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BIOGENIC AND DRUG CAPPED AuNps. AND THEIR BIOMEDICAL APPLICATION

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ABSTRACT

Development of green nano technology is generating interest of researchers toward eco friendly, safe, non-toxic, eco-friendly route of synthesis which can be used for the manufacture at a large scale. This is a simple, cost-effective, stable for long time and reproducible aqueous room temperature synthesis method to obtain a self-assembly of Au and Pd nanoparticles with the leaf extract of Hibiscus rosa. In the present study, we report the bio synthesis of aqueous stable gold and palladium nanoparticles by using petal extract of Hibiscus rosa as both reducing and capping agents.I have also synthesiszed Gold nanoparticle with the use of isonazide drug as redusing agents and PEG (poly Ethylene Glycol) as capping agents. The synthesized nanoparticles were characterized by UV/Vis, particle size analyzer (PSA) and transmission electron microscopy (TEM). We discuss here a quick, simple, economic, and eco friendly method through a completely green route for the selective detection. Furthermore this nanoparticles have applied in biological system like, gold nano particles for selective sensing of amino acid (L- dopa) potential antimicrobial agent and AuNps. nanoparticles applied for the catalyst study.

KEYWORDS: Gold Nanoparticles, Hibiscus rosa, AuNps, Amino Acids, Antimicrobial, Antioxident.

1. INTRODUCTION

The field of nanoscience has been established recently as a new interdisciplinary science which can be defined as a whole knowledge on fundamental properties of nano-size objects.[1] Size and shape of nanoparticles provide an efficient control over many of their physical and chemical properties.[2,3] and their potential application in optoelectronics,[4,5] recording media,[6,7] sensing devices,[8,9] medicine.[10-12] and catalysis.[13] Metallic nanoparticles with the unique optical and electrical properties have been widely investigated during the past decades.

The chemical synthesis methodologies include redox synthetic method, electrochemical method, photochemical method, seed-growth method, template synthesis, micro-emulsion template synthesis, and microwave synthesis, etc.[14-17] different reducing agents such as sodium borohydride, trisodium citrate, tannic acid, hydrazine, ascorbic acid, tartaric acid and human cells are used to reduce the gold halides ($HAuCl_4$) to colloid nanoparticles.[18-23] AuNPs can be used as multi labels in simultaneous optical and electron microscopy and as energy transfer assays for the detection of DNA, or proteins, or in optoelectronics, Heavy metallic cations determination, DNA detection, Protein analysis, cancer treatment.[24-27]

Hibiscus is a medicinal herb usually used effectively in native medicines against hypertension, pyrexia and liver disorder.[28] Hibiscus leaf extract contains antioxidant compounds and is applicable to prevent atherosclerosis in humans via its anti-hyperlipidaemic effect and anti-LDL oxidation.[29] Hypoglycemic activity of this extract has also been reported. Hibiscus leaf extract contains proteins, vitamin, organic acids (essentially malic acid), flavinoids and anthocyanins. The ability of hibiscus extract as a natural starch and sucrose blocker is found to lower starch and sucrose absorption when injested at reasonable doses by inhibiting amylase which in turn an influence the glycemic load favorably.[29] The antioxidant potential and anti-implantation activity of hibiscus extract were also studied.[30]

L-Dopa is one of the essential precursors used for the biosynthesis of dopamine. The enzyme aromatic-l-amino-acid decarboxylase present in brain converts L-Dopa into dopamine.[31] L-Dopa (3,4-dihydroxy-l-phenylalanine) is widely used as a source of dopamine in the treatment of most patients with Parkinson's disease

and epilepsy.[32] Several methods have been reported in literature for the determination of L-Dopa, such as titration,[33] spectrophotometry,[34,35] high-performance liquid chromatography (HPLC),[36] photo kinetic method,[37] capillary zone electrophoresis.[38] and glass carbon electrode.[39] Antioxidant compounds in food play an important role of a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases, including cancer and heart disease. The gold nanoparticles coated with torolex and chitosan enhance the DPPH radical scavenging activity. Gold, selenium, copper and platinum nanoparticles also exhibit the DPPH radical scavenging activity.[40-43] Palladium nanoparticles have been of increasing scientific interest as a catalyst for organic carbon– carbon bond formation reactions such as Stille and Suzuki cross coupling reactions., In general, palladium complexes and palladium nanoparticles have been used as catalysts for the carbon–carbon couplings which are among the most powerful methods in organic synthesis; the reactions have been performed by heating or refluxing.

INH-AuNps were found to be the selective and sensitive colourometry probe for L-Dopa. The simplicity, sensitivity and specificity for the detection of amino acids by the INH-AuNps, indicates that the synthesized nanoparticles can be used as nano biosensor and colorimetric probe for amino acids. The petal extract AuNps and INH-AuNps also were found very good antimicrobial agents against the microorganisma Furthermore, the antioxidant activity of INH-AuNps and petal extract AuNps with the DPPH assay has been studied.

2. MATERIALS AND METHODS

2.1 Materials

All metal salts including Auric Chloride ($HAuCl_4$), Palladium Acetate (Pd Ac_2) and Isoniazid analytical standard, ≥99% (TLC) were purchased from Sigma-Aldrich. Amino Acids Reference Standard Kit were purchased from the SRL(46832), Other reagents and solvents of analytical grade were purchased from commercial sources and used without further purification. All aqueous solutions were prepared from Millipore water (resistivity, 18 ΩX; Millipore Systems). TLC plates (TLC Silica gel 60 F254) fluorescence active was obtained from the Merck. Absorption spectra were studied on a Jasco V-570 UV-Vis recording spectrophotometer. pH of the solutions was measured using pH analyzer LI 614- Elico. The Malvern Zetasizer (Model; ZEN3600) was used in order to estimate the particle size (e.g., hydrodynamic diameter) and for the zeta potential measurements by laser Doppler electrophoresis as such without dilution. TEM images were recorded in MACK/*model* JEOL, JEM 2100 at an accelerated voltage of 200 kV. A drop of dilute solution of a sample in water on carbon coated copper grids was dried in vacuum and directly observed in the TEM. The antimicrobial susceptibility of nanoparticles was evaluated using the disc diffusion or Kirby-Bauer method and zones of inhibition were measured after 24 hours of incubation at 35 °C.

2.2. Synthesis of isonazide protected gold nanoparticles (Scheme 1)

10 mL (1mM) solution of $HAuCl_4$ (1a) was added to a 50mL of conical flask containing 10 mL of water and then 5 mL (1 mM) aqueous solution of isonazid and 5 mL (1 mM) aqueous solution of PEG(poly ethylene glycol) (1b) was added rapidly under vigorous stirring. isonazide and PEG stabilized gold colloids (INH-AuNps) (1c) were obtained immediately but vigorous stirring was continued for 5 minutes to ensure complete homogenization. The color of the solution changes from yellow to ruby red which indicates the successful formation of gold nanoparticles. This INH-AuNps solution was then subjected to repeated centrifugation (3 times) at 14000 RPM, washed with a copious amount of deionized water to remove uncoordinated molecules and again redispersed in 50 mL deionized water to get solution of INH-AuNps for further studies.

