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EXTRACELLULAR BIOSYNTHESIS OF GOLD NANO PARTICLES, CHARACTERIZATION AND MEDICAL APPLICATIONS - A REVIEW

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ABSTRACT

Gold is one of the metals to have been discovered; the history of its study and application spans at least several thousand years. The first data on colloidal gold can be found in treaties by Chinese Arabian and Indian scientists, who managed to obtain colloidal gold as early as in the V-IV centuries BC. The biological preparation of Gold nanoparticles from flowers, fruits, microorganisms and characterization of gold nanoparticles from different sources and applications of the gold nanoparticles in medicine through antibacterial activity against bacteria and fungi was discussed were presented in the article as a review.

Keywords: Gold nanoparticles, Characterization, Applications, Antibacterial activity, sources.

INTRODUCTION

Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other scientific fields, such as chemistry, biology, physics, materials science, and engineering conducted at the nanoscale, which is about 1 to 100 nanometers. Particles are further classified according to diameter. Coarse particles cover a range between 10,000 and 2,500 nanometers. Fine particles are sized between 2,500 and 100 nanometers. Ultrafine particles, or nanoparticles are sized between 1 and 100 nanometers.

The term nanoparticle is used to describe a wide variety of materials of submicron size. An internationally accepted definition for nanoparticles does not exist. It is used differently, according the context of the material being described. According to a recent definition suggested by British Standards Institution Nanoparticles are the particles with one or more dimensions at the nanoscale. They have defined the nanoscale as dimensions of the order of 100 nm or less. It is the critical length scale at which certain novel size related properties develop and the material start behaving differently than the molecules or bulk material [1]. Here novel properties refer to optical magnetic and electrical properties which typically appear in materials below 100 nm size. However, many a times, besides the strictly nano (1-100 nm) all submicron colloidal particles i.e. particles with at least one dimension in the scale of 1-1000 nm, also called mesoscale, are referred as nanoparticles, to include organic polymers and vesicles widely used in the area of drug delivery [2,3]. At this scale the properties of the matter are different from atomic or molecular properties which are governed by the laws of quantum mechanics, or the properties of bulk materials determined by the laws of classical physics. So the dimension of 1-100 nm may be considered as an intermediate state between atomic or molecular state and bulk state where materials exhibit some unexpected and unusual new properties which cannot be defined by the classical laws of physics [4]. It is these unusual properties which have attracted immense attention of researchers from almost every field science including biology and medicine.

Investigation of novel properties of nanoparticles and their application has become a very active area of research. Nanoparticle having one or more dimensions of the order of 100nm or less- have attracted considerable attraction due to their unusual and fascinating properties, with various applications, over their bulk counterparts. Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles. Though physical and chemical methods are more popular for nanoparticle synthesis, the use of toxic compounds limits their applications. The development of safe eco-friendly methods of biogenetic production are now of more interest due to the simplicity of the procedures and versatility. Due to their amenability to biological functionalization, the biological nanoparticles are finding important applications in the field of medicine. The antimicrobial potential of metal based nanoparticles has led to its incorporation in consume, health related and industrial products . Our area of interest lies in the biological synthesis of gold and silver nanoparticles.

Gold nanoparticles:

Gold is one of the metals to have been discovered; the history of its study and application spans at least several thousand years. The first data on colloidal gold can be found in treaties by Chinese Arabian and Indian scientists, who managed to obtain colloidal gold as early as in the V-IV centuries BC. They utilized it for medicinal purposes amongst other uses. Amongst an array of nanomaterials being investigated for applications in biology, Gold nanoparticles (GNP) are probably most extensively studied nanoparticles. In fact, gold nanoparticles have been in use since ancient times in one or another form, be it in traditional Chinese, Indian or European medicine or for decorative purpose in Lycurgus Cup. The work of Faraday has pointed towards specific optical properties of colloidal gold, long before the recognition of changing properties of matter at nanoscale. Gold in nanoscale display novel properties and have diverse activities that make it appropriate for therapeutic use and broad applications in Nanobiotechnology. Au particles are particularly and extensively exploited in organisms because of their biocompatibility. The range of GNP used in modern medical and biology studies is extremely wide. In particular, it comprises genomics, biosensorics, immunoanalysis, clinical chemistry, detection and photothermolysis of microorganisms and cancer cells; the targeted delivery of drugs, DNA and antigens; optical Bioimaging and the monitoring of cells and tissues using modern registration systems. It has been argued that gold nanoparticles could be used in almost all medical applications: diagnostics, therapy, prevention, and hygiene. The broad range of applications for GNP is based on their unique physical and chemical properties. In particular, the optical properties of GNP are determined by their Plasmon Resonance, which is associated with the collective excitation of conduction electrons and localized in the broader region, from the visible to the infrared (IR) region, depending on the particle size, shape, and structure. Gold nanoparticles (Au) generally are considered to be biologically inert but can be engineered to possess chemical or photo thermal functionality.

