International Journal of Pharmaceutical Development & Technology

e ISSN - 2248 - 910X

www.ijpdt.com

Print ISSN - 2248 - 9096

LEAF MEDIATED GOLD NANOPARTICLE FROM *Indigofera aspalathoides* ENHANCED ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

A. Murugan and A.J. A. Ranjith Singh*

Professor, Manonmanium Sundaranar University, Department of Microbiology, Tirunelveli, 627117, Tamilnadu, India.

ABSTRACT

Medicinal plants are widely used by the Indian population since it has no harmful side effects and low cost compared toother treatments. In the 21st century, nanotechnology field is expected to be the base for all the important technological innovations. From that, green synthesis of gold nanoparticle is gaining more momentum due to its commercial demand besides it plays a significant role in the medical field and biomedical applications. Sphericalgold nanoparticles isolated from the leaf extract of *Indigofera aspalathoides* were studied by Field Emission Scanning Electron Microscope (FESEM) and crystalline structure were studied by Selected Area Electron Diffraction (SAED), revealed the size of nanoparticles with 18-89 nm (HRTEM). The green synthesized IaGNPs considerably exhibited strong radical scavenging potential (77.54%) when compared to the aqueous leaf extract (66.09%). Further IaGNPs inhibited the growth of human pathogenic both Gram-positive *Staphylococcus aureus,* (23 mm) and Negative bacteria *Escherichiacoli* (19 mm).

Keywords: Gold nanoparticle, Antibacterial, DLS, Antioxidant, FESEM, SAED.

INTRODUCTION

Nanotechnology is one of the most active area of research in modern materials science, because of its modern applications and have emerged rapidly as one of the most promising multidisciplinary branch of sciences which embraces numerous diverse fields of science and technology ranging from agricultural, advanced materials, biomedical, chemical science, electronics, environmental, information technology, pharmaceutical, and textile as well as to generate new applications in biotechnology and nanomedicine. The smaller size and high surface of nanoparticles are the key factors which make them reliable to biomedical fields., due to its drug carrier properties [1].

Nanomaterials are capable to exhibit high drug loading and releasing capacity, abilitytotarget malignant cells and low toxicity, thus it I sappropriate for therapeutic applications [2]. Gold nanoparticles (AuNPs) have many potential applications in biological and biomedical fields due totheir high biocompatibility, stability and the distinct surface plasmon properties [3]. Colloidal IaGNPs have been recommended for diverse biomedical applications because of its unique surface, electronic and optical properties [4]. Synthesis of nanoparticles using plants are advantageous than other biological processes because it can be scaled up suitably for large - scale production . At present, green nanotechnology is quite new, the fullscope of technological improvement in the field of human health care products [5].

Plant-derived compounds identified as promising agents and it was successfully translated to marketable drugs. Whereas the cancer prevention field has developed, many researchers have turned and tuned towards plants to identify and isolate new potential bioactive compounds to analyse the chemo-preventive and chemotherapeutic efficacy [6]. In the traditional medicinal system, *Indigofera aspalathoides* was used for the treatment of leprosy, cancer and edematous tumors [7], decoction of leaves and flowers was used for treating various types of skin rashes.

Root was used in preparing medicated oil and externally applied for scabies, leprosy etc. The medicated oil prepared from its root was also given internally as powder for the treatment of leprosy, dermatitis and various forms of ulcers. The plant is mostly shrubs, though some are herbaceous and a few could grow up to 5-6 feet in height.Systematic screening of herbals may result in the discovery of novel effective compounds [8].

Human beings are often infected by microorganisms such as bacteria, molds, yeasts and viruses present in their living environments [9]. Multidrug resistance is the most important problem caused by the

Corresponding Author :- **A.J. A. Ranjith Singh** Email:- ranjitspkc@gmail.com

chemical antimicrobial agents. Their efficacy depends on the specific binding with surface of the microbial cell. Therefore, an alternative way to overcome the drug resistance is needed, especially in medical devices [10]. The detailed approach was considered to explore the potential of bioactive compound towards reduction and capping of gold nanoparticles. In this work, synthesis and its characterization was achieved their antibacterial activity and antioxidant ability was tested. Investigations of phytochemicals has been making rapid progress and becoming popular as sources of promising anticancer compounds [11]. In recent years, the prevention of many disorders such as cancer and cardiovascular diseases has been found to beconcomitant with the ingestion of fresh fruits, vegetables, tea or plant beverages that are rich in natural antioxidants [12].