2.2.1 Interaction of various amino acids with INH-AuNps by absorption spectroscopic measurements

1 mL (1 mM) aqueous solution of some random selective amino acids like arginine, cysteine, aspartic acid, glutamic acid, glutamine, leucine, methionine, thryonine, histidine, tryptophan and L-Dopa was added to 1mL (1 mM) solution of each INH-AuNps Furthermore, Absorption intensity of INH-AuNps interact rapidly in the presence of L-Dopa To evaluate their minimum detectable limit of L-Dopa was titrated against nanoparticles.

2. 3 Preparation of Plant Extration

(Hibiscus Rosa) **Rose mallow,** Jasud flower were collected from in botanical garden of Sarva Vidyalaya campus, Kadi, Gujarat. 100 gram of flower petals were washed with tap water ground and boiled with 500 ml of de-ionized water for 10 min. Finally the product was filtered and stored in freezer for further investigations. A 50% of flower petals extract was made up to 250 ml. Synthesis of nanoparticles using flower petals extracts, 1 ml of jasud flower petals extract was added to 100 ml of 1mM aqueous $HAuCl_4$/ Palladium acetate solution in a 250 ml Erlenmeyer flask. The flask was then kept overnight at room temperature. The Au nanoparticles solution thus obtained was purified by repeated centrifugation at 14,000 RPM for 15 min followed by re-dispersion of the pellet in de-ionized water **(Scheme 2).**

2.4 Stability Study of Nanoparticles

Effect of time and pH on stability of INH-AuNps

It has been found that pH has a great effect on the stability of nanoparticles. Therefore, the stability INH-AuNps was determined at different pH by measuring SPR. The pH of AuNps dispersion was adjusted using 0.1N hydrochloric acid and 0.1M sodium hydroxide solution (pH 4, 5, 6, 7, 8, 9 and 10) using calibrated pH

meter. Also the change in SPR of the INH-AuNps was recorded up to 60 days using UV-Visible spectroscopy.

2.5 Interaction of Amino acids with INH-AuNps by UV/Visible and spectroscopy measurements

We also investigated the interaction of amino acids with INH-AuNps by UV-Vis spectroscopy method. 1 mL (1 mM) aqueous solution of different amino acids like leucine, glutamic acid, glycine, tryptophan, aspartic acid, proline, cysteine was added to 1mL petal extract - AuNps. Individual set of experiment carried out with each amino acid reckoned that petal extract-AuNps showed hyper shift in surface plasmon resonance only in presence of L-Dopa.

2.6 Colorimetric detection of L-Dopa by using INH-AuNps

The metal nanoparticles are emerging as important type of colorimetric reporters because of their large extinction coefficients and tendency to agglomerate in the presence of analyte. Agglomeration leads to distinct colour change from red to blue.[53–57] and thereby making them very useful colorimetric sensing platforms. To investigate the colorimetric response of INH-AuNps, different amino acids like leucine, glutamic acid, glycine, tryptophan, aspartic acid, L-Dopa, proline, cysteine etc concentration were added to these nanoparticles. No visible colour change was observed with any of the metal ions except L-Dopa, which exhibits a sharp change in color from ruby red to purple, and finally to blue which can be easily judged by the naked eye (**Fig. 13**). The colour change with L-Dopa Amino Acids can be easily noticed even at nanomolar (nM) concentration in aqueous samples. It may be concluded that INH-AuNps can be used a remarkable selective colorimetric sensor for L-Dopa Amino Acids.In case of INH-PdNps their is no perfect change in colour change with anykind of Amino Acids.

2.7 Antioxident Study

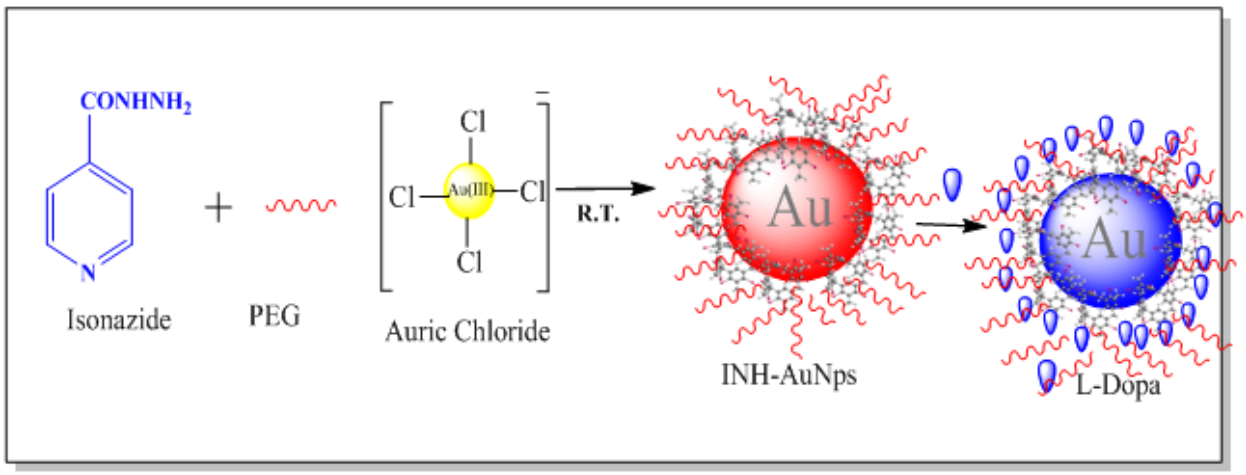
In vitro antioxidant assay by (1-1-diphenyl-2-picrylhydrazyl) DPPH radical quenching method

The antioxidant property of gold and palladium nanoparticles was evaluated by monitoring the ability of quenching synthetic stable DPPH radical into non-radical form.[44] The reaction mixture containing 2.7 mL of 2.5 mM DPPH was mixed with a solution containing (10, 50, 100, 200, 250, 300 µL) amount of nanoparticles and the total volume was kept constant, i.e. 3.0 mL. The reaction mixture was incubated at 37°C for 30 minutes and the colour change was measured spectrophotometrically at 518 nm. The radical-scavenging activity (RSA) was expressed in percentage of inhibition using the following equation:

