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ORIGINAL ARTICLE **OPEN ACCESS**

Comparative study of Biosynthesized silver and gold nanoparticles using Peel extract of *Momordica Charantia* and their anti-microbial efficacy

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ABSTRACT
Nanotechnology is a rising field in science and innovation, which can be connected to integrate new materials at the nanoscale level. The present study aimed at comparing the synthesis, characterization and in vitro microbial efficacy of synthesized silver and gold nanoparticles using peel extract of Momordica Charantia. Silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) were synthesized using aqueous extract of peel with solution of silver nitrate ($AgNO_3$, 1mM) and chloroauric acid ($HAuCl_4{\cdot}3H_2O$, 1mM) respectively. The synthesized nanoparticles were characterized using UV–visible spectrophotometry, Fourier transform infrared spectroscopy, scanning electron microscopy, energy-dispersive analysis of X-rays, X-ray diffraction, which confirmed the reduction of Ag^+ ions to Ag° and Au^{3+} ions to Au°. The in vitro antimicrobial efficacy of AgNPs, AuNPs and aqueous extract of peel confirmed by disc diffusion assay. The Zone of inhibition indicate that both silver and gold nanoparticles exhibited comparable antimicrobial efficacy.
Keywords:- Biosynthesis , AgNPs, AuNPs, streptomycin, Nystatin and Momordica charantia .

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INTRODUCTION
Nanoparticles have multifunctional properties and are very useful in various fields such as medicine, nutrition and energy [1]. Metal nanoparticle have found an increased interest because of their variation in magnetic, optical and electrical properties as they are dependent on their size, surface Plasmon, surface free energy and surface area. Hence, the size shape dependent properties of metal nanoparticles was used for technical applications ranging from, optics [2], electronics [3,4] sensing to catalysis [5].
Different types of nanomaterials have been synthesized including copper, zinc, titanium, gold and silver. But nanoparticles like gold and silver are currently under intensive study for applications in optoelectronic devices, highly sensitive chemical and biological sensors and as catalysts [6].
Silver and gold nanoparticles have several applications from electronics and catalysis to biology and therapy in biology, pharmaceuticals and medicine. The antimicrobial action of silver(Ag) ions is well-known but the antimicrobial action of elemental silver was developed in the form of nanoparticles. Silver nanoparticles have been tested for anti-microbial activity against yeasts, *E. coli, S. aureus* etc [6].
A matrix may help gold nanoparticles act as a ideal catalyst. The gold nanoparticles (AuNPs) have been used to alleviate unique interactions between DNA and anti-cancer drugs. Nanoscale drug delivery systems are able to boost pharmaceutical product distribution. This may result in beneficial use of metal nanoparticles in the relative biomedical field [7].
Momordica charantia is eaten throughout the world, the peels are usually discarded after consumption of the pulp. A few applications of *Momordica charantia* peels discussed in the literature include (i) Liver tonic (ii) Diabetes (iii) prevents skin problems (iv) Cancer (v) Heart diseases. In addition, bitter gourd peels which are naturally rich in alkaloids, tannin, steroids, and glycosides could be used in the synthesis of nanoparticles of silver and gold.
The present study focuses on the synthesis of silver and gold nanoparticles by a green biological process, using an extract derived from bitter gourd peel waste, and characterization of synthesized nanoparticles

using UV-visible spectroscopy, scanning electron microscope (SEM), energy dispersive X-ray spectroscopy (EDX), X-ray diffraction(XRD), and Fourier transforming infrared spectroscopy (FT-IR). In addition, their antimicrobial activity has been examined against various human pathogenic microorganisms.

MATERIAL AND METHODS

Both media and analytical reagents were bought at Hi-media pvt. Ltd. Ltd.

Source and Maintenance of bacterial culture-

Bacterial strains namely *Bacillus cereus* MCC 2039, *Pseudomonas aeroginosa* MCC 2080, *Esherichia coli* MCC 2246, *Staphylococcus aureus* MCC 2408 and *Klebsiella MCC 2570*, *Candida albicans MCC 1151* and *Aspergillus niger MCC 1138 were* obtained from the Culture Collection Center National Centre for Cell Science, Pune. The cultures were maintained by repeated subculturing on nutrient agar slants. 24 hour culture was prepared for each experimental procedure [8].