MATERIALS AND METHODS

Materials

Chloroauric acid, DPPH (1, 1 Di-phenyl picrylhydrazyl) were obtained from Sigma–Aldrich Chemicals.Mueller Hinton Agar (MHA) was obtained from Hi-Media. Fresh leaves of *Indigofera aspalathoides* were collected from University of Madras,Guindy campus,Chennai, Tamil Nadu, India. All glassware were sterilized by autoclave.

Preparation of plant extract and synthesis of goldnanoparticles

Fresh leaves of *I.aspalathoides* were washed several times with tap water to remove dust and dirt and it was cut into small pieces and8 g of leaves were boiled with 100 mL of double distilled water for 15 min and it was filtered using Whatmann No.1 filter paper. Synthesis of gold nanoparticles were done by reducing 1mM of chloroauric acid (195 µL) with50mL of leaf extract at room temperature.

Purification of gold nanoparticles

The completely phyto-reduced sample on treatment with acetone (1:4 proportion) undergoes aggregation which can then be separated by centrifugation and re-dispersion. The obtained pellet was washed and re-dispersed in sterile distilled water to produce nanoparticles free from biochemical constituents [13].

CHARACTERIZATION OF NANOPARTICLES

UV–vis spectral analysis

The reduction of pure gold ions was monitored by measuring theUV-vis spectrum of the reaction medium at 30 min after diluting a small aliquot of the sample with distilled water and a spectrum was read at a wide range of 200 to 800 nm (UV - Vis spec - Shimadzu).

X-ray Diffraction (XRD) analysis

To determine the crystal structure and chemical composition of the IaGNPs it was subjected to XRD. IaGNPs were prepared as a thin film on a glass slide and patterns were recorded in the 2θ region, from 10° to 70°.The XRD studies were conducted using X-ray diffractometer.

Fourier transform infra-red (FT-IR) spectroscopy

The FT-IR spectrum was recorded at diffuse reflectance mode with 4 cm^{-1} resolution in the mid-IR region between the wavenumbers 4000 and 400 cm-1 (Perkin elmer spectrum one instrument).

FESEM analysis of gold nanoparticles

Morphology of the synthesized gold nanoparticles were examined using Field Emission Scanning Electron Microscopy and using the similar instrument EDX analysis of the sample was performed using FESEM (HITACHI SU6600 FESEM) equipped with an EDAX attachment.

HR-TEM with EDS analysis

Green synthesized gold nanoparticles were washed and equally dispersed with sonication for 15 minutes. One drop of diluted sample were placed on Cu grid and allowed to dry in vacuo. After drying, the nanoparticles were visualized using High Resolution Transmission Electron Microscope.

Zeta potential measurement

Dynamic light scattering (DLS) were performed using Zetasizer (Malvern, UK), range between 0.1 and 10,000 nm. The measurement of zeta potential is based on the direction and velocity of particles. The size of the nanoparticles along with its polydispersity was determined using particle size analyser and it's based on measuring the time dependent, fluctuation of scattering of laser light by the nanoparticles undergoing Brownian motion.

DETERMINATION OF HYDROGEN DONATION ABILITY (DPPH ASSAY)

The ability of the IaGNPs to scavenge the stable free radical was assessed by the method of Leong &Shui[14].Briefly, a 0.1 mM solution of DPPH in methanol was prepared. An aliquot (20-100 µL) of IaGNPs was added to 3 mL of methanolic DPPH solution. Methanol alone served as blank and DPPH in methanol without IaGNPs served as positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 517 nm and radical scavenging activity was calculated as follows:

$FRSA = [(A_c – A_s)/A_c] \times 100$

Where A_c is absorbance of control and A_s is absorbance of tested sample after 60 min.