Wenxing Wang *et al.,* reported one-step method for the synthesis of biocompatible gold nanoparticles at room temperature by reducing $HAuCl_4$ with gallic acid in the presence of poly-(*N*-vinyl-2-pyrrolidone) (PVP) . The effect of the molar ratio of gallic acid to gold (*R*) on the size and shape of gold nanoparticles and the characteristic of gold nanoparticles was investigated by UV–vis and infrared spectroscopy, transmission electron microscopy, and X-ray diffraction. For an *R* value of about 0.4, the prepared gold nanoparticles were smaller in size and the most close to spherical in shape. At other molar ratios, particle sizes increased and various polyhedra were formed. A reaction mechanism for the reduction of $HAuCl_4$ of gallic acid is proposed. The incorporation of PVP effectively protected the surface of gold nanoparticles and improved their stability. The PVP-protected gold nanoparticles were modified with 3- and 5-alkanethiol-capped 12-base oligonucleotides, respectively, to form two different nucleic acid probes. The probes were successfully used to complex a 24-base complementary polynucleotide target in a tail-to-tail fashion.[1]

Kasthuri *et al*., investigates the anisotropic gold and spherical–quasi-spherical silver nanoparticles (NPs). These were synthesized by reducing aqueous chloroauric acid ($HAuCl_4$) and silver nitrate ($AgNO_3$) solution with the extract of phyllanthin at room temperature. The rate of reduction of $HAuCl_4$ is greater than the $AgNO_3$ at a constant amount of phyllanthin extract. The size and shape of the NPs can be controlled by varying the concentration of phyllanthin extract and thereby to tune their optical properties in the near-infrared region of the electromagnetic spectrum. The case of low concentration of extract with $HAuCl_4$ offers slow reduction rate along with the aid of electron-donating group containing extract leads to formation of hexagonal- or triangular-shaped gold NPs. Transmission electron microscopy (TEM) analysis revealed that the shape changes on the gold NPs from hexagonal to spherical particles with increasing initial concentration of phyllanthin extract. The Fourier transform infrared spectroscopy and thermogravimetric analysis reveal that the interaction between NPs and phyllanthin extract. The cyclic voltammograms of silver and gold NPs confirms the conversion of higher oxidation state to zero oxidation state. [2]

The synthesis of eco-friendly nanoparticles are an evergreen branch of Nanoscience for biomedical application was studied by Balaprasad Ankamwar *et al.,* On treating chloroauric acid solutions with *Terminalia catappa* (TC) leaf extract rapid reduction of chloroaurate ions is observed leading to the formation of highly stable gold nanoparticles in solution. TEM analysis of the gold nanoparticles indicated that they ranged in size from 10 to 35 nm with average size of 21.9 nm. [3].

Padma S Vankar *et al*., stated that the nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. The flower extract acts as a reducing agent and a encapsulating cage for the gold nanoparticles. The production of gold nanoparticles has been done by the controlled reduction of the Au_3^+ ion to Au_0. The formation of gold nanoparticles has been established by FT-IR and UV-Vis spectroscopy, as well as by TEM, XRD, EDAX and AFM. The study suggests that *M. jalapa* flowers can be a cheap source as a reductant in the production of gold nanoparticles. [4].

A. Thirumurugan *et al.,* studied the Biological synthesis of gold nanoparticles using *Azadirachta indica* plant leaf extract .The synthesized nanoparticles are confirmed by color changes and it was characterized by UV-visible spectroscopy. The plant based route could be considered to be an environmental friendly or green biological method of nanoparticles production. [5].

