition. DMSO has shown least inhibition (17.48%) against *Bacillus subtilis*, whereas DMSO, plant extract and gold chloride has not shown any zone of inhibition against both bacteria (Table-1). The results clearly indicated that the synthesized gold nanoparticles were on par with standard antibiotic in significantly ($P<0.05$) inhibiting the growth of the pathogen.

Antifungal activity

Antifungal activity was carried using the synthesized gold nanoparticles showed that, all the tested fungal phytopathogens were susceptible to gold nanoparticles synsthesized using aqueous leaf extract of *C. alata.* However, none of the synthesized nanoparticles surpassed the fungicide used in the present study in inhibiting the growth of the pathogen. Nonetheless, synthesized gold nanoparticles proved to be more effective against the *Aspergillus flavus* with percent inhibition of 53.11% (11.33mm) followed by *Didymella bryoniae* 50.07% (10.66mm) and *Fusarium oxysporum* 41.92% (8.66mm) compared to standard fungicide. DMSO has shown 10.92 % and 6.33% of inhibition against *Aspergillus flavus* and *Didymella bryoniae* respectively, but did not show any inhibition against *Fusarium oxysporum*. While aqueous plant extract and gold chloride shown 0% of inhibition (Table-2). The results of antifungal activities clearly showed that the synthesized gold nanoparticles were not on par with standard fungicides in inhibiting the growth of the pathogen.

Table 1: Antibacterial activity of synthesized gold nanoparticles.

Treatments	*Bacillus subtilis*		*Escherichia coli*	
	Inhibition zone in mm	% of inhibition	Inhibition zone in mm	% of inhibition
Plant extract	0.00 ± 0.00*	0.00	0.00 ± 0.00*	0.00
Gold Chloride	0.00 ± 0.00*	0.00	0.00 ± 0.00*	0.00
DMSO	2.33 ± 0.33**	17.48	0.00 ± 0.00*	0.00
Standard antibiotic	0.00 ± 0.00*	0.00	13.33 ± 0.33***	100.0
Synthesized Gold Nanoparticles	13.33 ± 0.33***	100.0	13.33 ± 0.33***	100.0

Results are mean of 3 replicates. The data were analysed using Post Hoc Tukey test. P<0.05. No. of *indicates the level of significance

Table 2: Antifungal activity of synthesized gold nanoparticles.

Treatments	*Aspergillus flavus*		*Fusarium oxysporum*		*Didymella bryoniae*	
	Zone of inhibition (in mm)	% of inhibition	Zone of inhibition (in mm)	% of inhibition	Zone of inhibition (in mm)	% of inhibition
Plant extract	0.00 ± 0.00*	0.00	0.00 ± 0.00*	0.00	0.00 ± 0.00*	0.00
Gold Chloride	0.00 ± 0.00*	0.00	0.00 ± 0.00*	0.00	0.00 ± 0.00*	0.00
DMSO	2.33 ± 0.33*	10.92	0.00 ± 0.00*	0.00	1.33 ± 0.88*	6.33
Fungicide	21.33 ± 0.88***	100.0	20.66 ± 0.33***	100.0	21.00 ± 0.57***	100.0
Synthesized Gold Nanoparticles	11.33 ± 0.66**	53.11	8.66 ± 0.33**	41.92	10.66 ± 0.33**	50.07

Results are mean of 3 replicates. The data were analysed using Post Hoc Tukey test. P<0.05. No. of *indicates the level of significance.

