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GREEN SYNTHESIS OF GOLD NANOPARTICLES USING *RUMEX NEPALENSIS* LEAF EXTRACT AND EVALUATION OF ANTIBACTERIAL EFFICACY

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ABSTRACT

The objective of current study is to synthesize biocompatible gold nanoparticles using *Rumex nepalensis* leaf extract and to evaluate their antibacterial activity. The biosynthesized gold nanoparticles were characterized by UV-vis spectroscopy, scanning electron microscopy (SEM), energy dispersive x-ray (EDX) and x-ray diffraction (XRD) analysis. SEM showed spherical in shape of nanoparticles and XRD revealed crystalline nature with an average size of 8.63 nm. Fourier transform infrared spectroscopy (FTIR) revealed the involvement of biomolecules in the reduction of gold ions to gold nanoparticles. Biosynthesized gold nanoparticles were applied against common pathogenic bacterial strains along with plant extracts (aqueous and methanolic). Gold nanoparticles demonstrated much higher activity as compared to both leaf extracts and positive control (Ampicillin).The current study demonstrated that an aqueous leaf extract of *R. nepalensis* can be utilised to fabricate gold nanoparticles with smaller spherical size and significant antibacterial activity. Moreover, this green approach to synthesize AuNPs using *R. nepalensis* leaf extract, being cost effective and eco-friendly, can be scaled up.

Key-words: Green synthesis, *Rumex nepalensis*, Gold nanoparticles, Characterization, Antibacterial activity

INTRODUCTION

The nanoparticles synthesis has received much attention of researchers recently, as they are parallel candidates to other conventional resources with diverse applications in the field of engineering and science (Azam *et al*., 2012). Larger surface area and smaller size of nanoparticles makes them appropriate for numerous biomedical usages (Koehler *et al*., 2001). Gold nanoparticles (AuNPs) are used extensively in biological and biomedical research (Rajendran, *et al*., 2019). Compared to bulk materials, gold nanoparticles exhibit unique catalytic, electronic and optical properties in smaller size ranging in 1-100 nm being nontoxic and biocompatible (Dash and Bag, 2014). Gold nanoparticles (AuNPs) are precious, inert and not easily oxidized when exposed to oxygen or highly acidic environment (Daniel and Astreu, 2004). Owing to their well-known applications in various fields such as microelectronics, biotechnology and medication, AuNPs synthesis has received intense research interests (Magudapathy *et al*., 2001). However, AuNPs are generally synthesized through several physical and biochemical techniques, which are costly and involve the utilization of poisonous substances that are not suitable for therapeutic applications (Korbekandi *et al*., 2009). AuNPs are also fabricated by using various microbes (Sehgal *et al*., 2018) and plant extracts (Klaus *et al*., 1999; Khalil *et al*., 2012). Among various biological systems, plant based green synthesis is most suitable as plant extracts reduce ionic metals into atomic structures at faster rate at normal an ambient temperature (Bhau *et al*., 2015). Green synthesis is a green and eco-friendly method used for production of large scale nanoparticles. The various phytochemicals in plant extract exhibit involvement in reduction and stabilization of nanoparticles (Dzimitrowicz *et al*., 2016). Plant based green synthesis need lesser incubation time as compared to microbes and at the same time can be scaled up for commercial production(Niraimathi *et al*., 2013).

It has been investigated that various human pathogenic bacteria enhanced their resistance against various synthetic drugs (Prema and Thangapandian, 2013). Due to development of resistant strains and increasing microbial resistance against metal ion and antibiotics, the nanoparticles are gained increasing interest in pharmaceutical field as they possess enhanced antibacterial potential due to larger surface area to volume ratio (Khalil *et al*., 2013). Recently, the green nanotechnology and nanosciences has established to offer and surpass opportunities for exploring the influence of metal nanoparticles for antimicrobial activities (Senthilkumar *et al*., 2017). Thus the present study was designed for eco-friendly and cost effective synthesis of AuNPs using *R. nepalensis* with antibacterial properties.