Preparation of peel extract

The peel selected for the synthesis of gold and silver nanoparticles is *M. charantia* (bitter gourd). The fresh *M. charantia* was collected from the local Jhansi market and peeled. Fresh peel extract were prepared by peeling the bitter gourd. The fresh and healthy bitter gourd fruit peels were collected rinsed thoroughly first with tap water for 5-10 min followed by distilled water for 10 min to remove all the dust and unwanted visible particles. 25 gm of papaya peel was taken with 50 ml double distilled water in soxhlet apparatus. Then it is boiled for 30 min in soxhlet and left at room temperature. The extract was filtered through whatmann filter paper No. 1 and used as such for gold(AuNPs) and silver(AgNPs) nanoparticles synthesis. The extract was freshly prepared for the biosynthesis of silver(AgNPs) and gold(AuNP) [9].

Synthesis of silver and gold nanoparticles

Silver(AgNPs) and gold(AuNPs) nanoparticles were synthesized by adding 10ml of aqueous peel extract of *M. charantia peel* to 40 ml of 1mM aqueous $AgNO_3$ solution and 40 ml of 1mM aqueous $HAuCl_4{\cdot}3H_2O$ solution at room temperature, respectively. The color change of the solution from light yellow to brown and pale yellow to purple signified the reduction of Ag^+ ions to Ag° and Au^{3+} ions to Au°, respectively. The formulation of the brown and purple solution provided the preliminary confirmation for the formation of Silver(AgNPs) and gold(AuNPs) nanoparticles peel-extract-mediated synthesis [10]. Nanoparticles of gold and silver were obtained by centrifuging the solution for 20 min at 10,000 rpm followed by repeated washing with water three times, and the lyophilized powder was processed for characterization.

Characterization of synthesized silver and gold Nanoparticles

The spectral response of synthesized AgNPs and AuNPs was studied using a UV–visible spectrophotometer (Thermo Scientific-multimode plate reader). Fourier transform infrared spectroscopy (FTIR) results were obtained from a Jasco 6300 spectrometer (KBr pellet) in the range of 400–4000 cm^{-1}. Using scanning electron microscopy (Hitachi SU6600, Japan), the shape and size of the nanoparticles were investigated. The nanoparticles chemical composition was analyzed using an energy-dispersive technique. To determine the crystal structure and phase of synthesized nanoparticles, Powder X-ray diffraction (XRD; Rigaku Japan) was performed using a X-ray diffractometer – Cu Kα radiation device.

Antibacterial assay

Disc diffusion test:

The in vitro antibacterial and anti fungal activity was evaluated by using agar disc diffusion assay with Muller Hinton Agar as growth media followed by measurement of zone of inhibition. Bacterial suspensions were prepared at a concentration of 0.5 McFarland Scale. Agar plates were inoculated, and plant peel extract, green synthesized AgNPs and AuNPs solutions disc placed in the agar medium .Then the plates of bacteria were incubated at 37 °C and of fungal at 28°C for 24 h and 48h respectively. Antibacterial and anti-fungal activity was dictated by zone of inhibition obtained.