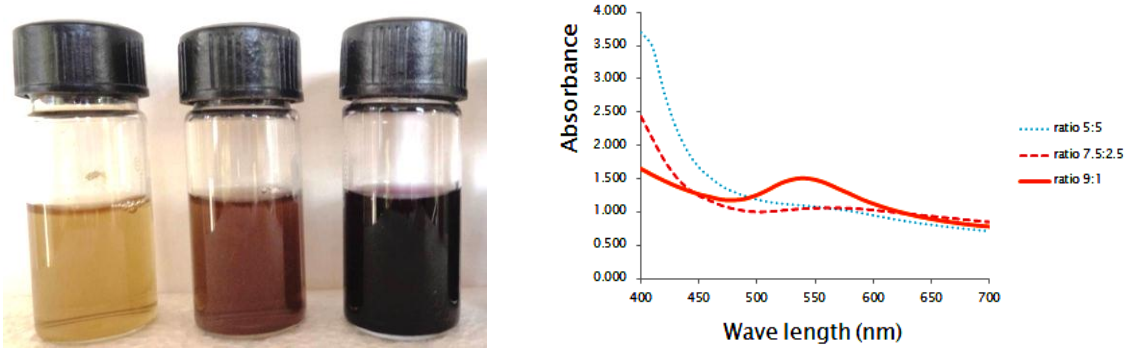

Fig. 1: (A) - Reaction mixtures with different ratio 1:1, 3:1 and 9:1 respectively. (B) - UV- absorption spectrum of reactions mixture.

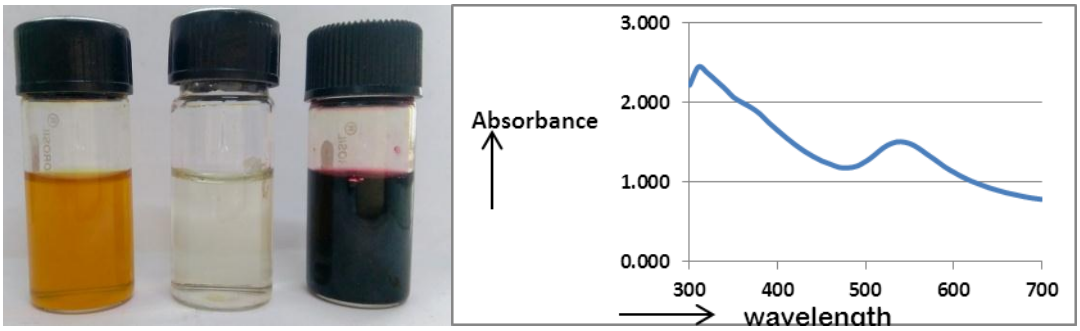

Fig. 2: (A) - Plant extract, gold chloride and reaction mixture with 9:1 ratio respectively. (B) - UV- absorption spectrum of reactions mixture with 9:1 ratio.

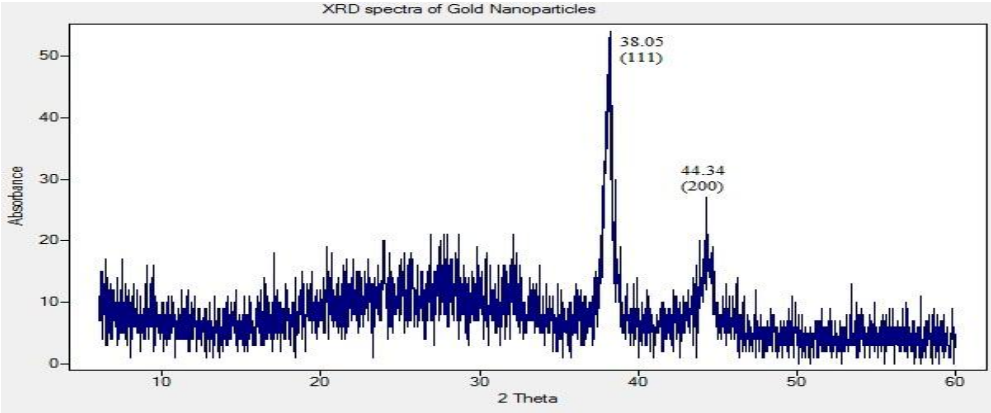

Fig. 3: XRD spectrum of synthesized gold nanoparticles.