MATERIALS AND METHODS

Materials

The tetrachlorogold (III) trihydrated yellow and Microbiology Agar were purchased from Merck & Company Inc. Methanol was purchased from Sigma Aldrich Corporation. Fresh leaf samples of *R. nepalensis* were collected from locality Chehla Campus University of Azad Jammu and Kashmir Muzaffarabad Pakistan.

Preparation of plant extract

The fresh leaf samples were washed with tape water and finally with distilled water thrice. Finely cut leaves were boiled in an electric oven for 10 minutes to make an aqueous extract. To prepare crude methanolic extract, shade dried powdered plant leaf material (100 g) was macerated with methanol (300 mL) (Alanis *et al*., 2005). To get dry filtrate, the extract was filtered and evaporated in water bath.

Biosynthesis of AuNPs

To synthesize AuNPs, aqueous leaf extract and 1mM gold chloride solution were homogenized at 1:1 by volume at room temperature. The first indication of AuNPs formation was evidenced by the change of colour from light yellow to ruby red. The colloidal mixture was centrifuged for 4 minutes at 16000 rpm to get pellets of AuNPs which were washed with deionized water and then with acetone.

Characterization

UV-Vis spectral analysis: UV-Vis spectral analysis of reaction mixture was carried out after 0, 12 and 24 h of reaction to observe the bio-reduction of gold ions using Lambda 950 UV/VIS spectrophotometer (PerkinElmer) at wavelength range from 400 nm to 800 nm.

***X-Ray Diffraction analysis*:** The size and crystallographic structure of AuNPs was determined by X-Ray diffraction analysis. Debye Scherrer formula ($D = 0.94\lambda/\beta\cos\theta$) was used to calculate crystalline size of AuNPs.

***Scanning Electron Microscopy*:** JEOL JSM-6490A Analytical Scanning Electron Microscope was used to determine the morphology of AuNPs. The sample was sonicated and then thin films were prepared on a carbon coated copper grid. Blotting paper was used to remove extra solution. Then the sample films were dried under mercury lamp for 10 minutes.

Energy dispersive X-ray spectroscopy: The elemental nature of AuNPs was confirmed by Energy dispersive X-ray spectroscopy using JEOL JSM-6490A Analytical Scanning Electron Microscope equipped with Thermo EDXs.

Fourier Transform Infra-red Spectroscopy: PerkinElmer Spectrum 100 FTIR Spectrometer was used for FTIR analysis to determine various functional groups adsorbed to surface of AuNPs involved in stabilization and capping.

Bacterial strains

The clinical strains of *Staphylococcus aureus* (gram positive) *Acetobacter sicerae, Pseudomonas aeruginosa, Escherichia coli and Enterobacter aerogenes* (gram negative) were used in the experiment. The bacterial strains were procured from Pathology Laboratory Combined Military Hospital Muzaffarabad Azad Jammu & Kashmir Pakistan.

Antibacterial activity

Disc diffusion method was used for antibacterial activity of AuNPs (Kelman *et al*., 1998). Ampicillin was used as a control. Two factorial completely randomized design with three replicates was used for antibacterial activity. The obtained results were presented as mean ± Standard Deviation. The level of significance was verified by using MSTAT-C software at p= 0.05. The mean value was separated by Duncan's Multiple Range test (Steel *et al*., 1997).

RESULTS

Biosynthesis of AuNPs

In the present study, AuNPs have been successfully synthesized using *R. nepalensis* leaf extract at an ambient temperature. The colour of the Au^{+} solution shifted from pale yellow to ruby red after addition of aqueous leaf extract due to phenomenon of surface Plasmon resonance (Fig. **1**).

UV-Vis spectral analysis

UV-Vis spectral analysis further supported the biosynthesis of AuNPs in solution. The spectrum displayed constant absorption peaks at 540 nm due to Surface Plasmon Resonance at different time intervals (Fig. **2**).

Fig. 1. Au+ Solution and synthesized AuNPs.