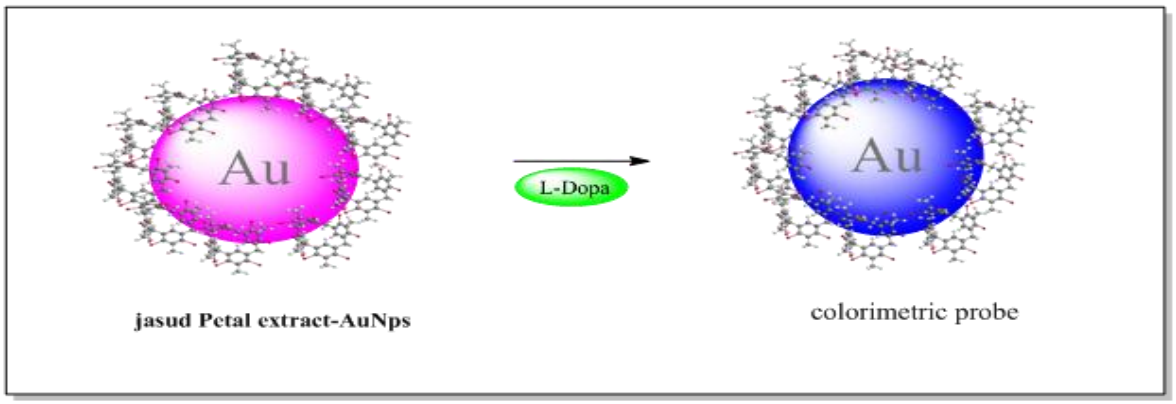
$$\%RSA = \frac{(A_{DPPH} - A_s)}{A_{DPPH}} \times 100 \quad \textbf{(Eq. 1)}$$

2.8 Antibacterial Activity of petal extract- AuNps (Scheme 3)

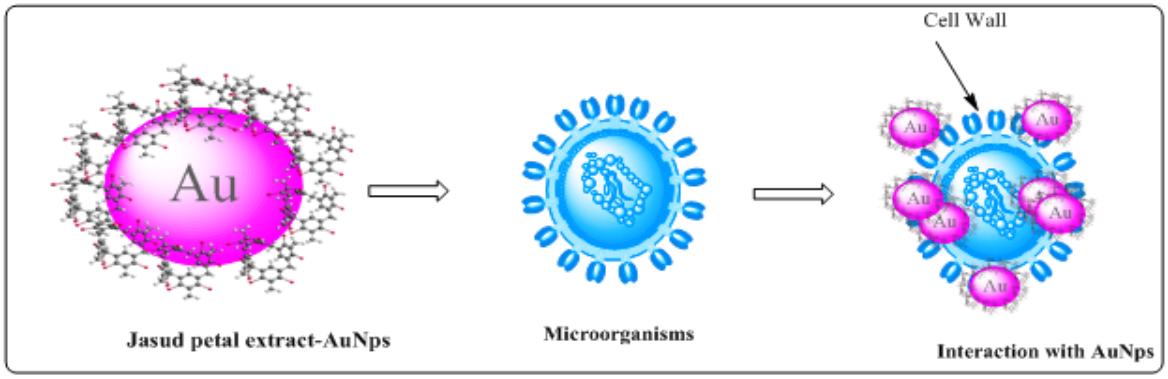
The antibacterial activity of gold nanoparticles was evaluated using the disc diffusion and Kirby-Bauer method.[45] The antimicrobial application of the INH-AuNPs and leaf extract- AuNps was carried out using both gram positive *Staphylococcus aureus, Bacillus subtilis* and gram negative *Escherichia coli* and Pseudomonas *aeruginosa*, The well diffusion method was used to study the antibacterial activity of the synthesized gold nanoparticles.[46,47] Sterile paper disc of 10 mm diameter containing gold nanoparticles and standard antibiotic **gentamicine** (100µg/ml) containing discs were placed in each plate as control. The plates were incubated at 30±4 ◦C overnight and the inhibition zones around the discs were measured.



Scheme 1: Schematic route of gold nanoparticles.



Scheme 2: Schematic representation of petal extract -AuNps and colorimetric probe for .dopa-L



Scheme 3: Proposed bacterial interaction with petal extract –AuNps.

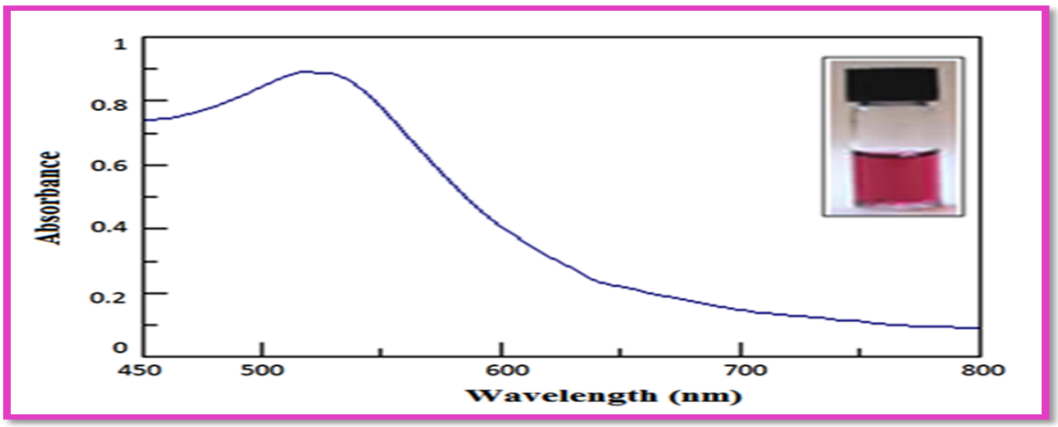


Figure 1: UV-Vis spectra of INH-AuNps.

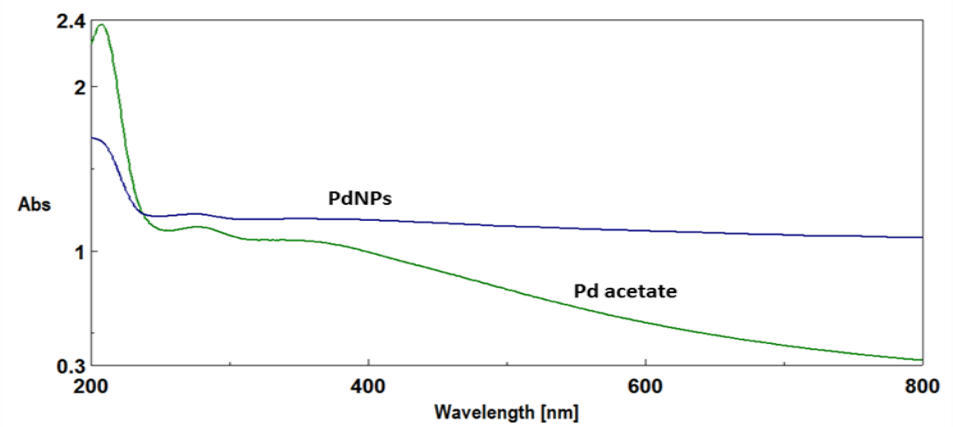


Figure 2: UV-Vis spectra of INH-PdNps.