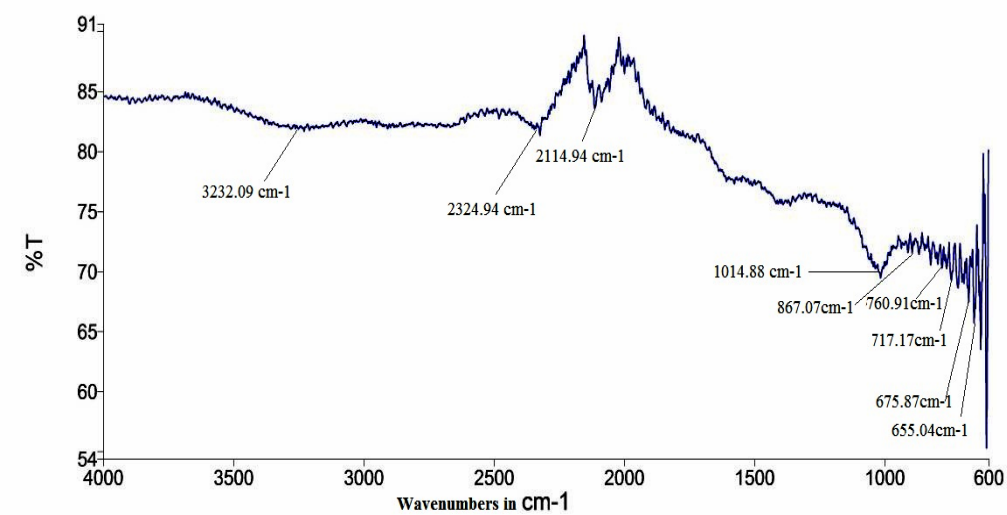

Fig. 4: FTIR spectrum of synthesized gold nanoparticles.

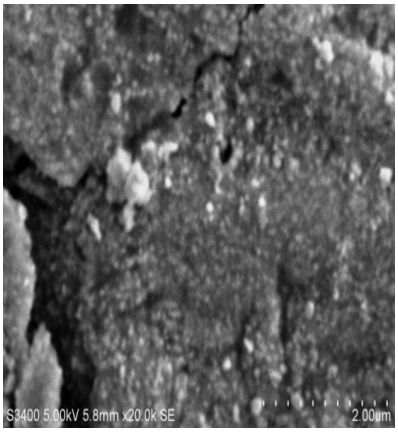

Fig. 5: SEM image of synthesized gold nanoparticles.

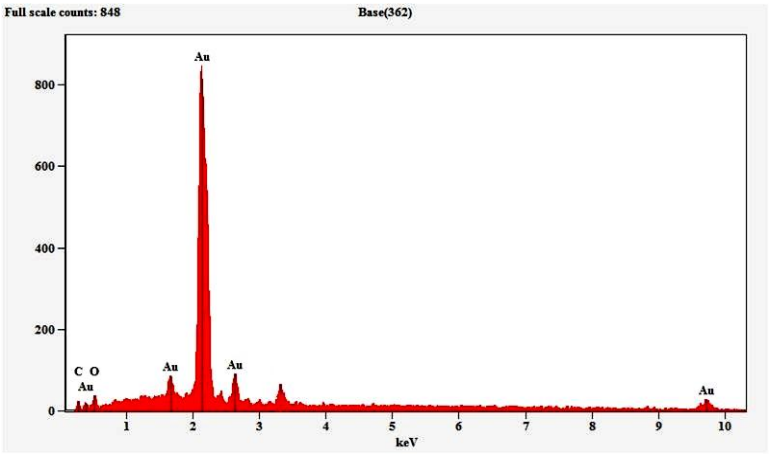

Fig. 6: EDAX of synthesized gold nanoparticles.

CONCLUSION

In the present study a successful effort was made to synthesize gold nanoparticles using *C. alata* leaf extract without any special condition like high pressure and temperature. Biomolecules present in the plant extract acted as reducing as well as capping agent in the reduction of Au^{+} to Au^{o}. This method of nanoparticle synthesis is an easy, simple, eco-friendly and economically feasible as no hazardous chemicals and solvents were used in the process. Nanoparticles synthesized were spherical in shape with average particle size of 12.3 nm and proved to be potential antimicrobial agent against selected phytopathogenic fungi and pathogenic bacteria. Since this method is simple it can be scaled-up and can be employed in the management of various plant and human diseases as alternative or in combination with conventional antimicrobials.

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