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Green Synthesis of Highly Stable Gold Nanoparticles using *Momordica charantia* as Nano fabricator

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ABSTRACT

The facile synthesis of ultra stable gold nanoparticles (GNPSs) is demonstrated using fruit peel extract of Momordica charantia. The best parameters for the synthesis of gold nanoparticles were pH10, high temperature (100^0C) and 100 ppm aurochlorate salt. The results were verified using UV-Vis spectroscopy, XRD and Transmission electron microscopy. The GNPSs were monodisperse and found to be 10-100 nm in size. The stability of the GNPs synthesised using biological protocols was found to be extremely high than the chemically synthesised GNPs when tested using 5M NaCl solution. The Nitrate reductase activity was found to be 0.1667 µmole/min/gram of plant tissue which got reduced to 0.0132 µmole/min/gram in the solution after the formation of gold nanoparticles. The protein content got depleted after the formation of GNPSs in the solution from 214.12 mg/ml to 64.42 mg/ml.

Keywords: Biosynthesis, *Momordica charantia*, Gold nanoparticles, Nitrate reductase.

INTRODUCTION

Synthesis of metal nanoparticles using biological systems as an efficient sink has grabbed exceptional attention due to their anomalous optical [1], chemical [2], photo electrochemical [3], and electronic [4] properties. Unlike chemical protocols which demands expensive instruments and results in release of inimical chemicals, biological method is more facile, eco-friendly and results in more monodispersed nanoparticles. Gold salt when exposed to aqueous extracts of plant have resulted in the intra-cellular as well as extra cellular formation of metal nanoparticles. The rate of formation for nanoparticles and therefore the size of the nanoparticles could, to an extent, be manipulated by controlling parameters such as pH, temperature, substrate concentration and exposure time to substrate [5].The reason for selecting plant for Biosynthesis is because they contain reducing agents such as Citric acid, Ascorbic acids, flavonoids, reductases and dehydrogenases and extracellular electron shuttlers that may play an important role in biosynthesis of metal nano particles.

In the present work, use of clear aqueous extracts of fruit of *Momordica charantia* is tried. *Momordica charantia*, commonly known as Karela or bitter gourd is a tropical and subtropical vine of the family Cucurbitaceae. It contains an array of biologically active plant chemicals including triterpenes, proteins and steroids. Various parameters like optimum reaction temperature, pH, and time required for the synthesis of metal nanoparticles, concentration of gold salts as well as plant extracts were taken into consideration. Monodispersity and stability of the Metal nanoparticles over a stipulated period of time were also one of the important considerations.

MATERIALS AND METHODS

Materials: *Momordica charantia*, commonly known as bitter gourd was procured from local market. Gold aurochlorate was procured from sigma Aldrich, USA. The experiments were performed in double distilled water (18 MΩ). The glasswares were washed with aqua regia to remove the traces of metal contaminant. In order to record the temperature, thermocouple was used.

Preparation of Plant Extract: To prepare the extract, 10 gram peel of *Momordica charantia* fruit was crushed in 10 ml of distilled water using mortar and pestle. The extract obtained was centrifuged at 10,000 rpm for 15 minutes. The supernatant was used as reducing agent for synthesis of gold nanoparticles. In order to retain the activity of the enzymes and other factors such as glutathione and Phytochelatins, the extract was made in ice box.

Procedure for Biosynthesis of the Gold Nanoparticles: Clear extracts of *Momordica charantia* was used for the biosynthesis of gold nano particles (GNPs). A stock solution of 50,000 ppm aurochlorate was prepared and diluted as per the pre-requisite of the experiment. The required amount of aurochlorate salt was added in a boiling solution of reaction vessel containing plant extract. In order to optimise the nanoparticle formation, the impacts of pH (4, 6,8,10 & inherent) on synthesis of GNPs were studied at low temperature (30^0C) and high temperature (100^0C). The parameters obtained from the above two experiments were kept constant to comprehend the impact of temperature and salt concentration on the optical as well as morphological features of GNPs.

Characterization of the Biosynthesized Gold Nanoparticles

UV- Vis Spectroscopy of the gold nanoparticles- The UV-Vis spectra of the GNPS formed were recorded using dual beam spectroscopy Lambda 25 Perkin Elmer, USA. High quality quartz cuvette (Perkin Elmer optics, USA) was used as a vessel to record the spectra.