ASSESSMENT OF ANTIBACTERIAL ACTIVITY

The antibacterial activity of green synthesized IaGNPs were tested against six bacterial isolates using Agar well diffusion method [15]. Mueller Hinton Agar plates were inoculated with 100 µL of standardized culture (1.5×10^8 CFU/ml) of each bacterium (in triplicates) and

spread with sterile swabs. 6mm wells were made using sterile cork borer and different aliquots were added (25, 50, 75and 100 µL) into the wells. The plates were left 10 minutes at room temperature to allow diffusion of samples. After incubation for 24 h at 37˚C, the plates were observed. Zone of inhibition was measured and expressed in millimetres as well as the average diameter of inhibition zone was taken for evaluating the antibacterial activity of the extracts.

RESULTS AND DISCUSSION

UV–visible spectral analysis

The leaf extract of I.*aspalathoides* was mixed with $HAuCl_4$(0.1 mM) solution, the reduction of gold ions was confirmed after 30 min with the gradual appearance of yellow to pink colour and the surface plasmon resonance (SPR) of the IaGNPs formed at 536 nm(Fig. 1). UV–vis spectroscopy is an efficient technique to determine the formation and stability of AuNPs. The Plasmon bands were broad with and tail in the longer wavelength region that extends well into the infrared region in colloidal solution. Similar was the findings of [16] and [17], who had reported that the natural extract act as a reducing agent for synthesis of nanoparticles. The intensity of surface plasmon peak was directly proportional to the density of the nanoparticles in solution [18].

FTIR Analysis of aqueous and synthesized IaGNPs

FTIR study were carried out to identify the potential biomolecules present in the *I.aspalathoides* leaf extracts which is responsible for reduction and capping of the bio-reduced gold nanoparticles. The aqueous leaf extract of *I.aspalathoides* showed absorptions peak at3241.75 (N-H), 2922.59 (C-H), 2114.58 (C=N), 1624.73 (C-C), 811.88 (C-Cl) cm^{-1} (Fig.2 a).The aqueous extract showed characteristic absorption bands for flavonoidal derivatives[19].The peak at 3241.75 cm^{-1} stretch with N-H of 1˚, 2˚ amines and amides group, band at 2922.59 cm^{-1}stretch with C-H alkanes group, peak at 2114.58 cm^{-1} stretch with C=N corresponds to nitriles group. The peak 1624.73 cm^{-1} stretch with C-C in ring aromatic group and 811.88 peak corresponds to alkyl and aldehyde group respectively. Spectrum of synthesized IaGNPs revealed absorption bands at 3290.93, 2123.24, 1668.12, 1402.96 and 1028.24 cm^{-1}(Fig.2 b).

The absence of C=O group and shift of a C=C stretching to 1402.96 cm^{-1} comparison with that of aqueous extract showed the development of reduction of AuNPs. After reduction, the oxidized biomolecules were capped on the IaGNPs and exposed their peaks in IR spectrum.The bonds which we mentioned above was commonly took place in proteins which indicated the increase in the stability of synthesized gold nanoparticles. The mechanism of reduction of gold salts (Au^{3+}) to gold nanoparticles (Au^0) using phyto-molecules have been recently reported by [20]. The hydroxyl and carbonyl groups of flavonols derivatives and other bioactive molecules in water extracts first bound with gold ions (Au^{3+}) to form gold complexes.

XRD analysis synthesized IaGNPs

The XRD patterns of nanoparticles exhibited size-dependent features leading to peak height and width at 2θ=38.19and 46.23 (Fig-3). Two Bragg's reflections were observed in the (111) and (200) of face-centeredcubic (fcc) structure was observed and it was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS No. 00-004-0784), concluding that the synthesized gold nanoparticles were pure crystalline gold. The similar results were reported by [13].