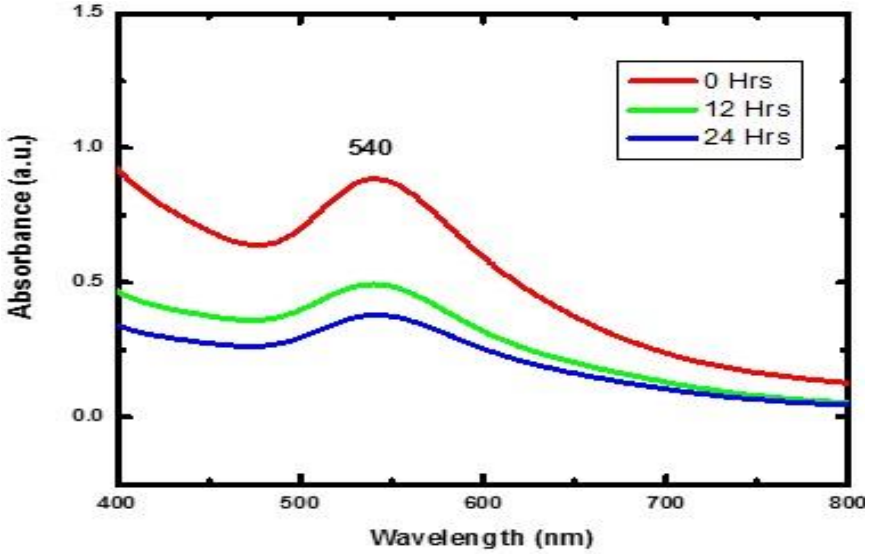

Fig. 2. UV-Vis Spectrum of AuNPs.

X-Ray Diffraction Analysis (XRD)

The crystalline nature and size of AuNPs was determined by X-ray Diffraction Analysis (XRD). XRD pattern of synthesized AuNPs showed number of Bragg's reflections that may be indexed on the basis of face centered cubic structure of gold (Fig. **3**). Characteristics absorption peaks for AuNPs values 2 θ = 38.0577, 64.4868 and 77.8306 for indexing angle of reference plane (111), (220) and (311). Debye Scherer formula ($D= 0.94\lambda/\beta\cos\theta$) was used to calculate the crystalline size of AuNPs. The size of AuNPs was found to be in between the 6.03 nm and 11.03 nm.

Scanning Electron Microscopy (SEM)

The SEM image showed the high density of agglomerated gold nanoparticles. The agglomerated AuNPs were spherical in shape and were clustered (Fig. **4**).

EDX Analysis

Energy dispersive x-ray spectrum further supported the fabrication of AuNPs by plant extract. EDX spectrum clearly evidenced the formation of elemental gold with weight percentage of 65.42 % (Fig. **5**).

Fourier Transform Infra-Red Spectroscopy (FTIR)

The involvement of biomolecules in reduction and capping of AuNPs was confirmed by FTIR analysis. The absorption peaks were located at about 708, 882,1118 1429,1559, 1558,2028 and 3403 cm^{-1} in the region 400-4000 cm^{-1} (Fig. **6**).