Transmission electron micrographic Analysis-To elucidate the morphology of the GNPS biosynthesised using *Momordica charantia* plant extract high resolution transmission electron microscope (HRTEM), Carl Zeiss Micro imaging, GmbH, Germany, was used. Sample was ultrasonicated for 15 minutes and then coated on ultraclean carbon coated copper grid for analysis. The SAED pattern of the gold nanoparticles indicates presence of crystalline GNPS as deciphered using the diffraction pattern using X-rays.

X-Ray diffraction studies (XRD)-To peep into the crystallinity and the lattice properties of the GNPs, XRD (P Analytical, Philips PW 1830, The Netherlands) operating at 40 kV and a current of 30 mA with Cu Kα radiation (λ = 1.5404 Å) was used. The colloidal suspension containing metal nanoparticles was dirtied on a small glass slab.

Nitrate Reductase Assay and Total protein Estimation – was done to assay the possible role of nitrate reductase as Reducing agent, using standard Vega and Cardenas [6] method with few variations and the total protein content was estimated using Bradford's method.

RESULTS AND DISCUSSION

Impact of different pH on formation of GNPs at 30 and 100°C are presented in table -1 which shows that pH 10 at 100°C gives best results and hence the further optimisation of other parameters was done using same above parameters.

Visual observations: After addition of the gold salt solution in plant extract, the colour changed from colourless to wine red indicating the formation of GNPs [7] . This is due to the fabrication of GNPs with the molecular assistance of biological reducing agents present in the plant extract. The tenure for the appearance of the colour varied drastically with the temperatures. At 30°C, more than 24 hours were required for the complete reduction; whereas at 100°C the colour appeared in less than 5 seconds. The clear colloidal suspension of GNPs were stable for more than two months at 4°C.The GNPs fabricated at higher pH values (6, 8 & 10) were exceptionally stable in contrast to those synthesized at acidic pH. The stability of the GNPs synthesized at pH 10 at 100°C was reluctant for coagulation even after addition of several millilitres of 5M NaCl solution (Data not shown).

UV-Vis Spectroscopic analysis: As shown in UV-Visible spectra (fig .1), the SPR bands centered between 500-600 nm confirms the formation of GNPs in the solution. The appearance of the peak is due to the size dependant quantum mechanical phenomenon called Surface Plasmon Resonance (SPR).This effect becomes influential when the De-Broglie wavelength of the valence electrons becomes equal to or less than the size of the particle (less than 50nm) [8]. At 30°C, the SPR band of the GNPs synthesized at pH 4, 6, 8, 10 and inherent were found to be centered between 536 to 550 nm. The most influential pH affecting the synthesis of GNPs was found to be 10.This can be

speculated because of a sharp peak centered at 536 nm. As per the previous studies, the size of the nanoparticles exhibiting a peak at this wavelength is between 30-40 nm. There was a minor blue shift in SPR of GNPs synthesized at inherent pH 5.6 (from 536 nm to 532 nm) and another peak appeared in near infra red region (670 nm) as shown in table 1.The appearance of dual peak at 532 and 670 nm may be due to [9].

- **Formation of anisotropic nanoparticles**
- **Agglomeration of the nanoparticles**
- **The combined effect of both the phenomenon**

Table – 1: Impact of pH and Temperature on the Biosynthesis of GNPs using 100 ppm Aurochlorate and *Momordica charantia* fruit peel extract

Ph	Temperature	
	30˚C	**100˚C**
4	• Change in colour in 24 h • Peak of moderate intensity at 540 nm • XRD Crystalline structure	• Change in colour in < 5 sec • No peak ,flat absorption spectra • XRD Crystalline structure
6	• Change in colour in 24 h • Peak at 536 nm • XRD Crystalline structure	• Change in colour in < 5 sec • Broad curve at 531 nm • XRD Crystalline structure
8	• Change in colour in 24 h • Peak of moderate intensity at 541 nm • XRD Crystalline structure	• Change in colour in < 5 sec • Good peak of moderate intensity at 540 nm • XRD Crystalline structure
10	• Change in colour in 24 h • Sharp peak at 536 nm • XRD Crystalline structure • **TEM-spherical polydispersed nanoparticles**	• Change in colour in < 5 sec • Sharp intense peak at 551 nm • XRD Crystalline structure • **TEM-Spherical nanoparticles with less monodispersity**
pH of plant extract 5.6	• Change in colour in 24 h • Dual peak at 532nm and 670 nm • XRD Crystalline structure	• Change in colour in < 5 sec • Good peak at 532 nm • XRD Crystalline structure