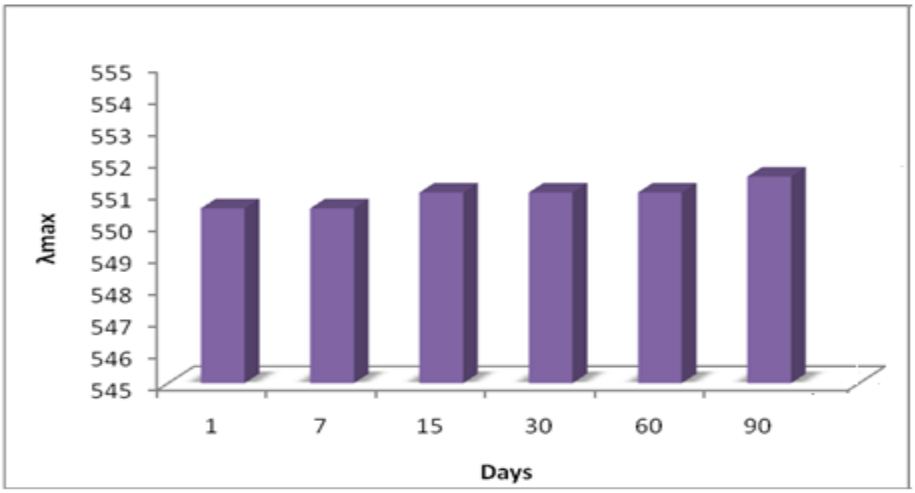


Figure 3: Stability of AuNps more than 90 days.

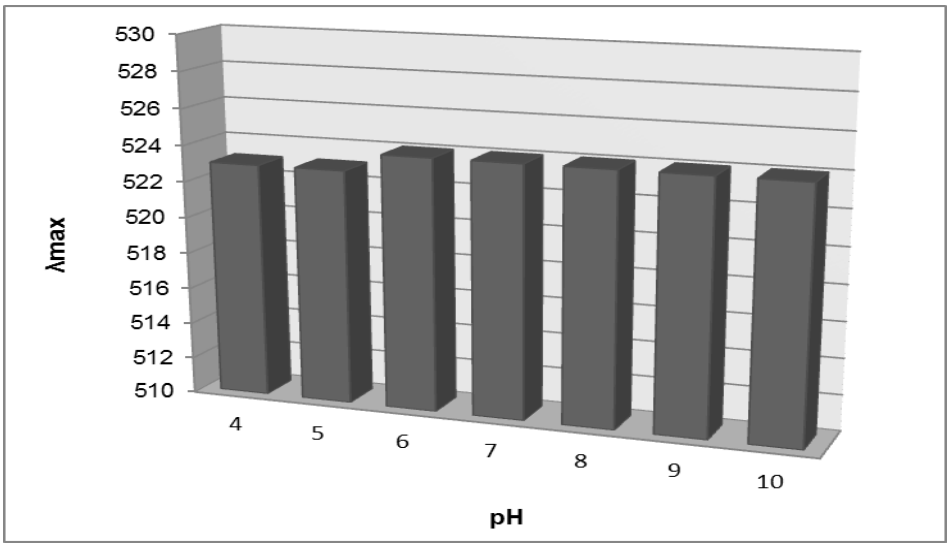
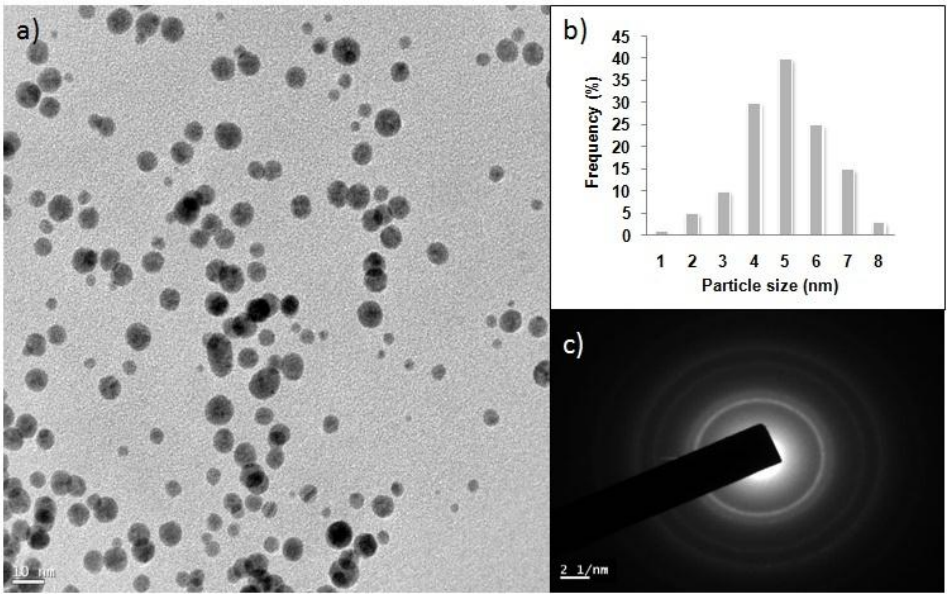
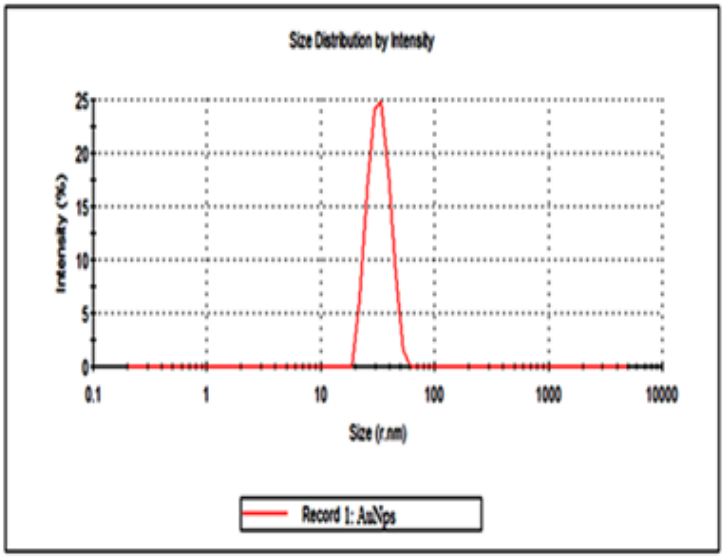


Figure 4: Graph represents stability of INH-AuNps at varied pH (4-10 pH).