Biosynthesis of nanoparticles has arisen as a promising alternative to conventional synthetic methodologies owing to its eco-friendly advantages was reported by Yao Zhou et al., This research, for the first time from the standpoint of statistics, confirmed an electrostatic force or ionic bond-based interaction between the chloroauric ions and the involved bioconstituents and manifested that reducing sugars and flavonoids were both important reductants responsible for conversion of Au (III) to Au (0). The result also demonstrated that the proteins were not the reducing agents, yet they might be protection agents in the biosynthesis of gold nanoparticles (GNPs). Besides, a significant linear relationship was found between the anti-oxidant ability of the floor broths and their capability to reduce Au (III) into Au (0). Furthermore, the preliminary investigation gold nanospheres with a higher degree of homogeneity in size tended to be promoted by foliar broths containing higher content of reducing sugars/ flavonoids and proteins. Otherwise, i.e., for those broths with lower content of the above biocompounds, sphere GNPs of wider size distribution or even gold nanotriangles tended to be fabricated. [6] M. Banoee1 *et al.,* stated that the ethanol extract of black tea and its tannin free fraction used for green synthesis of gold nanoparticles. All the extracts were used separately for the synthesis of gold nanoparticles through the reduction of aqueous $AuCl_4^-$. Transmission electron microscopy and visible absorption spectroscopy confirmed the reduction of gold ions to gold nanoparticles. The ethanol extract of black tea and its tannin free ethanol extract produced gold nanoparticles in the size ranges of 2.5-27.5 nm and 1.25-17.5 nm with an average size of 10 nm and 3 nm, respectively. The prepared colloid gold nanoparticles, using the ethanol extract of black tea, did not show the appropriate stability during storage time (24 hours) at 4°C. In contrast, gold colloids, which were synthesized by a tannin free fraction showed no particle aggregation during short and long storage times at the same conditions. To the best of our knowledge, this is the first report on the rapid synthesis of gold nanoparticles using an ethanol extract of black tea and its tannin free fraction. [7].

In the present research program, cost effective and environment friendly gold nanoparticless were Synthe- sized using the onion (*Allium cepa*) extract as the reducing agent which was investigated by Umesh Kumar *et al.,*. The nanoparticless were characterized using UV-visible, XRD, TEM, SEM, and methods. The absorption peak at 540 nm was found to be broaden with increase in time indicating the polydispersity nature of the nanoparticles. The XRD results suggested that the crystallization of the bio-organic phase occurs on the surface of the gold nanoparticles or vice versa. The broadening of peaks in the XRD patterns was attributed to particle size effects. The internalization of nanoparticles within cells could occur via processes including phagocytosis, fluid-phase endocytosis and receptor mediated endocytosis. [8].

Ratul Kumar et al., explores the reducing and capping potentials of an ethanolic flower extract of the plant *Nyctanthes arbortristis* for the synthesis of gold nanoparticles. The extract at different volume fractions were stirred with $HAuCl_4$ aqueous solution at 80°C for 30 min. The UV–Vis spectroscopic analysis of the reaction products confirmed successful reduction of Au_3+ ions to gold nanoparticles. Transmission electron microscope (TEM) revealed dominant spherical morphology of the gold nanoparticles with an average diameter of 19.8 ± 5.0 nm. X-ray diffraction (XRD) study confirmed the crystalline nature of the synthesized particles. Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) analysis of the purified and lyophilized gold nanoparticles confirmed the surface adsorption of biomolecules during preparation and caused long-term (6 months) stability. Low reaction temperature (25 °C) favored anisotropy. The strong reducing power of the flower extract can also be tested in the green synthesis of other metallic nanoparticles. [9].