Table – 2: Impact of different temperatures on the Biosynthesis of GNPs using 100 ppm Aurochlorate and *Momordica charantia* fruit peel extract at pH 10.

Temperature	Observations			
	Visual	**UV-Vis Peak**	**XRD**	**TEM**
4 ºC	Change in colour in > 24 h	Flat absorption spectra	Crystalline	-
RT (28 ± 2ºC)	Change in colour in < 24 h	Minor peak at 675 nm	Crystalline	Spherical poly dispersed nanoparticles.
60 ºC	Change in colour within 10 min	Broad peak at 545 nm	Crystalline	Roughly spherical monodispersed nanoparticles
100 ºC	Change in colour in <5 sec	Sharp peak at 538 nm	Crystalline	Spherical nanoparticles with less monodispersity

Table- 3: Impact of aurochlorate concentration on synthesis of GNPs using extract of *Momordica charantia* at pH 10

Concentration of Aurochlorate	Observation	
	Visual	**UV-Vis Peak**
50 ppm	Change in colour in < 5 sec	Flat absorption spectra
100 ppm*	Change in colour in < 5 sec	Sharp peak at 537 nm
150 ppm	Change in colour in < 5 sec	Sharp peak at 540 nm
200 ppm	Change in colour in <5 sec	Broad peak at 535 nm
250 ppm	Change in colour in< 5 sec	Medium intensity peak at 548 nm

**TEM of the sample exhibited Spherical gold nanoparticles of size less than 50 nm.*

Due to the combined impact of agglomeration and orientation of the nanoparticles in the solution, there was a red shift observed at pH values 4 and 8 with respect to 536 nm observed at pH 10 (table 1).There was no peak shift observed at pH 6.The GNPs formed in the solution at alkaline pH were highly stable as predicted by addition of 5M NaCl. However, the GNPs synthesized at pH 10 were exceptionally stable. The stability of the nanoparticles at pH 10 may be due to the optimal activity of the enzymes and capping proteins. Moreover, the electrical double layers (such as Stern and Goy- Chapman) around the nanoparticles must be more stabilised at this pH.

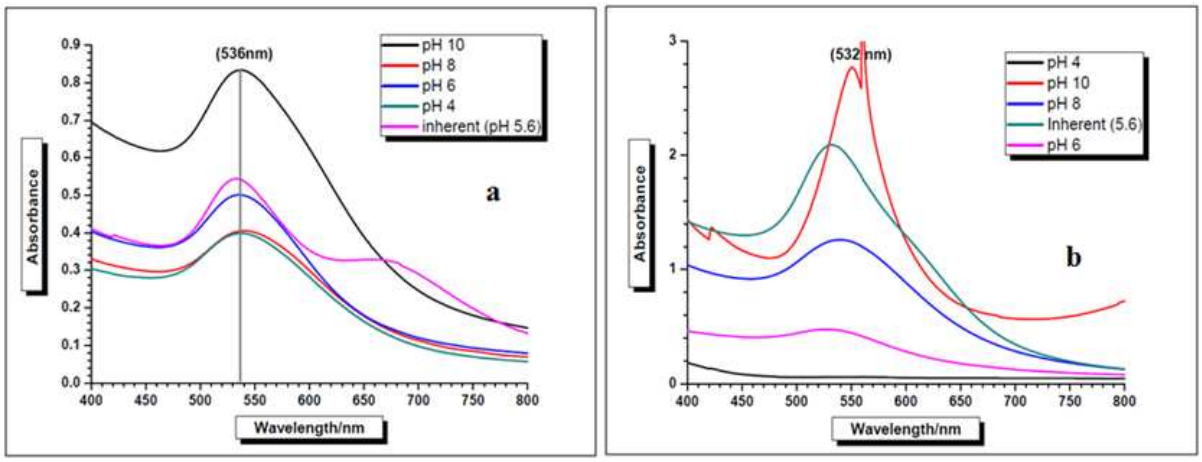

Figure- 1: Impact of temperature and pH on synthesis on GNPs using extract of *M. charantia*; as recorded by the UV-Vis spectroscopy of the sample synthesized at (a) 30°C (b) 100°C.