L-dopa



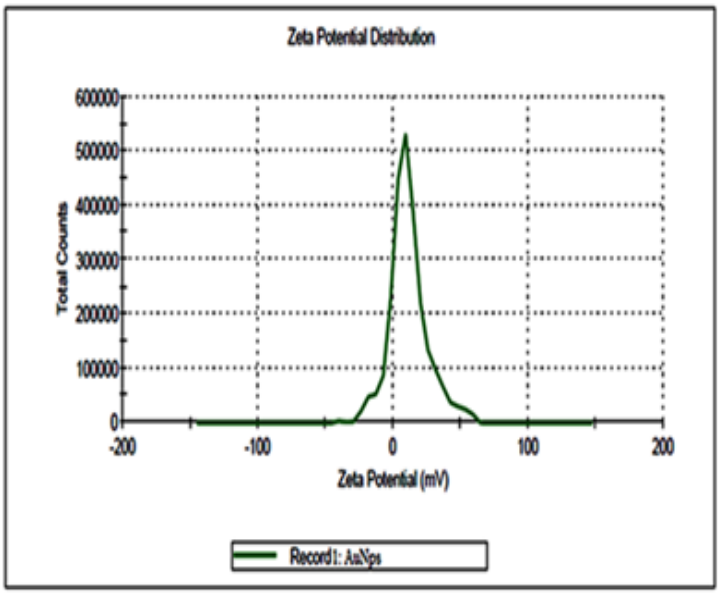
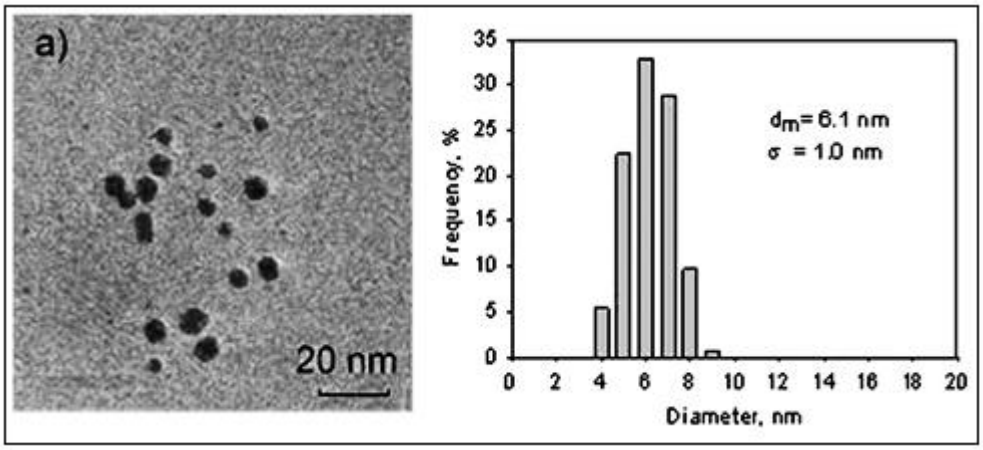


Figure 5: a) TEM image of INH- AuNps, b) particles size distribution graph c) electron differaction pattern And d) Size distribution by particles size analyzer, Zeta potential graph of gold.



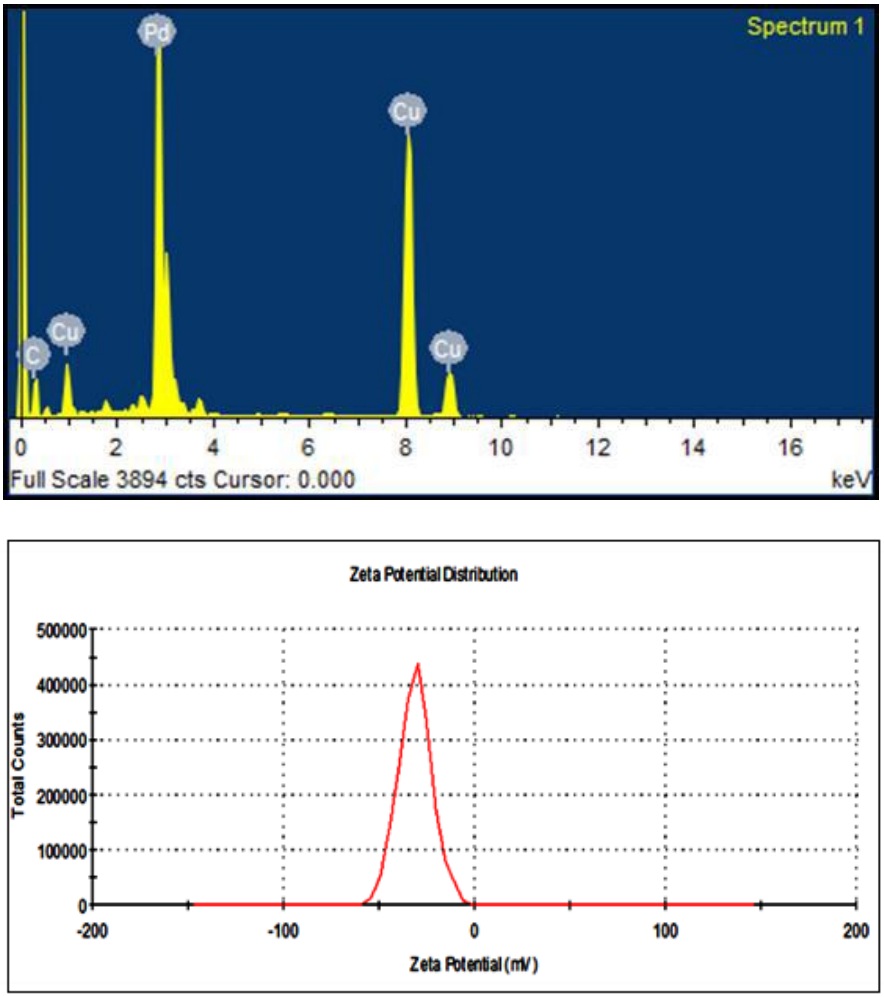


Figure 6: a) TEM images, histograms and electron differaction pattern of Palladium nanoparticles. b) Zeta potential graph of Palladium nanoparticles.