FESEM analysis of gold nanoparticles

The dimension and morphology of IaGNPs were studied at different diameter -1 µm and 500 nm were examined by FESEM (Fig. 4a, b). EDX analysis of IaGNPs provided in (Fig. 4c) showed strong signals for elemental and other signals of metals. The gold nanocrystals displayed an optical absorption band peak at 1.51keV. EDX analysis suggested that bioactive compounds were absorbed on the surface of the IaGNPs and they are strongly responsible for the stability of the biosynthesized IaGNPs which is accordance with the earlier reports of [21].

HRTEM analysis of gold nanoparticles

The crystalline nature of the green synthesized IaGNPs was confirmed by TEM images (Fig. 5a-d). The obtained nanoparticles were poly dispersed in nature with sizes ranging between 51 and 57 nm. From the SAED pattern (Fig. 5e) diffraction rings were indexed to the (111), (200), diffraction planes of the cubic structure of Au were in agreement with that of XRD pattern obtained. TEM-EDS analysis (Fig. 5f), showed strong gold signals along with signals of copper and carbon which might have originated from the bioactive compounds bound to the surface of the gold nanoparticles. Similar was the results of [17].

DLS and Zeta potential analysis

The results of zeta potential value is indicating the surface charge of the nanoparticles. The size distribution Vs intensity graph has been displayed (Fig. 6a, b). The average size for biosynthesis of IaGNPs was 51 nm. The particle size was larger and more poly dispersed compared to the TEM result.The average size of the IaGNPs was also measured by DLS measurement. There was a little variation between the zeta potential value and concentration dependent ZP values was demonstrated *vice versa*. In DLS, the optimal measurements were highly dependent on the sample and their size. If the sample is too dilute, there may be not enough scattering events to make a proper measurement[22].

ANTIBACTERIAL ACTIVITY

The therapeutic potential of IaGNPs have been explored by *in vitro* antibacterial assay. Phyto-fabricated

gold nanoparticles exhibited dose-dependent antibacterial activity against all the test organisms. The maximum zone of inhibition obtained were against *E. coli* (23 mm) and followed by *P. aeruginosa* (21mm) *S, typhi* (19mm) and *S. aureus* (18mm) respectively. Concentration of gold nanoparticle were limited (500 µg), because higher dosage will lead to the toxic towards host pathogens. Whereas, the least activity was obtained in 25 µg/mL against *S. aureus* (6 mm) and also absence of zone of inhibition were recorded against both in *S. typhi*and *P. aeruginosa* at 25 µg/mL concentration. From these results it was concluded that an increase in the concentration of IaGNPs might be helpful for the scientific communities to overcome from certain bacterial diseases.

Earlier findings (Zhao and Nalwa, 2007) stated that gold nanoparticles will bind into the nucleus itself which allows them to diffuse through the nuclear pores. The variations in zone of inhibition might be due to the bacterial cell wall composition [23].The synthesized AuNPs from *Menthapiperita* was active against Gram negative (*E. coli*) and Gram positive (*S. aureus*) microorganisms.

ANTIOXIDANT ACTIVITY

The *in vitro* free radical scavenging activity of both aqueous extract and IaGNPs was performed using (Leong &Shui, 2005). The green synthesized IaGNPs exhibited better results (77.54 ± 4.19) when compared to that of aqueous extract (66.09 ± 4.14), but the standard (BHT) showed improved results when compared to both aqueous extract and gold nanoparticles. The antioxidant properties of *I.aspalathoides* and its role against diseases associated with oxidative stress as well as the composition of phenolics and flavonoids compounds would have contributed to the antioxidant activities of the plant[24].

The DPPH radical scavenging of HAuCl4 showed low percent of inhibition when compared to the gold nanoparticles which might be due to less catalytic activity of salts and less solubility of metal oxides[25]. The earlier report indicated that the methanolic extract of *S. monoica* stem possessed 116.22% of radical- scavenging activity at 800 µg/mL. When compare to the findings of [25], the present study revealed (66.09 %) and (77.54 %) of radical scavenging activity both in aqueous and IaGNPs at 100 µg/mL.