At 100°C, the SPR band was centered at 532 nm at pH 10 was more stable for over a week(Fig 1 b). At pH value 4 and 6 a flat spectrum and a broad hump was observed respectively.This indicates the formation of larger and polydispersed GNPs in the solution.This may also indicate the agglomeration of the nanoparticle. This may be due to the destruction of the enzymes and capping agent present in the plants which catalyse the capping of the gold and other metal nanoaprticles in order to make them stable in the solution. Moreover, the dielectrics of the medium at high temperature and destabilisation of the electrical double layers may also be additive factor in aggglomeration of the GNPs.

The possible impact of temperature and concentration of the aurochlorate salt in catalysing the formation of GNPs in aqeous extract of *Momordica charantia* was studied at pre-optimised pH value (pH 10) at 100ºC.From the UV-Visible spectra displayed in fig 2.a, its can be clearly comprehended that the most favourable temperature is 100ºC.The finding is in agreement with our previous results.At 100ºC a sharp peak was observed at 538 nm which depicts the formation of 20-30 nm nanoparticle as per the previous studies.At all the other temperatures (4, 28±2 & 37º C) flat spectra were observed (Fig.2.a).A possible explanation for the above phenomenon may be due to the activity of capping proteins,enzymes as well as the thermodynamic stability of the electrical double layers around the GNPs.

The most efficient concentration of aurochlorate salt was found to be 100 ppm as depicted by a sharp peak at 537 nm (Fig 2.b).There was a minor red shift in SPR bands observed in GNPs synthesized at other concentrations.This red shift is due to the increase in the particle size as well as decrease in the inter particle distance of GNPs in the solution.

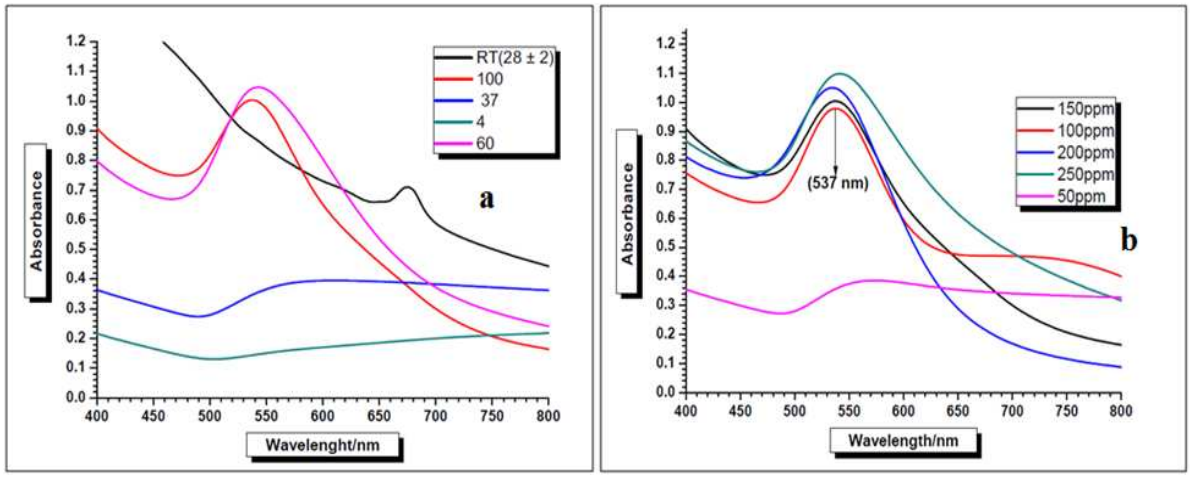

Figure- 2: (a) Impact of temperature on synthesis of GNPs using extract of *M. charantia* at pH 10,100 ppm aurochlorate; (b) Impact of aurochlorate concentration on synthesis of GNPs using extract of *M. charantia* at pH 10.