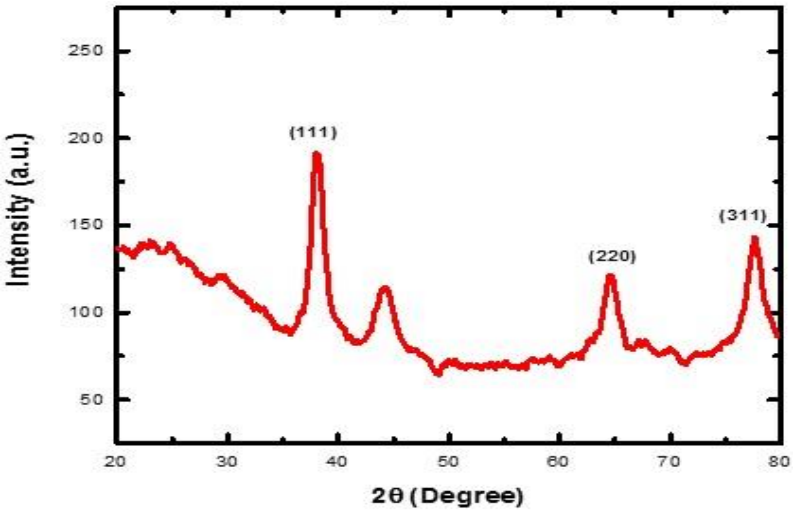

Figure 3. XRD spectrum of AuNPs

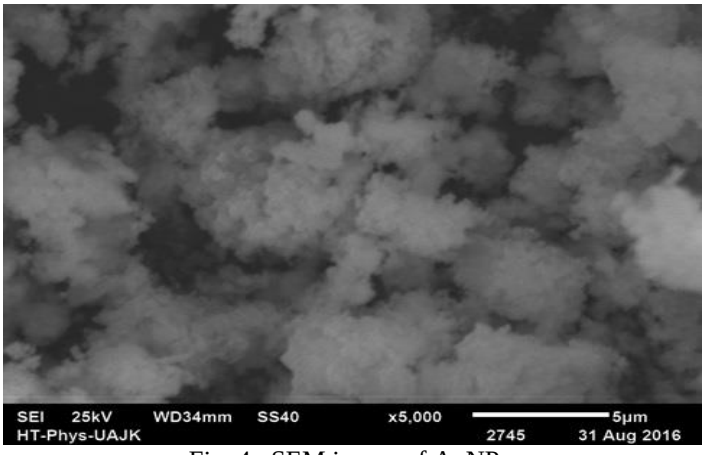

Fig. 4. SEM image of AuNPs.

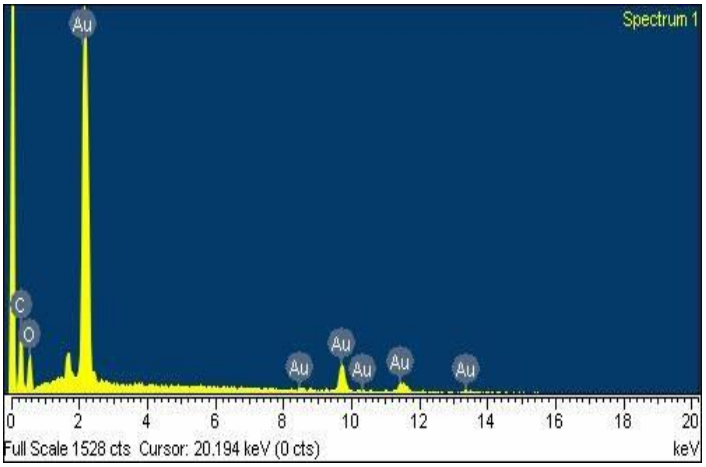

Fig. 5. EDX spectrum of AuNPs.