RESULTS AND DISCUSSION

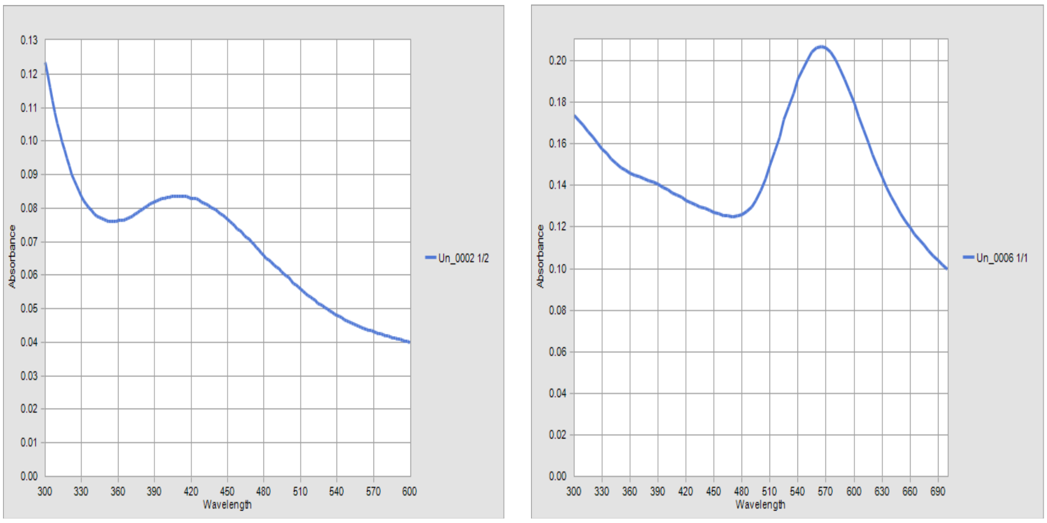
Spectroscopic characterization

***UV–visible spectroscopy*:**

The UV–visible spectrum of the synthesized silver and gold nanoparticles is shown in figure 2. The surface Plasmon resonance (SPR) for the synthesized silver nanoparticles occurred at 430 nm, while the SPR for gold nanoparticles occurred at 550 nm which is indicative of the fact that they belong to the range of SPR specific for silver and gold nanoparticles [11].

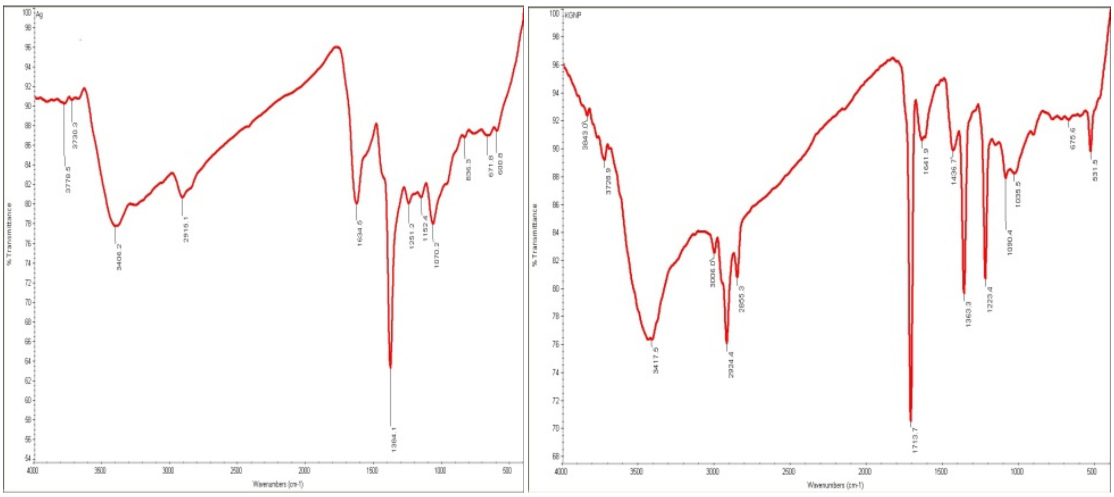
(A) (B)

Fig. 1 Showing Silver nanocolloids (A) and gold nanocolloids (B) made by *M. charantia* peel extract