Fig 1. Dendrogram depicts UV–vis absorption spectrum of aqueous leaf extract a) and reaction mixture (b)

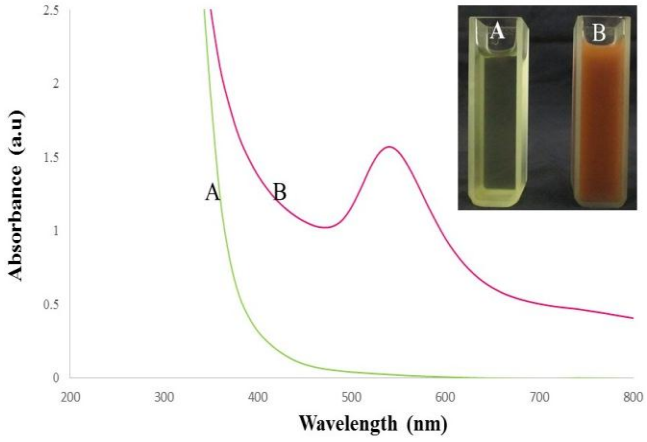


Fig 2. FT-IR spectrum of a) aqueous and b) synthesized IaGNPs

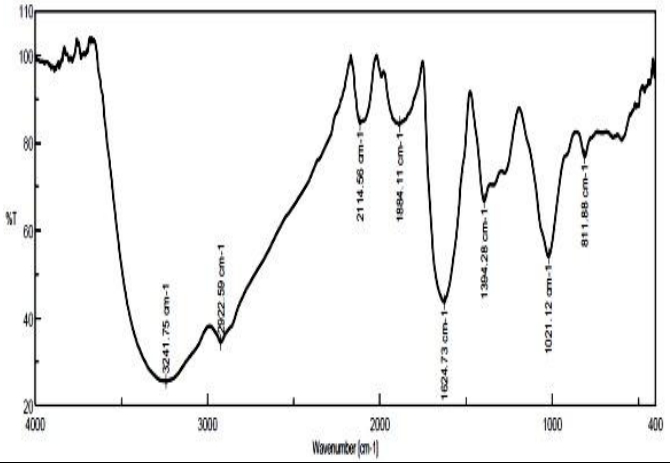


Fig 3. Dendrogram showing X-ray diffraction pattern of gold nanoparticles

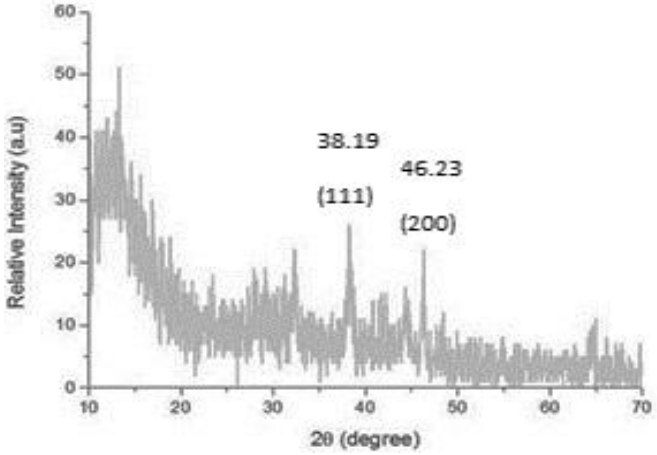


Fig 4. Morphological studies of IaGNPs at different magnifications (a) 1.00 µm, (b) 500 µm and (c) represents elemental composition using EDX