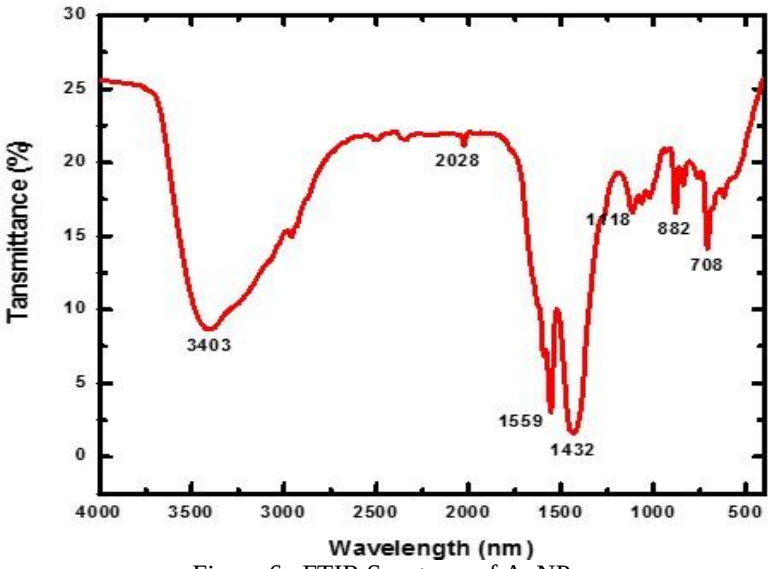

Figure 6. FTIR Spectrum of AuNPs

Antibacterial Activity

In present study, the antibacterial activity of AuNPs (10ppm, 30ppm and 40ppm) and aqueous and methanolic extract of *R. nepalensis* was evaluated against a gram positive bacterium (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Acenetobacter baumannii* and *Enterobacter aerogenes*). The plant leaf extracts and AuNPs concentration significantly inhibited the growth of bacterial species (Table 1). Maximum mean zone of inhibition (12.3 mm) was recorded in *P. aeruginosa* which was significantly different from other bacterial strain. Different concentrations of AuNPs and extracts significantly affected the growth of bacterial strains. The maximum zone of inhibition (13.2 mm) was recorded in bacteria treated with 40 ppm AuNPs which was significantly different from the strains treated with extracts and AuNPs (10 and 30 ppm) (Table 1). A statistically significant interaction was found between extracts and bacterial strains. Highest zone of inhibition (14.3 mm) was recorded for *P. aeruginosa* at 40 ppm AuNPs concentration which was non-significantly different from *A. sicerae* at similar concentration. The minimum zone of inhibition (9 mm) was recorded for *E. aerogenes* when aqueous extract was used.

Table 1. Antibacterial assay of AuNPs and R. nepalensis extracts (Mean ± S.D).

Bacterial species	**Control**	**Aqueous Extract**	**Methanolic Extract**	**AuNPs (10 ppm)**	**AuNPs (30 ppm)**	**AuNPs (40 ppm)**	**Means**
A. sicerae	10.3 ± 1.52d-h	10.6 ± 2c-h	11 ± 1.0b-h	9.3 ± 0.5g-h	12 ± 1.0a-g	14.3 ± 1.1a	11.2 ± 1.18B
S. aureus	9.6 ± 1.15f-h	11 ± 2.0b-h	13 ± 1.0a-d	10.6 ± 1.5c-h	10.6 ± 1.5c-h	13 ± 1.0a-d	11.3 ± 1.35AB
P.aeruginosa	9.3 ± 1.52gh	12.3 ± 2a-f	12.6 ± 2a-e	12.3 ± 1.5a-f	13.3 ± 1.52a-c	14.3 ± 1.5a	12.3 ± 1.67A
E. coli	10 ± 1.0e-h	12.3 ± 1a-f	13.3 ± 1.5a-c	11 ± 2.0 b-h	10.6 ± 1.1c-h	12.2 ± 1.52a-f	11.6 ± 1.35AB
E. aerogenes	9.6 ± 0.5f-h	9 ± 1.0 h	12.3 ± 1a-f	12.6 ± 1.5a-e	13.6 ± 1.5ab	12 ± 1.0a-g	11.5 ± 1.0AB
Means	9.8 ± 1.1D	11.0 ± 1.6C	12.4 ± 1.3AB	11.2 ± 1.4C	12 ± 1.3BC	13.2 ± 1.2A*	

* Any two means carrying the same letter(s) in a columns or rows are non-significantly different at *P*= 0.05 by Duncan's Multiple Range Test (Small letters show interaction between bacterial species and treatments and capital letters show their means)

DISCUSSION

AuNPs have received tremendous attraction of researcher due various application including cosmetics and medicine, therefore it is important to introduce environmental friendly protocols for synthesize of AuNPs. The transition in colour was first indication of AuNPs synthesis (Fujiwara *et al*., 2007). It is well known that AuNPs exhibits special optical properties directly related to Surface Plasmon Resonance which is highly dependent on the morphology of the nanoparticles (Lokina and Narayanan, 2013). UV-Vis spectrum of biosynthesized AuNPs revealed strong absorption bands at 540 nm due to Surface Plasmon Resonance (Konen-Adiguzel *et al*., 2018). It was observed that the peak intensity decreased with the passage of time due to decrease in reduction rate (Wang *et*