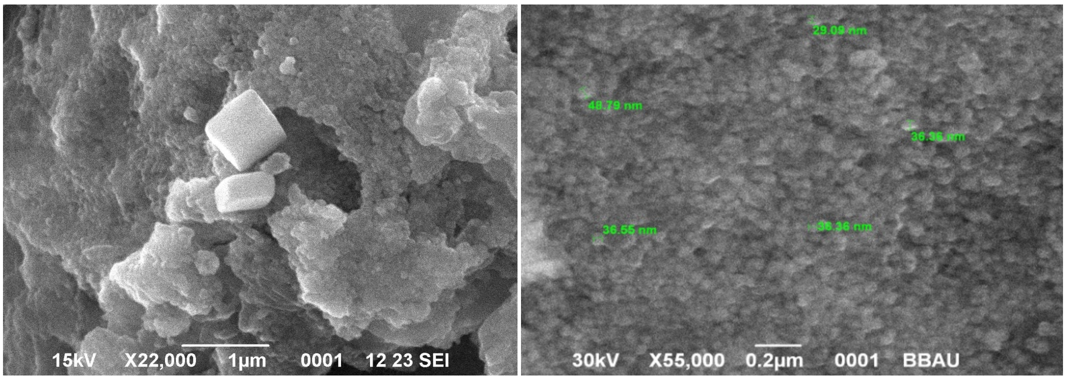
(A) (B)

Fig. 2. Showing UV –visible absorption spectra of silver and gold nanoparticles synthesized from *M. charantia* peel extract



(A) (B)

Fig. 3. Showing FTIR of Silver (A) and gold (B) nanoparticles Synthesized from *M. charantia* peel extract.