Yi Xia Zhang *et al.,* discussed a new method of one-pot biosynthesizing of gold nanoparticles (GNPs), using chloroplasts as reductants and stabilizers. The as-prepared GNPs were characterized by ultraviolet visible spectroscopy, transmission electron microscopy, X-ray powder diffraction, and Fourier transform infrared spectroscopy (FTIR). The cytotoxicity of the GNPs was evaluated using the 3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) method against gastric mucous cell line GES-1 and gastric cancer cell line MGC-803. Rhodamine 6G as a Raman probe was used for investigating surface-enhanced Raman spectroscopy (SERS) enhancement of GNPs. The transmission electron microscopy results indicated that the GNPs were spherical in structure and almost 20 nm in diameter. Ultraviolet visible spectroscopy exhibited an absorption peak at 545 nm. The GNPs exhibited high crystallinity, with the (111) plane as the predominant orientation, clarified by X-ray powder diffraction. In addition, a potential mechanism was proposed to interpret the formation process of GNPs, mainly based on the analysis of FTIR results. The FTIR spectrum confirmed that the GNPs were carried by N–H groups. Toxicological assays of as-prepared GNPs revealed that the green GNPs were nontoxic. SERS analysis revealed that the GNPs without any treatment could substantially enhance the Raman signals of Rhodamine 6G. The Raman enhancement factor was calculated to be nearly 1010 orders of magnitude. In conclusion, the GNPs with good biocompatibility and excellent SERS effect were successfully synthesized using chloroplasts. These biogenetic GNPs have great potential for Ultrasensitive detection of biomarkers in vitro and in vivo based on SERS. [10].

Metal nanoparticles, in general, and gold nanoparticles, in particular, are very attractive because of their size and shape dependent properties. Biosynthesis of anisotropic gold nanoparticles using an aqueous extract of *Madhuca longifolia* and their potential as IR blockers has been demonstrated. The tyrosine residue was identified as the active functional group for gold ion reduction. was studied by A. Mohammed Fayaz *et al.,* and were characterized by UV–Vis spectrophotometer, FTIR, TEM and HrTEM. The presence of proteins was identified by FTIR, SDS-PAGE, UV–Vis and fluorescence spectroscopy. The micrograph revealed the formation of anisotropic gold nanoaprticles. The biologically synthesized gold nanotriangles can be easily coated in the glass windows which are highly efficient in absorbing IR radiations. [11].

Sontara Konwar Boruah *et al.,* stated the green synthesis of gold nanoparticles (AuNPs) by using a simple, fastest, low cost, eco-friendly technique. The green synthesis of AuNPs was done by using fresh young leaves and leaf buds of tea (*Camellia Sinensis*). Reduction of $HAuCl_4$ by polyphenols present in young leaves and leaf buds of tea extract at room temperature provides AuNPs ($Au_3+\rightarrow Au_0$). The UV-Visible absorption spectrum of AuNPs in tea extract shows two bands at around 534 and 752 nm, which results from transverse and longitudinal surface plasmon resonance (SPR) respectively. In fluorescence spectroscopy study, AuNPs in tea extract shows fluorescence emission at 450 and 705 nm when excited at 350 nm. The kinetics of the reaction rate ($Au_3+ \rightarrow Au_0$) with respect to time was studied with the help of UV-Visible and fluorescence spectroscopy. The reaction rate evaluated with the help of UV-Visible and fluorescence spectroscopy was found to be almost similar in results. The kinetics of the reaction also suggest that the reaction was fast and completed in 28 minutes. The amount of tea extract determines the core size of the AuNPs. The core size of the AuNPs decreases as the amount of tea extract increases and it causes the blue-shift of SPR band. The physical properties, particle size and morphology of AuNPs were characterized using X-ray diffractometer (XRD), field emission scanning electron microscope (FESEM) and high resolution-transmission electron microscopy (HR-TEM) techniques. The AuNPs size in the range ~2. 94-45.58 nm with an average of 13.14 nm. [12].

The synthesis of eco-friendly nanoparticles are an evergreen branch of Nanoscience for biomedical application was reported by Nagaraj B, Krishnamurthy Nb *et al.,* Low cost of synthesis and non toxicity are the main features which make it more attractive potential option for the biomedical field. The synthesized nanoparticles are confirmed by color changes and it has been characterized by UV-visible spectroscopy. The UV- visible spectra indicate a strong Plasmon resonance that is located at ~550 nm. The presence of this strong broad plasmon peak has been well documented for various Me- NPs, with sizes ranging all the way from 2 to 100 nm. The morphology and size of the biologically synthesized gold nanoparticles were determined using TEM. The images clearly show that the average size of the nanotriangles is about 200 nm, while, the spherical like particles show very small size about 5-10 nm. The study also shows that gold nanoparticles with antibiotic show more inhibitory zones than compared to the standard antibiotics. [13].