al., 20116). The size of AuNPs was found to be 6.03 nm, 8.83 nm and 11.03 nm which is consistent with previous investigation (Dorosti and Jamshi, 2016; Ghosh *et al*., 2012; Umamaheswari *et al*., 2014). The smaller size enhances the surface area and hence is more active. At higher magnification SEM image showed that AuNPs are spherical in shape and clustered due to agglomeration (Senthilkumar *et al*., 2017). The elemental analysis determines the purity of AuNPs. The sharp optical absorbance peaks in EDX spectrum at 3 KeV (Fig. **5**) indicated the presence of elemental gold nanoparticles (Li *et al*., 2010). In the present, EDX analysis revealed 65.42 %. of AuNPs by weight percentage in *Rumex* mediated nanoparticles. The weak absorbance peaks for C and O suggested presence of mixed precipitates from plant extract adsorbed to the surface of AuNPs (Mittal *et al*., 2013). The FTIR peaks are correlated to certain functional group of *R. nepalensis* bioactive compounds that involved in the green synthesis of gold nanoparticles. Previous study suggests that these bioactive compounds are thought to be responsible for coating of AuNPs and hence play role in their stabilization (Nath and Banerji, 2013).

The infectious microbes can contaminate water and food consumed by human and all living beings. AuNPs exhibit their antibacterial activity as they get attached to the cell surface. This kind of interaction with bacterial cell leads to the damage and structural changes which ultimately affecting normal functionality of the cell. Nanoparticles suppression of respiratory chain enzymes and cell permeability which leads to cell death (Rai *et al*., 2009; Sharma *et al*., 2009; Velammal *et al*., 2016). The gram positive bacteria such as *Staphylococcus aureus* and gram negative bacteria such as *Pseudomonas aeruginosa* have been found to develop resistance against conventional antibiotics (Frieri *et al*., 2017). The inhibitory effect of gold nanoparticles at increased concentration showed better result (Mohamed *et al*., 2017). In previous reports, higher concentrations of AuNPs showed similar results (Rajeshkumar *et al*., 2013; Katas *et al*., 2019). Similarly in a previous report, maximum zone of inhibition (14 mm) was observed at AuNPs concentration of 4000 ppm was used but no antibacterial activity was observed below concentration of 65.5 ppm (Nazari *et al*., 2012) whereas in present study, lower concentration of AuNPs (10, 30, 40 ppm) showed significant antibacterial activity against selected bacterial strains. Therefore, *R. nepalensis* mediated AuNPs are more effective against human pathogen specifically at lower concentrations. Further study needs to be done on engineering and mechanism of action of AuNPs against human pathogenic bacteria.

Conclusion

AuNPs with smaller size can be synthesized in an energy efficient and cost effective manner using *R. nepalensis* leaf extract at an ambient temperature. These green based AuNPs exhibit higher antibacterial property especially against gram negative bacteria that may develop resistance to other conventional antibiotics.

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REFERENCES

Alanis, A. D., F. Calzada, J. A. Cervantes, J. Torres and G. M. Ceballos (2005). Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. *Journal of Ethnopharmacology*, 100(1-2): 153-157.

Azam, A., A. S. Ahmed, M. Oves, M. S. Khan, S. S. Habib and A. Memic (2012). Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. *International Journal of Nanomedicine*, 7: 6003

Bhau, B. S., S. Ghosh, S. Puri, B. Borah, D. K. Sarmah and R. Khan (2015). Green synthesis of gold nanoparticles from the leaf extract of *Nepenthes khasiana* and antimicrobial assay. *Advanced Material Letters*, 6 (1): 55-58.

Daniel, M. and D. Astruc (2004). Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical Reviews*, 104 (1): 293-346.

Dash, S. S. and B. G. Bag (2014). Synthesis of gold nanoparticles using renewable Punica granatum juice and study of its catalytic activity. *Applied Nanoscience*, 41: 55-59.

Dorosti, N. and F. Jamshidi (2016). Plant-mediated gold nanoparticles by *Dracocephalum kotschyi* as anticholinesterase agent: synthesis, characterization, and evaluation of anticancer and antibacterial activity. *Journal of Applied Biomedicine*, 14(3): 235-245.