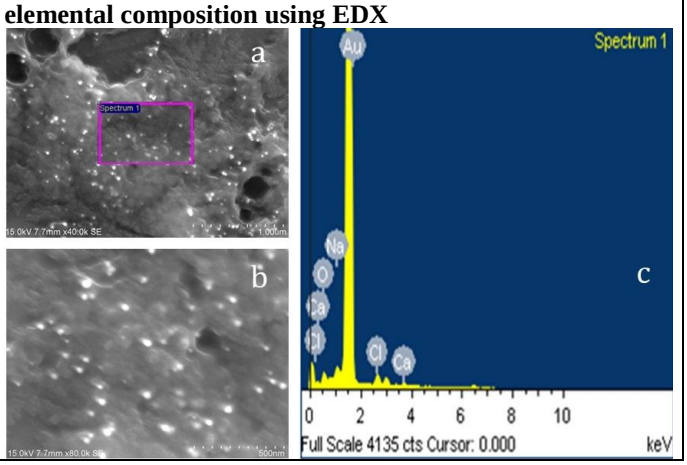


Fig 5. TEM micrograph of IaGNPs at different magnifications: (a) the scale bar denotes 20 nm, (b) 50 nm, (c) 100 nm, (d) 200 nm, (e) represents SAED pattern and (f) depicts EDX result of IaGNPs.

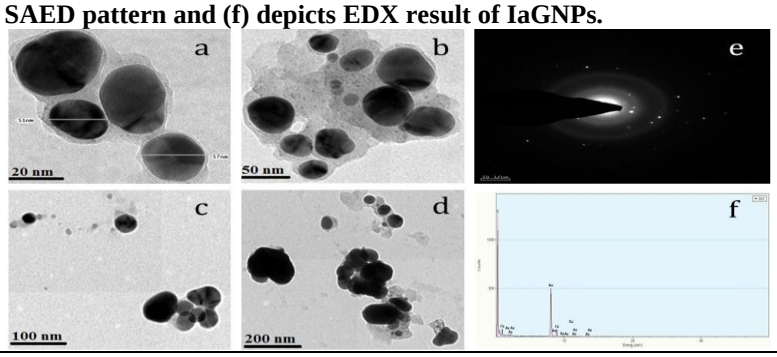
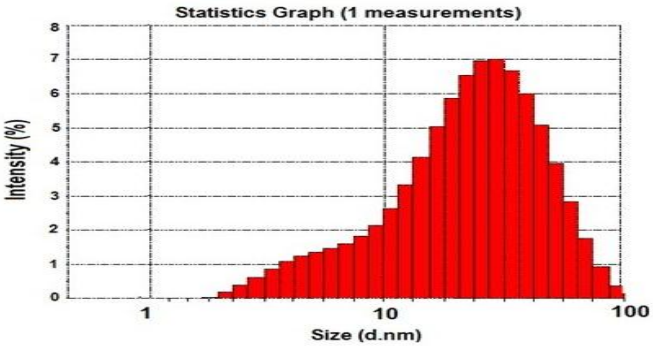


Fig 6. DLS measurement to determine the size distribution (a) and Particle size distribution of IaGNPs (b)



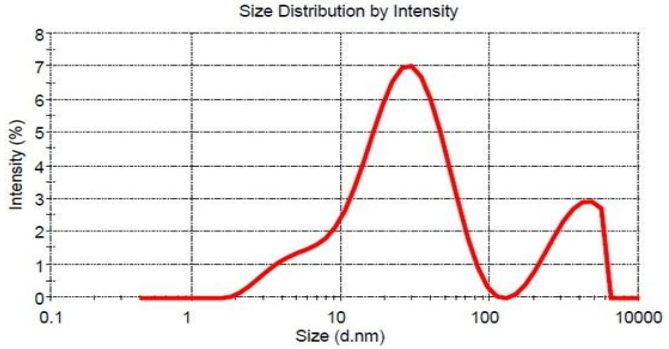


Fig 7. Illustration of the antibacterial activity of *I. aspalathoides*mediated GNPs against various human clinical pathogens using agar well diffusion method.