Javad Karimi *et al*., reports the biosynthesis of gold nanoparticles using dried flowers extracts of *Achillea wilhelmsii* as the reducing agent. Rapid reduction of gold ions was observed leading to the formation of gold nanoparticles in solution. The formation of gold nanoparticles was confirmed by the presence of an absorption peak at 580 nm using UV–visible spectrophotometry. The size and morphology of gold nanoparticles were monitored scanning electron microscopy. Analysis of these particles showed an average size of 70 nm. Fourier transform infrared spectroscopy revealed possible involvement of reductive groups on the surfaces of nanoparticles. [14].

The facile synthesis of ultra stable gold nanoparticles (GNPSs) was demonstrated by Sunil Pandey *et al.,* using a 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 synthesized using biological protocols was found to be extremely higher than the chemically synthesized 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. [15].

Radhika Rajasree SR *et al.,* worked on the extracellular synthesis of gold nanoparticles made by making use of *Pseudomonas fluorescens.* Methods: The nanoparticles obtained were characterized by UV–vis, transmission electron microscopy (TEM), Scanning electron microscopy (SEM) and FTIR spectroscopy. The results: Synthesized nanoparticle size ranged from 50-70 nm. FTIR spectrum indicates that the biomolecule cap the nanoparticles. Hence the present study enlightens the green chemistry approach on the production of gold nanoparticles using a microorganism. In comparison to chemical synthesis, the synthesis of gold nanoparticles by microbial source is the most reliable method of production and yield. [16].

Plant mediated synthesis of metallic nanoparticles is an increasing commercial demand due to the wide applicability in various areas such as electronics, catalysis, chemistry, energy, cosmetics and medicine. Nagaraj Basavegowda1 *et al.,* investigated the synthesis of gold nanoparticles done by using fruit extracts of *Ananas comosus* (L). Nanoparticles were characterized by using UV visible absorption spectra. Their morphology, elemental composition and crystalline phase were determined by scanning electron microscopy, energy dispersive X-ray spectroscopy and selected area electron diffraction. FT-IR analysis was used to confirm the presence of gold nanoparticles in the extracts. The synthesized gold nanoparticles were generally found to be effective as antimicrobial agents against some important human pathogens like *E. coli* and *Streptobacillus Sp.* Which are affecting and cause diseases like food poisoning and rat-bite fever to human beings respectively. [17] Biosynthesis of gold nanoparticles using Aloe barbedensis leaf extract was studied by Sunil Pandey *et al.,* and considered different parameter like pH (3, 4, 6, 8, 10), Temperature (4, 30, 60 and 100^0C) and concentration of aurochlorate solution. At lower pH and temperatures, formation of hexagonal and triangles of gold nanoparticles occurred, as viewed by TEM. The leaf extract was so efficient that change in color of reactant was noticed in < 2 minutes of interaction with aurochloratesolution at both low and high temperatures. Formation of nanoparticles was characterized by UV-Vis Spectroscopy, XRD and TEM; which confirmed the synthesis of monodispersed gold nanoparticles. Gold nanoparticles synthesisedusing A. barbadensis was extremely stable as compared to chemically synthesized Gold nanoparticles synthesized using using citrate reduction (chemical) method. [18].

Biological methods of reduction of metal ions using plants or microorganisms are often preferred because they are clean, non-toxic, safe, biocompatible and environmentally acceptable. Pranav Vasanthi Bathrinarayanan *et al.,* presented that *Aspergillus fumigatus* was used for the intracellular synthesis of gold nanoparticles. Stable nanoparticles were produced when an aqueous solution of chloroauric acid ($HAuCl_4$) was reduced by *A. Fumigatus* biomass as the reducing agent. Production of nanoparticles was confirmed by the color change from yellow to pinkish violet after approximately 72 h of reaction. The produced nanoparticles were then characterized by Fourier Transform Infra Red Spectroscopy, Scanning Electron Microscope (SEM), Energy Dispersive Spectroscopy (EDS) and X-ray Diffraction spectroscopy (XRD). The SEM images of samples revealed that the nanoparticles were spherical, irregularly shaped with no definite morphology. Biosynthesized gold nanoparticles were ranged in size from 85.1 to 210nm. The presence of gold nanoparticle was confirmed by EDS analysis. Crystalline nature and face centered cubic structure of synthesized gold nanoparticle was confirmed by XRD pattern. [19].