(A) **(B)**

Fig. 4 Showing SEM micrographs of the silver(A) and gold(B) nanocolloids

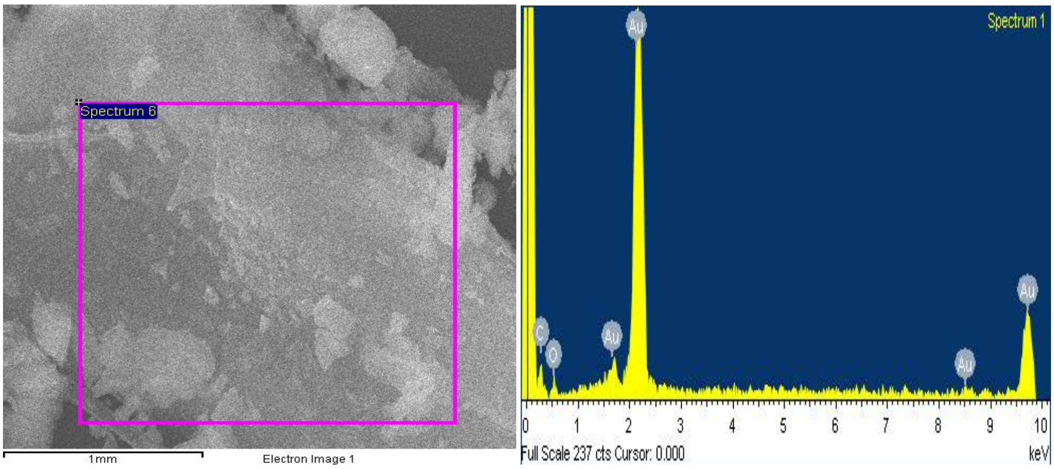


Fig. 5 (A) Showing EDX of silver nanoparticles

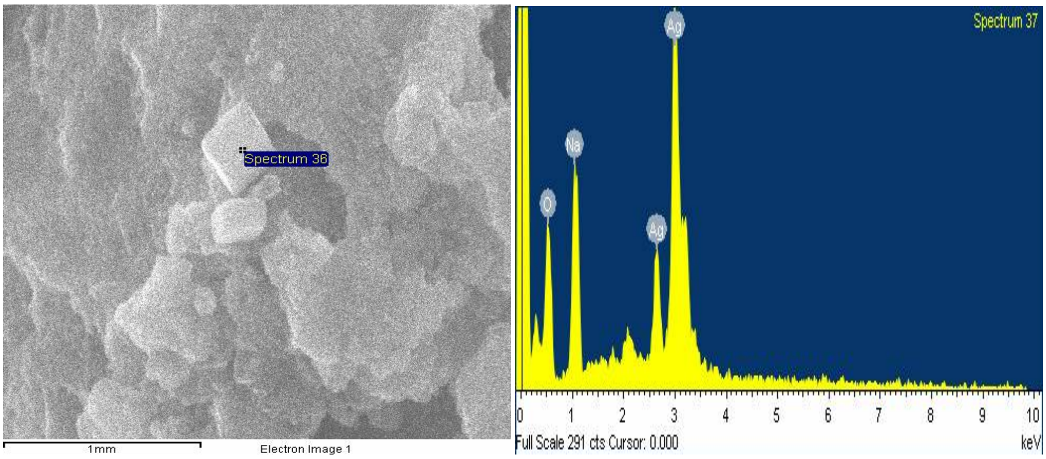
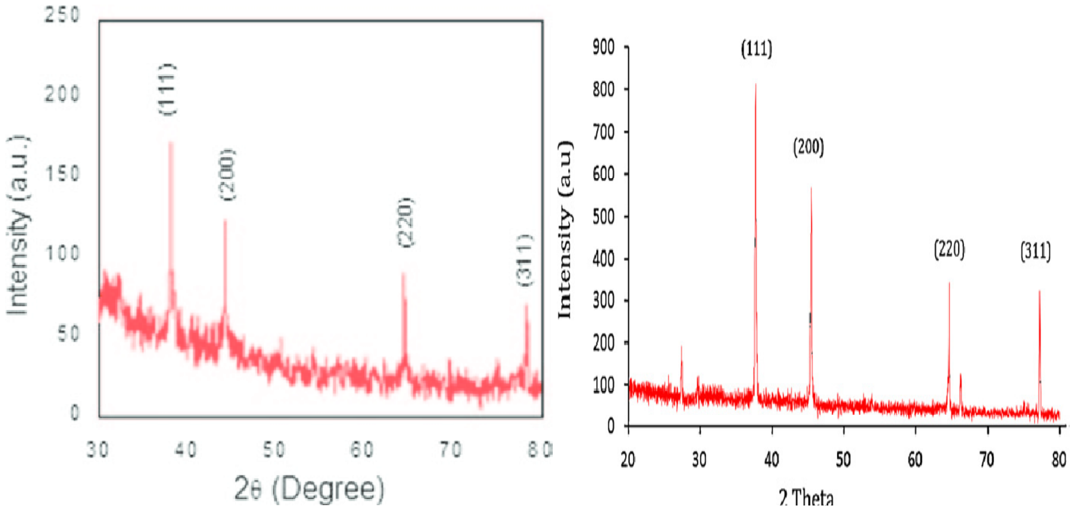


Fig. 5 (B) Showing EDX of gold nanoparticles



(A) (B)

Fig. 6 Showing XRD pattern of Silver (A) and Gold (B) nanoparticles biosynthesized by *M. charantia* peel extract

Table 1. Zone of Inhibition of silver and gold nanoparticles against different bacterial and fungal cultures.

Zone of inhibition →	Silver nanoparticle	Gold nanoparticle	streptomycin	Conjugate with AgNPs	Conjugate with AuNPs
Bacterial species and Fungal Species ↓					
Pseudomonas aeruginosa	27mm	20mm	15mm	30mm	20mm
Staphylococcus aureus	33mm	30mm	25mm	35mm	30mm
E.coli	20mm	15mm	10mm	25mm	18mm
Bacillus subtilis	28mm	25mm	20mm	30mm	28mm
Klebsiella pneumonia	28mm	25mm	20mm	30mm	25mm
Aspergillus niger	20mm	15mm	17mm	25mm	17mm
Candida albicans	22mm	17mm	22m	25mm	17mm