Dzimitrowicz, A., P. Jamróz, I. Sergiel, T. Kozlecki and P. Pohl (2016). Preparation and characterization of gold nanoparticles prepared with aqueous extracts of Lamiaceae plants and the effect of follow-up treatment with atmospheric pressure glow microdischarge. *Arabian Journal of Chemistry*,

Frieri, M., K. Kumar and A. Boutin (2017). Antibiotic resistance. *Journal of infection and public health*, 10(4): 369-378.

Fujiwara, K., A. Ramesh, T. Maki, H. Hasegawa and K. Ueda (2007). Adsorption of platinum (IV), palladium (II) and gold (III) from aqueous solutions onto l-lysine modified crosslinked chitosan resin. *Journal of Hazardous Materials*, 146 (1-2): 39-50.

Ghosh, S., S. Patil, M. Ahire, R. Kitture, D. D. Gurav, A. M. Jabgunde, S. Kale, K. Pardesi, V. Shinde, J. Bellare and D.D. Dhavale (2012). Gnidia glauca flower extract mediated synthesis of gold nanoparticles and evaluation of its chemocatalytic potential. *Journal of Nanobiotechnology*, 10(1): 17.

Katas, H., C. S. Lim, A. Y. H. N. Azlan, F. Buang and M. F. M. Busra (2019). Antibacterial activity of biosynthesized gold nanoparticles using biomolecules from *Lignosus rhinocerotis* and chitosan. *Saudi Pharmaceutical Journal*, 27 (2): 283-292.

Kelman, D., A. Kushmaro, Y. Loya, Y. Kashman and Y. Benayahu (1998). Antimicrobial activity of a Red Sea soft coral, *Parerythropodium fulvum*: reproductive and developmental considerations. *Marine Ecology Progress Series*, 169: 87-95.

Khalil, K. A., H. Fouad, T. Elsarnagawy and F. N. Almajhdi (2013). Preparation and characterization of electrospun PLGA/silver composite nanofibers for biomedical applications.*International Journal of Electrochemical Science*, 8(3): 3483-3493.

Khalil, M.H ., E. H. Ismail and F. El-Magdoub (2012). Biosynthesis of Au nanoparticles using olive leaf extract: 1st nano updates."*Arabian Journal of Chemistry*, 5(4): 431-437.

Klaus, T., R. Joerger, E. Olsson and G. C. Granqvist (1999). Silver-based crystalline nanoparticles, microbially fabricated. *Proceedings of the National Academy of Sciences*, 96 (24): 13611-13614.

Koehler J. M., A.Csáki, J. Reichert, R. Möller, W. Straube and W. Fritzsche (2001). Selective labeling of oligonucleotide monolayers by metallic nanobeads for fast optical readout of DNA-chips. *Sensors Actuators B: Chemical*, 76:166-172.

Könen-Adıgüzel S., A.O. Adıgüzel, H. Ay, S. Alpdoğan, N. Şahin, A. Çaputçu, R.S. Ergene, H.M. Gübür and M. Tunçer (2018). Genotoxic, cytotoxic, antimicrobial and antioxidant properties of gold nanoparticles synthesized by Nocardia sp. GTS18 using response surface methodology. *Material Research Express*, 5(11): 115402.

Korbekandi, H., S. Iravani and S. Abbasi (2009). Production of nanoparticles using organisms. *Critical Reviews in Biotechnology*, 29 (4): 279-306.

Li, W. R., X. B. Xie, Q. S. Shi, H. Y. Zeng, O. Y. Y.Sheng and Y. B. Chen (2010). Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. *Applied Microbiology and Biotechnology*, 85 (4): 1115-1122.

Lokina S. and V. Narayanan (2013). Antimicrobial and anticancer activity of gold nanoparticles synthesized from grapes fruit extract. *Chemical Science Transactions*, 2: 105-110.

Magudapathy, P., P. Gangopadhyay, B. K. Panigrahi, K. G. M. Nair and S. Dhara (2001). Electrical transport studies of Ag nanoclusters embedded in glass matrix. *Physica B: Condensed Matter*, 299 (1-2): 142-146.