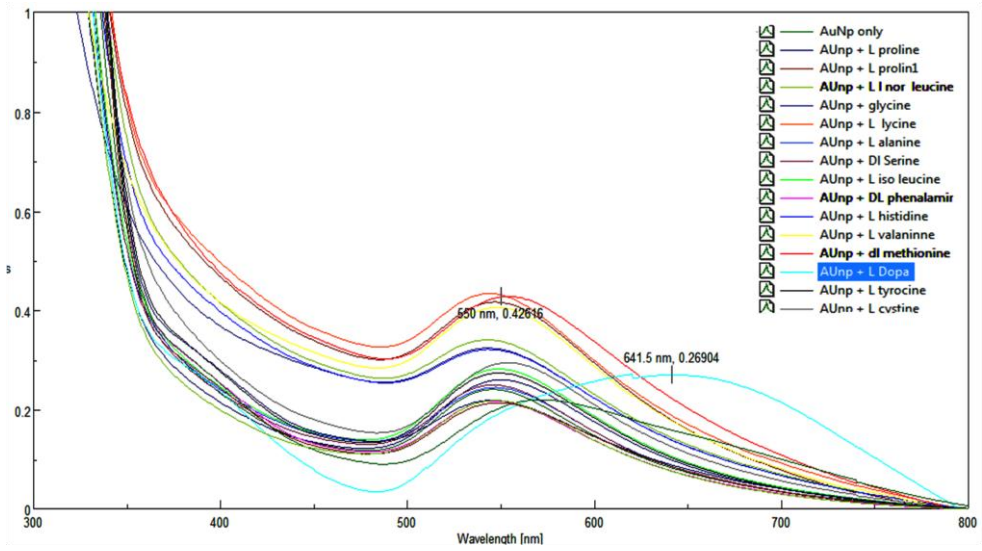
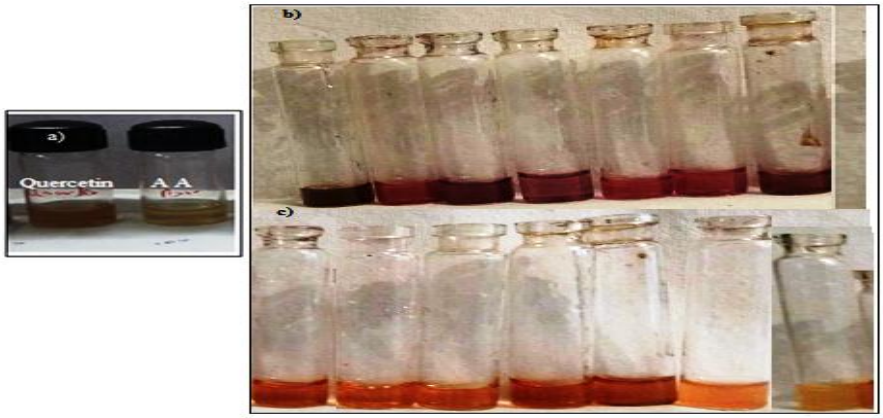


Figure 7: Interaction between amino acids with INH-AuNps by absorption spectroscopy.



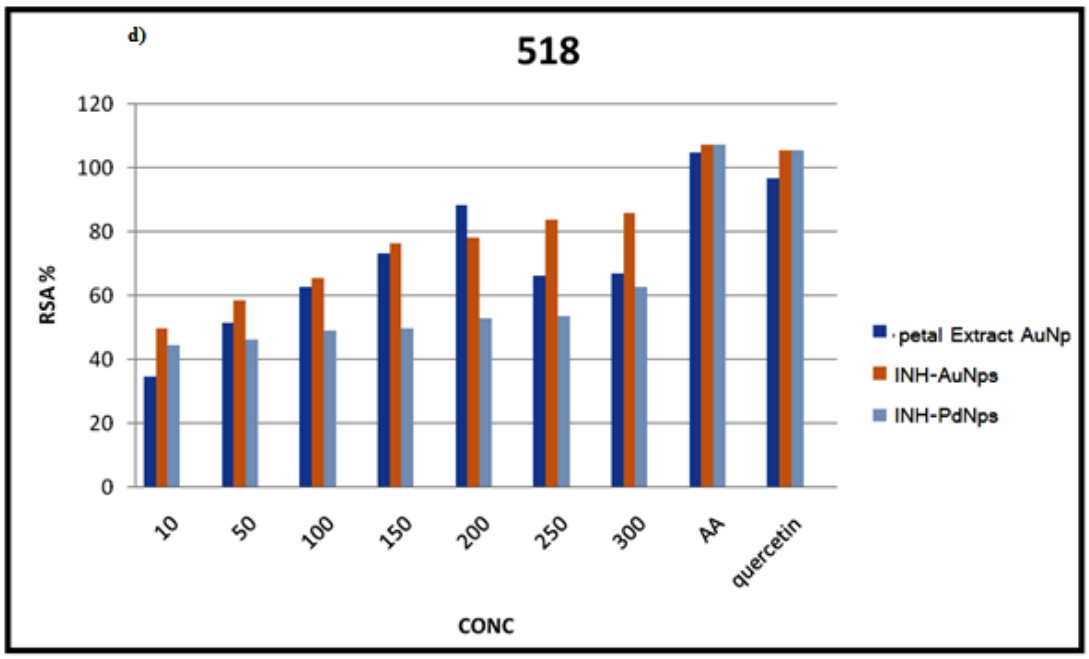
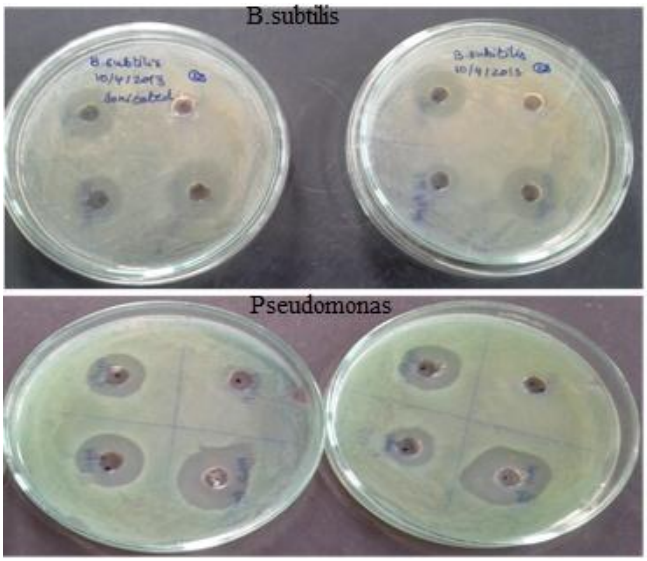


Figure 8: Antioxidant assay of DPPH with different concentration of: a) Standard (quercetin and ascorbic Acid), b) INH**- AuNps, c) INH-pdNps d) DPPH antioxidant assay at (λmax 518).**