Leaf extract of *Adathoda vasica* a tropical shrub was used as reducing agent to convert gold ions into monodispersed gold nano particles was investigated by Sunil Pandey *et al*., using Parameters like pH, temperature, concentration of reactants viz. Gold salt and leaf extract were standardized to produce gold nano particles of 10 -20 nm size. The best parameters obtained were high temperature (80 and 100ºC), pH 6 and 50 ppm of aurochlorate. The characterization was based on observations made using UV-Vis spectroscopy, XRD and HRTEM. The reducing agents for synthesis of gold nano particles were speculated to be nitrate reductase and glutathione. [20].

In this work green synthesis of gold nano particle using various plant extracts and spices extracts was done, in which extracts reduces aqueous $HAuCl_4.3H_2O$ to Au_0 and stabilized by crystalline phase. The synthesized nano particle is confirmed by the change of color of Auric chloride which is yellow in color, and growth of nano particle was monitored by Sumit. S. Lal *et al.,* with surface plasmon behavior using UV-Vis Spectroscopy and concerned pH were determined. Furthermore, this green synthesis approach is a rapid and better alternative to chemical synthesis and also effective for the large scale synthesis of gold nanoparticles. [21].

The optical properties and morphology were verified using UV-Vis spectroscopy and High Resolution Transmission electron microscopy. HRTEM micrographs as well as the SPR peaks of UV-Vis spectra showed that the size of the GNPs ranged from 25-30 nm. . The crystalline nature of gold nanoparticles was examined by Ashmi Mewada *et al*., using XRD and was found to be face centered cubic. FTIR analyses of such GNPs possess sulphydryl, amido, carbonyl functional groups which depicts the presence of peptides involved in capping the nanoparticle leading to its stability. Moreover, the stability of GNPs synthesized from *Pseudomonas denitrificans* exudates was compared with chemically synthesized GNPs with the help of 5M NaCl. An analysis of nitrate reductase activity in microbial exudates exhibited its involvement in the synthesis of GNPs. [22].

The biological synthesis of Gold nanoparticles (GNPs) using *Nocardia farcinica* exudates present in the medium, as reducing and stabilizing agent was reported by Goldie Oza *et al.,* On treating *Nocardia farcinica* extract with gold salt, rapid reduction of gold ions was observed resulting in the formation of highly stable GNPs. The inherent pH of the bacterial exudates, high temperature (100º C) and 100 ppm aqueous solutions of aurochlorate were found to be the optimum parameters for synthesis of GNPs. The results were verified using UV-Visible spectroscopy, Transmission electron microscopy (TEM) and X-ray diffraction (XRD) studies TEM images show monodispersed spherical gold nanoparticles of 15-20 nm diameters. Moreover, the nitrate reductase activity of exudates was found to get reduced from 1.020 mmole/min/ml to 0.14 mmole/min/gram when added to the aqueous solution of aurochlorate that resulted in the bio fabrication of gold nanoparticles. [23].

Nagajyothi *et al.,* reported the synthesis of highly dispersed gold nanoparticles (AuNPs) using Dried flower extract of *Carthamus tinctorius* as the reducing agent. After exposing the gold ions to flower extract, rapid reduction of gold ions is observed leading to the formation of AuNPs in solution. UV-VIS spectrum of the aqueous medium containing AuNPs obtained after 25 min (at 40 °C) and after 10 hrs (at room temperature) of the reaction, showed absorption peaks at around 414 and 410 nm respectively. Scanning electron microscopy (SEM) micrograph analysis of the AuNPs indicated that they were well-dispersed This green synthetic method is proved to be simple, eco-friendly and there is no need of using toxic chemicals. AuNPs show the antibacterial activity against the S aureus (KCTC 1916), *E. coli* (KCTC 2441), *B. Substilis* (lab culture) and *C. albicans* (lab culture) to the best of our knowledge. This is the first report on synthesis of gold nanoparticles using *Carthamus tinctorius* flower extract. [24].

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