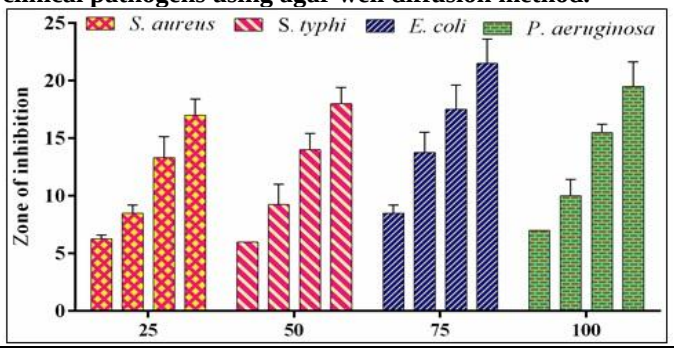


Fig 8. *In vitro* antioxidant activity of IaGNPs using DPPH.

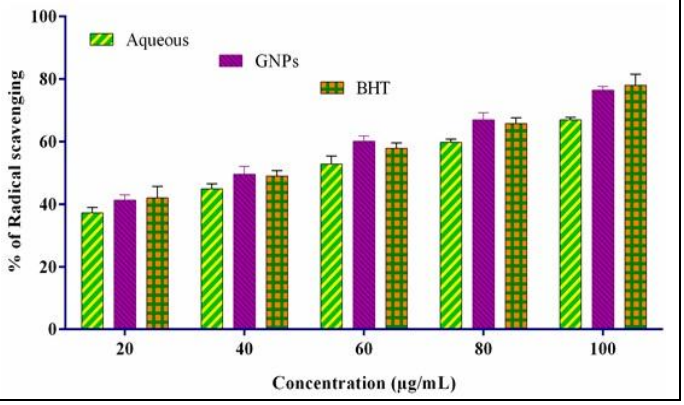


Table 1. Preliminary screening of phytochemicals from *Indigofera aspalathoides*

S. No	Name of Phytochemicals	Inference
1	Acids	+
2	Alkaloids	-
3	Carbohydrates	+++
4	Cardiac glycosides	+++
5	Coumarins	+
6	Cyanin	-
7	Flavonoids	+++
8	Glycosides	+++
9	Phenols	++

CONCLUSION

The nano-revolution explains significant role of plants for green synthesis of nanoparticles. The present study focussed towards green chemistry approach with eco-friendly nature for synthesis of gold nanoparticles using aqueous leaf extract of *I.aspalathoides*. The phytochemicals such as cardiac glycosides, carbohydrates, flavonoids and phenols acted as reducing and capping agents for the

preparation of AuNPs. UV-Vis spectra and DLS analysis are the important factors to confirm concentration of reducing agent and reaction time of the nanoparticles. The synthesized IaGNPs were stable for one month without aggregation and mostly spherical with an average size of 18.75 nm and it could offer a massive scope for use in medical field as an efficient antimicrobial agent. Besides, it is cost- effective, eco-friendly, non-toxic and easily renewable.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Director, Manonmanium Sundaranar University, Tirunelveli for providing the laboratory facilities and Nanoscience and Nanotechnology, University of Madras, Guindy Campus, Chennai–600025, for providing instrumentation facilities.