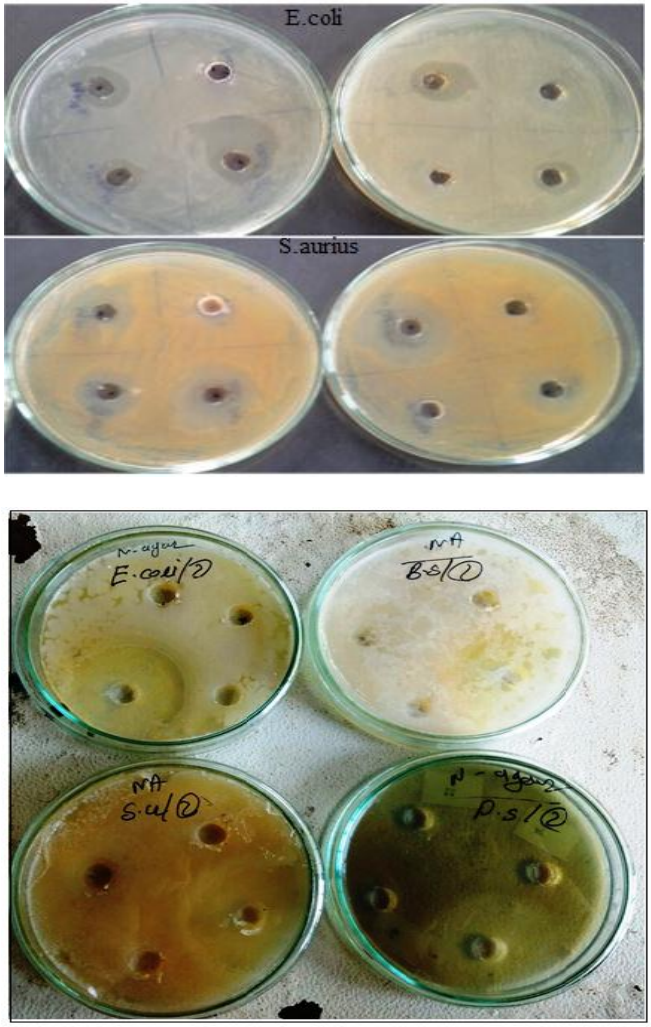


Figure 9: Antimicrobial Activity of AuNps & PdNps.

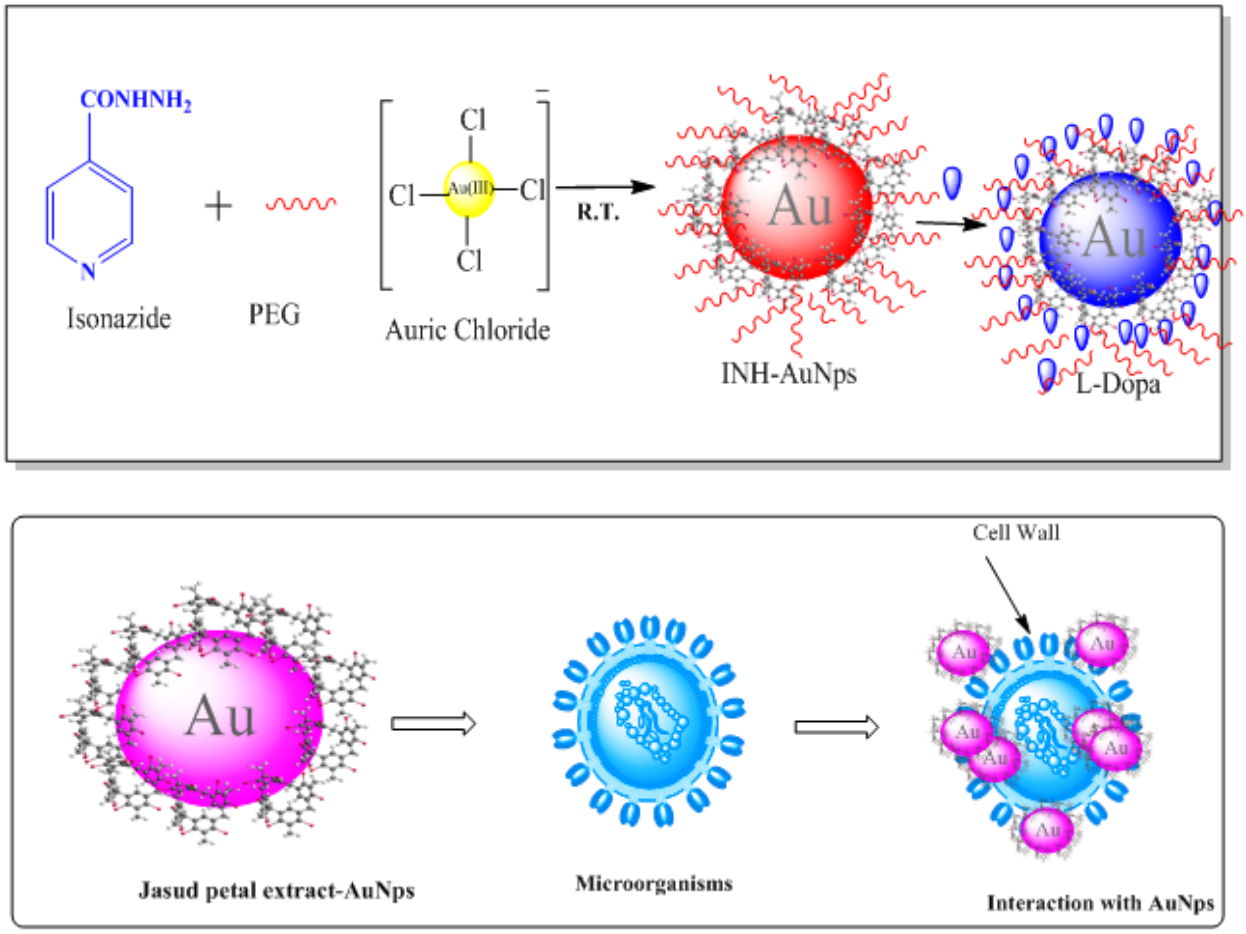
Figure 10: Scolorometric detection of amino acid with gold nanoparticle.

Table 1: Antimicrobial activity (Zone of inhibition in mm) of Compounds 1-2.

Name of compound	Zone of inhibition (mm)							
	B.subtilis		*S.aureus*		*E.coli*		*Pseudomonas*	
	100 ppm	100 ppm (sonicated)	100 ppm	100 ppm (sonicated)	100 ppm	100 ppm (sonicated)	100 ppm	100 ppm (sonicated)
Control	0	1	0	1	0	1	0	1
A*	13	16	16	7	11	13	15	15
1	9	12	8	12	5	8	8	13
2	11	12	8	13	6	10	9	14
3	05	06	04	05	04	07	06	05

Control Water
A* = **gentamicine** (Antibiotic control)
(1) Petal extract only.
(2) ePtal extract -AuNps.
(3) Petal extract –PdNps.

- Gold and Pd nanoparticle were synthesized by Isonazide (INH) and Hibiscus rosa leaf extract using as reducing, capping and stabilizing agent.
- The nanoparticle are water dispersed and stable
- INH-AuNps, INH-PdNps and biosynthesized -AuNps have also been studied for their antioxidant activity and antimicrobial Activity.
- The selective detection of amino acids by the INH-AuNps via absorption spectroscopy and colorimetric indicate that the synthesized nanoparticles can be used as nano biosensor, colorimetric detection with a potential prospect in the biomedical analysis.