Mittal, A. K., Y. Chisti and U. C. Banerjee (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, 31 (2): 346-356.

Mohamed, M. M., S. A. Fouad, H. A. Elshoky, G. M. Mohammed and T. A. Salaheldin (2017). Antibacterial effect of gold nanoparticles against *Corynebacterium pseudotuberculosis*." *International Journal of Veterinary Science and Medicine*, 5 (1): 23-29.

Nath, D. and P. Banerjee (2013). Green nanotechnology–a new hope for medical biology. *Environmental Toxicology and Pharmacology*, 36 (3): 997-1014.

Nazari, Z. E., M. Banoee, A. A. Sepahi, F. Rafii and A. R. Shahverdi (2012). The combination effects of trivalent gold ions and gold nanoparticles with different antibiotics against resistant *Pseudomonas aeruginosa*. *Gold Bulletin*, 45 (2): 53-59.

Niraimathi, K. L., V. Sudha, R. Lavanya and P. Brindha (2013). Biosynthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) extract and their antimicrobial, antioxidant activities. *Colloids and Surfaces B: Biointerfaces*, 102: 288-291.

Pasca, R. D., A. Mocanu, S. C. Cobzac, I. Petean, O. Horovitz and M. Tomoaia-Cotisel (2014). Biogenic syntheses of gold nanoparticles using plant extracts. *Particulate Science and Technology*, 32(2): 131-137.

Prema, P. and S. Thangapandiyan (2013). In-vitro antibacterial activity of gold nanoparticles capped with polysaccharide stabilizing agents. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(1): 310-314.

Rai, M., A. Yadav and A. Gade (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances,* 27 (1): 76-83.

Rajendran, G., T. Rajamuthuramalingam, D. M. I. Jesse and K. Kathiravan (2019). Synthesis and characterization of biocompatible acetaminophen stabilized gold nanoparticles. *Materials Research Express,* 6 (9): 095043.

Rajeshkumar, S., C. Malarkodi, M. Vanaja, G. Gnanajobitha, K. Paulkumar, C. Kannan and G. Annadurai (2013). Antibacterial activity of algae mediated synthesis of gold nanoparticles from Turbinaria conoides. *Der Pharma Chemica*, 5 (2): 224-229.

Sehgal, N., K. Soni, N. Gupta and K. Kanchan (2018). Microorganism assisted synthesis of gold nanoparticles: a review. *Asian Journal of Biomedicsl Pharmceutical Sciences,* 8 (64): 22-29.

Senthilkumar S., L. Kashinath, M. Ashok and A. Rajendran (2017). Antibacterial properties and mechanism of gold nanoparticles obtained from *Pergularia daemia* leaf extract. *Journal of Nanomedicine Research,* 6: 1-5.

Sharma, V. K., R. A. Yngard and Y. Lin (2009). Silver nanoparticles: green synthesis and their antimicrobial activities. *Advances in Colloid and Interface Science*, 145 (1-2): 83-96.

Steel R. G., J. H.Torrie and D.A. Dickey (1997). Principles and procedures of statistics a biometrical approach. 3rd edition McGraw Hill Book Incorporation, New York, pp. 400-428.

Umamaheswari K, R. Baskar, K. Chandru, N. Rajendiran and S. Chandirasekar (2014). Antibacterial activity of gold nanoparticles and their toxicity assessment. *BMC Infectious Disease,* 14: 64.

Velammal, S. P., T. A. Devi and T. P. Amaladhas (2016). Antioxidant, antimicrobial and cytotoxic activities of silver and gold nanoparticles synthesized using *Plumbago zeylanica* bark. *Journal of Nanostructure in Chemistry,* 6 (3): 247-260.

Wang, C., R. Mathiyalagan, Y. J. Kim, V. Castro-Aceituno, P. Singh, S. Ahn, D. Wang and D. C. Yang (2016). Rapid green synthesis of silver and gold nanoparticles using *Dendropanax morbifera* leaf extract and their anticancer activities. *International Journal of Nanomedicine,* 11: 3691.

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