Stability of Biogenic Vs chemically synthesized Gold nanoparticles

To solve the problem of agglomeration of GNPs in solution, particularly when it is suspended in high salt concentration for clinical uses such as drug delivery, the stability of biological nanoparticles was tested against very high salt concentration. As shown in the figure 3 a, there was a red shift of 28.02 nm after addition of approximately 5 ml of 5M NaCl. In stark contrast to this, the shift in chemically synthesized nanoparticles using same parameters was found to be 130.67 nm after addition of merely 100µl of 5 M NaCl. This exceptional stability of biogenic nanoparticles can be attributed to protection of GNPs by intelligent capping proteins. Under optimal ionic strength of the solution these proteins avoid the columbic attraction between the nanoparticles by maintaining suitable surface potentials.

High resolution transmission electron microscopic (HRTEM) studies:

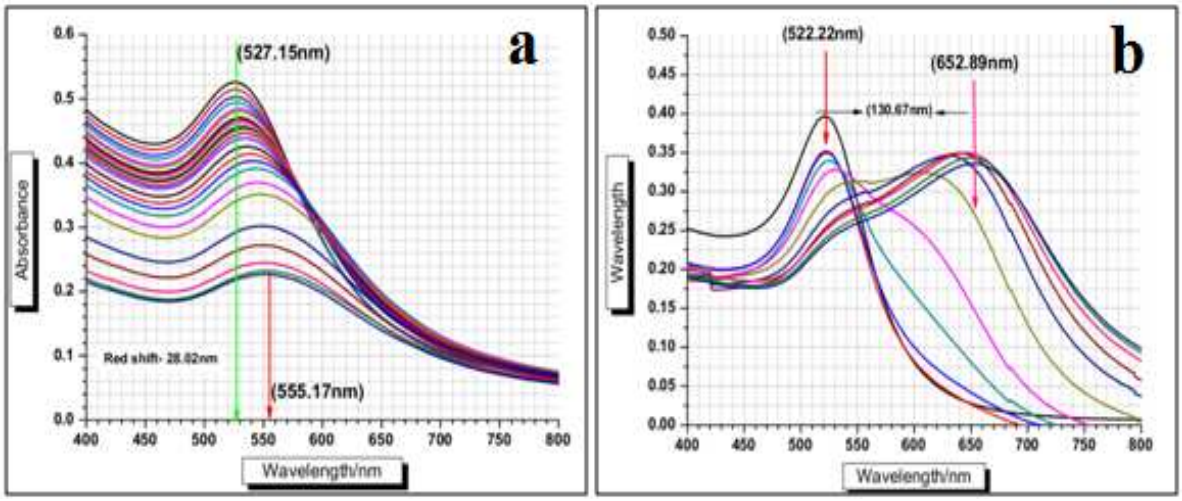

Figure3: UV-Visible spectra showing the stability of (a) Biogenic nanoparticles using *M. charantia* synthesized at pH 10, 100° C & 100 ppm of aurochlorate (b) Chemically synthesized nanoparticles using the same parameters used for biogenic nanoparticles.

TEM image of the GNPs synthesised at 30°C at pH 10 shows the presence of spherical polydispersed nanoparticles ranging from 10 to 100 nm (fig 4.a). The results were in accordance with the UV-Vis spectroscopy. The TEM image of the gold nanoparticles synthesised at 60°C at pH 10 shows (fig. 4.b) nanoparticles which are monodisperse and roughly spherical ranging from 30-100 nm. Some uniquely shaped nanoparticle can also be seen in the image. This may be due to growth at unusual facets (any of 111, 200) which results in the formation of anisotropic (non-spherical) nanoparticles [9. At very high temperature such as 100 °C, pH 10 was most competent parameter. As depicted in HRTEM image (fig 4.c), the nanoparticles are spherical with less monodispersity. Agglomeration in the nanoparticles can be seen due to the high temperature which results in the destruction of the stabilising protein. The SAED pattern shows that the GNPs is crystalline in nature (Fig 4. d).