CONFLICT OF INTEREST

No interest

REFERENCES

1. Chatterjee K, Sarkar S,RaoK.J, Paria S. Core shell nanoparticles in biomedical applications. *Adv. Colloid Interface Sci*, 1, 2014, 34.
2. Vimala, K Sundarraj S, Paulpandi M, Vengatesan S, Kannan S. Green synthesized doxorubicin loaded zinc oxide nanoparticles regulates the Bax and Bcl-2 expression in breast and colon carcinoma. *Process Biochem*., 49, 2014, 160–172.
3. GunasekaranYWS. Spectroscopic and microscopic investigation of gold nanoparticle nucleation and growth mechanisms using gelatin as a stabilizer, 2012.
4. Leu JG, Chen SA, Chen HM, Wu M, Hung CF, Yao YD. The effects of gold nanoparticles in wound healing with antioxidant epigallocatechin gallate and α-lipoic acid. *Nanomedicine*, 8, 2012, 767–75.
5. Mohanpuria P, Rana NK, Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications. *J. Nanoparticle Res*, 10, 2007, 507–517.
6. Aqil F, Munagala R, Jeyabalan J, Vadhanam MV. Bioavailability of phytochemicals and its enhancement by drug delivery systems. *Cancer Lett*, 334, 2013, 133–141.
7. Balandrin MF, KjockeAJ, Wurtele E. Natural plant chemicals: sources of industrial and mechanical materials. *Science*, 228, 1985, 1154-1160.
8. Kirtikar KR and Basu BD. Glossary of Indian medicinal plants. *Periodical Experts*, 1975, 338.
9. Lee H, Ryu D, Choi S, Lee D. Antibacterial Activity of Silver-nanoparticles Against Staphylococcus aureus and Escherichia coli. *IJPR*, 39, 2011, 77–85.
10. Kim JS, Kuk E, Yu N, Kim J, Park SJ, Lee J. Antimicrobial effects of silver nanoparticles. *JAS*, 3, 2007, 95–101.
11. Afolabi EA, Ibukun EO, Akinmoladun, Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. *Sci. Res. Essay*, 2, 2007, 163 – 166.
12. Anand P, Kunnumakkara AB, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST. Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res*., 25, 2008, 097–116.
13. Aarthi FA, Arumugam R, Saravanan S, Anantharaman P. Phytofabrication of gold nanoparticles assisted by leaves of Suaeda monoica and its free radical scavenging property. *J. Photochem. Photobiol. B Biol.*, 135, 2014, 75–80.
14. Leong L and Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. *Food. Chem.*, 76, 2002, 69–75.
15. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J. Ethnopharmacol.*, 74, 2001, 113–123.
16. Jayaseelan C, Ramkumar R, Rahuman AA, Perumal P. Green synthesis of gold nanoparticles using seed aqueous extract of Abelmoschus esculentus and its antifungal activity. *Ind. Crops Prod.*, 45, 2013, 423–429.
17. Annamalai A, Christina VLP, Sudha D, Kalpana M, Lakshmi PTV. Green synthesis , characterization and antimicrobial activity of Au NPs using Euphorbia hirta L . leaf extract. *Coll. Surf. B Biointerfac.*, 108, 2013, 60–65.
18. Ahmad A, Mukherjee M, Senapati S, Mandal D, Khan MI, Kumar R. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. *Coll. Surfac. B Biointerfac*, 28, 2003, 313–318.
19. Basavegowda N, Idhayadhulla A, Lee YR. Phyto-synthesis of gold nanoparticles using fruit extract of Hovenia dulcis and their biological activities. *Ind. Crops Prod.*, 52, 2014, 745–751.
20. Babu BJ, Sharma P,Saranya S, Bora U. Synthesis of gold nanoparticles using ethonolic leaf extract of Bacopa monnieri and UV irradiation. *Mater. Lett*, 93, 2013, 431–434.
21. Kunjiappan S, *et al.* A green chemistry approach for the synthesis and characterization of bioactive gold nanoparticles using Azolla microphylla methanol extract. *Front. Mater. Sci.*, 8, 2014, 123–135.
22. Lim J,Yeap SP, Che HX, LowSC. Characterization of magnetic nanoparticle by dynamic light scattering. *Nanoscale Res. Lett.*, 8, 2013, 381.
23. AyazAhmed K, Subramanian S, Sivasubramanian A, Veerappan G,Veerappan A. Preparation of gold nanoparticles using Salicornia brachiata plant extract and evaluation of catalytic and antibacterial activity., Spectrochim. *Acta. A. Mol. Biomol. Spectrosc*, 130, 2014, 54–58.

24. Kamdem JP, Adeniran A, Boligon AA, Klimaczewski CV, Elekofehinti OO, Hassan W. Antioxidant activity, genotoxicity and cytotoxicity evaluation of lemon balm (Melissa officinalis L.) ethanolic extract: Its potential role in neuroprotection. *Ind. Crops Prod.*, 51, 2013, 26–34.
25. Ramamurthy CH, Padma M, Mariya samadanam MD, Mareeswaran R, Suyavaran A, Kumar MS. The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. *Colloids Surf. B. Biointerfaces*, 102, 2013, 808–15.