FTIR: FTIR spectrum of *M.charantia* peel extract, after synthesis of AuNP and AgNP, is shown in figure 3. Characteristic vibrations after reduction of Ag+ ions by *M. charantia* peel extract were found to be 3406.2, 2915.1, 1384.1, 1634.5, 1251.2, 1152.4, 1070.2, 836.3, 671.8, 600.8 and Au3+ ions by *M. charantia* peel extract were found to be 2927.4,2855.3,3417.5,1713.7,1641.9,1436.7,1223.4,1035.5,675.6,531.5, respectively. Figure shows vibrations attributed to functional groups like alcohols and phenols (broad O–H stretching), alkynes (C≡C), saturated aldehydes (strong C=O bending), primary and secondary amines or amides (weak NH2 bending), alkanes (medium CH2 and CH3 deformation bending), carboxylic acid derivatives (medium O–C bending) and disulphide (weak S–S bending) confirmed that the phytoconstituents present in the peel extract were responsible for the reduction of silver and gold ions to silver and gold nanoparticles . Similarly, FTIR spectra were recorded after plant leaves reduced Ag^{+} ions and Au^{3+} ions [12,13].

Morphological characterization

SEM: SEM analysis of synthesized AgNPs and AuNPs are shown in figure 4. AgNPs showed the presence of cuboidal and irregular particles and the particle size ranged from 43.5 to 69 nm. Similarly, synthesized AuNPs revealed the presence of polydispersed spherical nanoparticles with the particle size ranging from 36.6 to 45 nm. The results are in concurrence to the presence of cuboidal silver nanoparticlesi using *Glycyrrhiza glabra* root extract and the presence of spherical gold nanoparticlesi using *Pistia stratiotes* L. has been reported by Kasthuri J *et al*. and Dinesh *et al*. [14,15] respectively.

Chemical characterization

Energy dispersive X-rays: EDAX of synthesized silver and gold nanoparticles is shown in figure 5 (A) and (B). The spectrum confirmed the occurrence of a strong peak for elemental silver at approx. 3 keV and the presence of a strong absorption peak for elemental gold at approximately 2 keV. The peaks of oxygen and carbon may be due to biomolecules attached to the surface of the synthesized nanoparticles . Dinesh *et al.*

confirmed the presence of a silver peak using *G. glabra* root extract and Anuradha *et al*. confirmed the presence of a gold peak using *P. stratiotes* L [15,16].

Structural characterization

XRD: The XRD pattern of synthesized AgNPs and AuNPs is shown in figure 6. The XRD patterns at 38.250, 44.29, 64.54, 77.45 and 81.65 corresponding to the lattice planes [(111), (200), (220), (311), (222)] and 38.04, 44.12, 64.52, 77.49 and 81.53 corresponding to the lattice planes [(111), (200), (220), (311), (222)] are in accordance with the reported pattern (JCPDS 04-0783) and (JCPDS 04- 0784), which confirmed that the green synthesized AgNPs and AuNPs are nanocrystalline with fcc crystal structure, respectively. A similar pattern of XRD for silver and gold nanoparticles has been reported by Shankar *et al* [12].

Disc diffusion assay:

Antibacterial studies were conducted against different Gram-negative and Gram-positive bacteria. The results of the well-diffusion method showed that green-AgNP and AuNP had different magnitude of antibacterial activity, depending on the kind of nanoparticles (Table 1).

The AgNPs has the larger inhibition zone in comparison with an inhibition zone of AuNP. Streptomycin and Nystatin was used as a positive control for both types of the bacterial and fungal cultures respectively [17,18]. Zone of inhibition was not obtainted in the case of peel extract. Significant synergistic effect of antibiotic and Ag nanoparticles has been observed and the condition was not same in gold.

CONCLUSION

A simple, cost-effective synthesis of non-toxic gold and silver nanoparticles has been performed by means of aqueous extract of peel of *M charantia* and their spectroscopic, morphological, chemical, structural characterization and *in microbial* efficacy on different gram positive, gram negative and fungus have been studied and compared. The presence of phytoconstituents such as proteins, phenols, tannins and flavonoids in the aqueous extract might be responsible for the synthesis of AgNP and AuNP. The green synthesized silver and gold nanoparticles were found to be stable and the key ingredients responsible for reduction should be isolated and quantified. Further, this study suggests that both gold nanoparticles and silver synthesized using aqueous peel extract can be used as a potential agent in anti-microbial assay. Yet, the means of action and specific activity of synthesized silver and gold nanoparticles and aqueous peel extract against different pathogenic bacteria need to be thoroughly investigated.

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