X- Ray Diffraction studies (XRD):

To elucidate crystallinity and the lattice properties of the GNPs, XRD (P Analytical, Philips, Netherlands) was performed. The colloidal suspension containing metal nanoparticles was dirtied on a small glass slab. XRD spectra confirm the GNPS formation and also the presence of 111 facets (Fig. 5). X-ray diffraction (XRD) analysis of a droplet of the mixture on a glass cube showed intense peaks at (111), (200), (220) and (311). Bragg reflections in the 2 θ range 30°-80 ° as shown in figure 4; this is in agreement with the previous data available on gold nanocrystals [10].

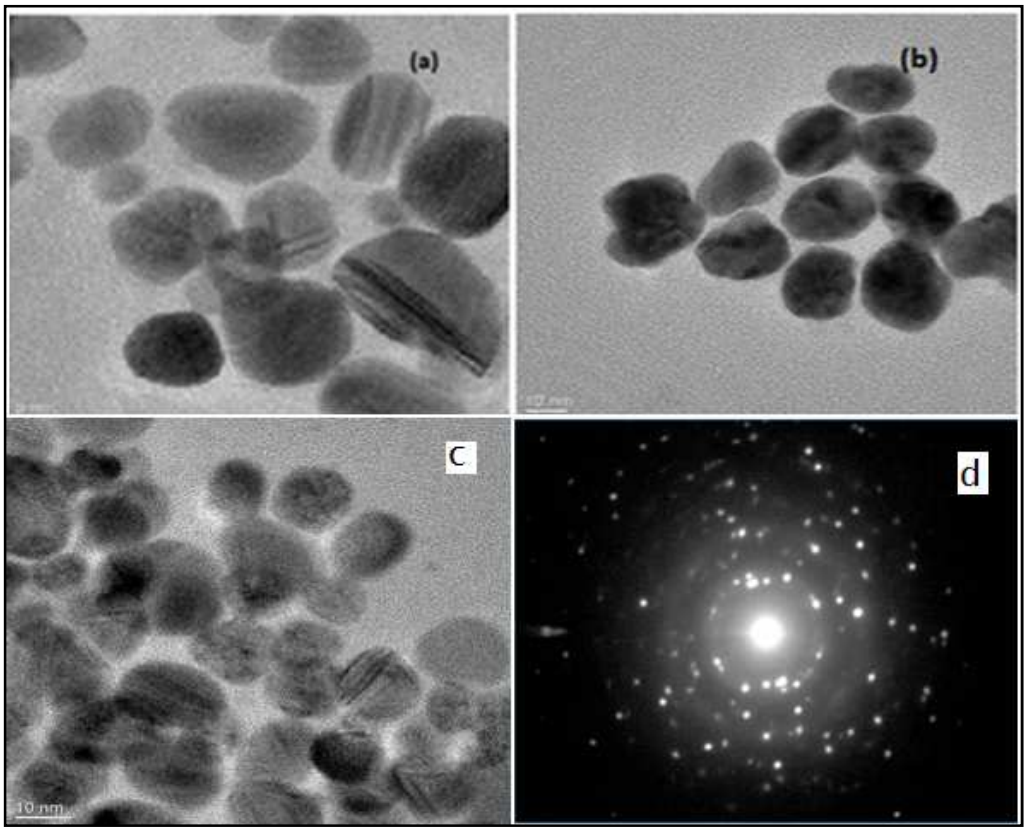

Figure- 4: HRTEM of GNPs synthesized using extracts of *M. charantia* at 30°C, pH 10 (b) 60°C, pH 10 and (c) 100°C, pH 10 and (d) The selective area energy dispersion (SAED) pattern showing that the nanoparticles are crystalline.