3. RESULT AND DISCUSSION

3.1 Spectroscopic Study of Gold Nanoparticles

The as-prepared petal extract metal Nanoparticles (gold, and palladium) and INH-AuNp, INH-PdNps were primarily characterized by UV-Visible spectroscopy, one of the most widely used techniques for the structural characterization of nanoparticles. There is also a well established relationship between nanoparticles size and SPR band position i.e. with an increase in size of particle, plasmon resonance band shifting to the red and vice versa.[48] The synthesized metal Nanoparticles (Gold, & palladium) showed a single surface plasmon band at 525, and 550 nm respectively **Figure. 1,2.** And it remained same for more than three months **Figure. 3** indicating the presence of highly stable and spherical metal nanoparticles.[49] The pH dependent stability of jasud petal extract metal nanoparticle was studied at different pH from 4 to 7 by observing their absorption spectrum. No considerable change in absorption spectra of nanoparticles was observed at different pH range from 4 to 7 **Figure 4.** It was concluded that the maximum relative intensity of gold nanoparticels was 4.5 pH and hence, this pH was selected for further absorption related studies.

TEM and Particle size analyzer observations with their zeta potential values

The size distribution of the Au nanoparticles was characterized by two techniques, Transmission electron microscopy (TEM) and particle size analyzer (PSA). TEM image of (AuNps) and (PdNps) as shown in **Figure. 5** and **Figure.6** respectevly depicts that the synthesized gold nanoparticles are more or less mono dispersed with spherical or roughly spherical shape and

had an average diameter of 5 nm with majority of particles within the size range of 3 to 7nm. Also no aggregation or formation of large particles was observed by TEM image. As evident from the image **Figure. 5c** Selected area electron diffraction study (SAED) showed the presence of the (111) plane for gold nanoparticles. Hence, from the electron diffraction pattern it is clear that the particles are crystalline in nature.

The size distribution of the gold nanoparticles was also measured by Malvern's particle size analyzer (PSA). As shown in the **Figure. 5(a)** the particles are homogeneously monodispersed with an average particle size in the range of 15-22 nm. The statistical graph and size distribution Vs intensity has been shown in **Figure. 5(b)** the overall particles charge in a particular medium is denoted as their zeta potential value which is responsible for deciding the fate of stability. Here, synthesized gold nanoparticles had a 12 ± 4 MeV zeta potential values, PdNps had -20 ± 4 which is sufficient to keep the particles away from aggregation and maintained the stability **Figure. 6(a-c)**. The synthesized gold nanoparticles (INH-AuNps) were found to be the selective and sensitive probe, for the detection of L-Dopa **(Scheme 1)**, From the absorption spectra, it was observed that INH-AuNps showed a red shift in surface plasmon resonance only in the presence of L-Dopa in **Figure. 7**, The maximum absorption spectra of INH-AuNps was observed at 550 nm to 641.5 nm. To ensure high selectivity of synthesized nanoparticles for amino acids, the interaction of nanoparticles with different amino acids such as arginine, cysteine, aspartic acid, glutamic acid, glutamine, leucine, methionine, thryonine, histidine, tryptophan, and L-Dopa has been studied under similar conditions. The initial concentration of amino acids was taken 1 nM, which gradually increased to 100 µM and it was observed that the Red shift of INH-AuNps with the increasing concentration of L-Dopa **Figure. 7.** INH-AuNps were also found to be colorimetric chemosensors for selective signaling of L-dopa. **Figure. 10.**

3.2 Antioxidant activity of Gold Nanoparticles

Free radical is an unstable atom or molecule with an outer most electron unpaired and is highly reactive. The free radicals always strive to form a stable bond, by gaining or losing an unpaired electron. Nanoparticles of gold and silver based on reaction conditions are ready to accept/donate an electron to quench radicals.[42,44,50]

The DPPH is reduced by accepting the electron from nanoparticles, the DPPH reducing ability of gold and **palladium** nanoparticles was quantified spectrophotometrically by changing the DPPH colour from purple to yellow Figure. **8**. with standard antioxidant reference materials, quercetin and ascorbic acid (AA) were conducted Figure. **8**a. Although the antioxidant activity of **IN**H-A**u**Nps Figure. **8**b and **INH-Pd**Nps Figure. **8**c is slightly lower than that of standard antioxidant quercetin and ascorbic acid (AA), it is therefore, reasonable to propose that **IN**H-AuNps and **IN**H-**Pd**Nps hold the potential of their use as a good antioxidant agent. Figure. **8d.**

3.3 Antimicrobial study

Mechanism of action of gold nanoparticles

The Gold nanoparticles show efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release gold ions in the bacterial cells, which enhance their bactericidal activity (Scheme 2).[51-54]

The antimicrobial activity of INH-AuNps and *Hibiscus Rosa* Petal extract-AuNps is slightly less than that of standard gentamicine therefore it is reasonable to propose that INH-AuNps hold the potential of their use as good antibacterial agent Table 1. The INH-AuNps also exhibited the antibacterial activity against both gram positive *Staphylococcus aureus, Bacillus subtilis* and gram negative *Escherichia coli* and Pseudomonas *aeruginosa* and formed the zone of inhibition Figure. 9. Several researchers have reported the possible inhibitory action of gold nanoparticles on various bacterial strains,[55] reported that gold nanoparticles were preferentially bound and localized on the membrane of E. coli cells. The dissipation of the proton motive force of the membrane in E. coli occurs when nanomoles concentration of gold nanoparticles were given.[56] Bacterial growth inhibition around the well is due to the release of diffusible inhibitory compounds from gold nanoparticles. it can be concluded that synthesized gold nanoparticles had significant antibacterial action on both the Gram classes of bacteria.[57]

CONCLUSION

In summary, a rapid, simple and one pot synthesis of AuNps and PdNps was developed using *Hibiscus Rosa* petal extract and Isonazide drug with PEG act as a reducing, stabilizing as well as capping agent respectively.The Biogenic and drug capped nanoparticles were found to be highly stable over a long period of time.

In addition, the interaction of different amino acids like Arginine, Cysteine, Aspartic Acid, Glutamic Acid, Glutamine, Leucine, Methionine, Thryonine, dopamine, L-dopa Tryptophan with synthesized gold nanoparticles (INH-AuNps) have been studied by Uv-vis spectroscopy. Using INH-AuNps as a selective and sensitive probe, L-dopa could be detected at a minimum concentration level of 10 µM in a facile way of complexation of spectroscopy. INH-AuNps were found be colorimetric chemosensors for selective signaling of L-dopa.

Further, the AuNps, PdNp showed high antimicrobial and bactericidal activity against bacteria such as

Escherichia coli, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas* found highly strain. In addition, the antioxidant activity of INH-AuNPs and INH-PdNps were also successfully evaluated to show free radical scavenging activity up to 75 % in 35 minutes, which is relatively higher in comparison to other metal nanoparticles.

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