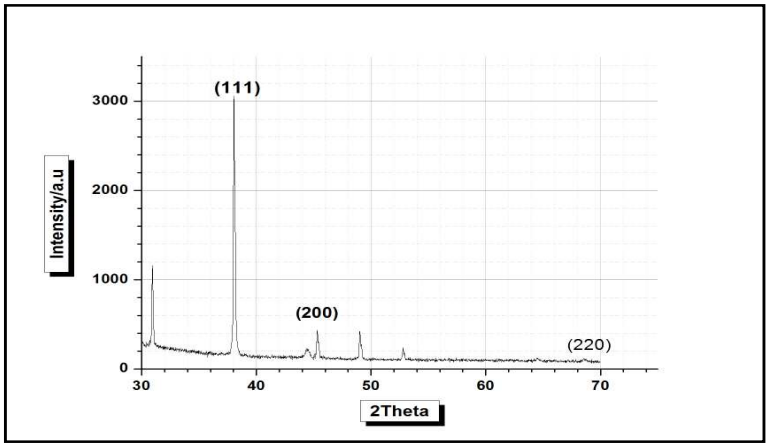

Figure- 5: X-ray diffraction pattern of gold nanoparticles biosynthesised using *Momordica Charantia*

Nitrate Reductase Assay and Total Protein Estimation: It was found that the fruit peel extract of *Momordica charantia* exhibited the Nitrate reductase activity as 0.1667 µmole/min/gram of plant tissue, which got reduced to 0.1654 µmole/min/gram of plant tissue when it was subjected to 100° C.(Fig.6 a) After the formation of GNPs, the nitrate reductase activity was again assayed in the reactant mixture which showed a substantial decrease (0.0132 µmole/min/gram) in the solutions having gold nanoparticles as compared to nitrate reductase activity in plant extracts without gold nanoparticles. This result validates the possible involvement of reductases in the reduction of gold ion to GNPs. Total Protein concentration in *Momordica charantia* (peel extract) was found to be 214.12 mg/ml and in gold nanoparticles synthesized from *Momordica charantia* peel was found to be 64.42 mg/ml it means concentration of protein reduced after gold nanoparticle synthesis.(Fig.6 b)

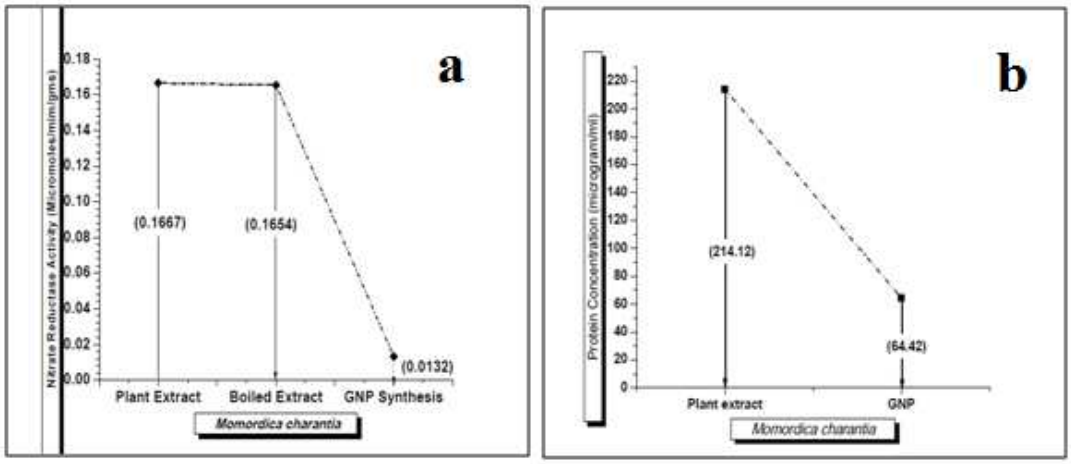

Figure 6: (a) Nitrate reductase activity of *Momordica charantia* peel extract, Boiled plant extract and gold nanoparticles respectively in µmoles/min/gm (b) Total protein concentration in *Momordica charantia* plant extract and gold nanoparticles respectively in mg/ml

CONCLUSION

The optimum conditions for biosynthesis of crystalline GNPs are observed to be at pH 10,100°C and 100 ppm aurochlorate. The GNPs synthesized using *Momordica charantia* can be efficiently used for surface orchestrations using plethora chemical linkers used for drug delivery owing to its excellent stability in high salt concentrations. Moreover, such capped nanoparticles can also be used without attachment of any linker for synaphic delivery of active pharmaceuticals ingredients to specific organs. Depletion of the Nitrate reductase activity in the solution containing GNPs confirms its molecular role in nanoparticle biosynthesis.

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