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Phytosynthesis of Silver and Gold Nanoparticles

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Abstract: *Nanoparticles are extensively used in biological and medical research due to their unique properties. Use of such nanoparticles in biological & medicinal field gives rise to the concept of biomedical nanotechnology, bio nanotechnology & nanomedicines. Phytosynthesis of nanoparticles is an emerging area in plant science research. Different plants are used for this purpose being it is the most eco friendly and convenient method of synthesizing nano scale particles of different salts. The plants are their potent sources of many valuable bioactive constituents and these constituents contributes reduction of salt in the system. In present work, fruit peel and plant bark of Punica granatum plant was taken as an experimental system for Phytosynthesis of silver and gold nanoparticles from silver nitrates and gold chloride salt.*

Keywords: Nanoparticles

I. INTRODUCTION

A nanometer is one-billionth of a meter. Norio Taniguichi coined the term "nano-technology" in 1974. Nobel Laureate Dr. Horst Stormer said that, “the nanoscale is more interesting than the atomic scale because the nanoscale is the first point where we can assemble something- it’s not until we start putting atoms together that we can make anything useful”. Biologists, chemists, physicists and engineers are all evolved in the study of substances at the nanoscale. Dr. Stormer hopes that the different disciplines develop a common language and communicate with one another without a solid background in multiple sciences one cannot understand the world of nanotechnology. Nano biotechnology is the merger of two distant fields of nanotechnology and biotechnology. Nanoparticles are extensively used in biological and medical research communities for various applications. Use of such nanoparticles in biological and medical fields gives rise to the concept of biomedical nanotechnology, bionanotechnology and nanomedicine. The integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles. Biological systems especially algal materials, fungal forms and Angiospermic plants of medicinal importance are considered as the most ecofriendly and nontoxic systems with superiority of applications over physical and chemical methods of nanoparticle synthesis.

Nanocages, Nanofibers, Nanotubes and Nanodots are the specific types of nanostructures. Nanocages are hollow porous gold nanoparticles ranging in size from 10nm-150 nm. These are the product of reaction of silver nanoparticles with chloroauric acid ($HAuCl_4$) in boiling water. Nanocages show different properties than their building molecules. E.g. Gold Nanoparticles show absorbance in the visible spectrum of light while gold Nanocages absorb light in the near infrared region. Nanofibers are specially designed fibers with diameter less than 100 nanometers. They can be produced by interfacial polymerization and electro spinning. Carbon Nanofibers are graphitized

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fibers and are shaped by catalytic synthesis. Napkins with Nanofibers contain antibodies against numerous biohazards and chemicals that signal by changing color. These napkins are chiefly used in identifying the bacteria in kitchens. Nanotubes are nanoscale tube-like structures whose diameter ranges from 0.1 to 100 nm and length is much greater. Such nanotubes which exhibit extraordinary strength and unique electrical properties are efficient conductors of heat. Nanodots or Nanoparticles that consist of homogenous materials, especially those that are almost spherical or cubical in shape.

The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications. Thus far, the integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles. Several proteins make nanomaterials suitable for bio tagging or labeling. In order to interact with biological targets, a biological or molecular coating or layer acting as a bioinorganic interface should be attached to the nanoparticles. These biological coatings include antibodies, biopolymers like collagen, or monolayers of small molecules making the nanoparticles biocompatible. This is the reason why the biological metal nanoparticle synthetic methods are favored over physical and chemical methods. As the biosynthetic methods utilize natural solvents for the production of metal nanoparticles they are biocompatible and can be utilized in medicine. Also, nanoparticles have a further advantage over larger macromolecules as they are better suitable for intravenous delivery.

The shape of the nanoparticles is more often spherical but cylindrical, plate-like and other shapes are possible. The size and size distribution might be important in some cases, for example if penetration through a pore structure of a cellular membrane is required. The size and size distribution are becoming extremely critical when quantum-sized effects are used to control material properties. A tight control of an average particle size and a narrow distribution of sizes allow creating very efficient fluorescent probes that emit narrow light in a very wide range of wavelengths. This helps with creating biomarkers with many and well distinguished colors. The core itself might have several layers and be multifunctional. For example, combining magnetic and luminescent layers one can both detect and manipulate the particles.

In medicine, nanoparticles first found use in the diagnosis of tumors in the spleen and liver using magnetic resonance tomography. In cancer therapy a major difficulty is to destroy tumor cells without harming the normal tissues. Radiotherapy attempts to focus irradiation on the tumor but nevertheless damages healthy tissues which cannot always be protected in the desired way. Magnetic drug targeting employing nanoparticles as the carrier is a promising cancer treatment avoiding side effects of conventional chemotherapy (Akerman et.al.2006). There is also a very significant role in hyperthermia in cancer drug delivery. There is increasing evidence that hyperthermia at 40-43^0 Celsius enhances the uptake of therapeutic agents into cancer cells and provides an opportunity for improved targeted drug delivery (Kocbek et.al.2001). Using nanoparticles for drug delivery of anticancer agents has significant advantages such as the ability to target specific locations in the body, the reduction of the overall quantity of drug used, and the potential to reduce the concentration of the drug at non target sites resulting in fewer unpleasant side effects (Joenathan et.al.2006). The use of nanoparticles as drug delivery vehicles for anticancer therapeutics has great potential to revolutionize (Faroji and wipf et.al.2009) the future of cancer

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therapy. As tumor architecture causes nanoparticles to preferentially accumulate at the tumor site, their use as drug delivery vectors results in the localization of a greater amount of the drug load at the tumor site; thus improving cancer therapy and reducing the harmful nano specific side effects of chemotherapeutics.in addition, formulation of these nanoparticles with imaging contrast agents provides a very efficient system for cancer diagnostics.

Pomegranate fruit, technically a berry, are globose and up to 6 inches wide. Inside they contain up to six hundred seeds surrounded by transparent sacs that are red when ripe. The seeds are divided into clumps by yellow membranes. Pomegranate aril juice provides about 16% of an adult's daily vitamin C requirement per 100 ml serving, and is a good source of vitamin B5 (pantothenic acid), potassium and polyphenols, such as tannins and flavonoids. Pomegranates are listed as high-fiber in some charts of nutritional value. That fiber, however, is entirely contained in the edible seeds which also supply unsaturated oils.

The most abundant polyphenols in pomegranate juice are the hydrolysable tannins called ellagitannins formed when ellagic acid binds with a carbohydrate. Punicalagins are tannins with free-radical scavenging properties in laboratory experiments and with potential human effects. Punicalagins are absorbed into the human body and may have dietary value as antioxidants, but conclusive proof of efficacy in humans has not yet been shown. During intestinal metabolism by bacteria, ellagitannins and punicalagins are converted to urolithins which have unknown biological activity in vivo. Other phytochemicals include polyphenolic catechins, gallocatechins, and anthocyanins, such as prodelphinidins, delphinidin, cyanidin, and pelargonidin. The ORAC (antioxidant capacity) of pomegranate juice was measured at 2,860 units per 100 grams.

II. MATERIALS AND METHODS

2.1 Collections of Plant Materials

The bark and fruit peels of the *Punica granatum* (pomegranate) tree were collected from the fruit market and the dust particles were removed. The plant materials are kept in the oven for 24 hours at 40^0C. After drying plant materials are converted into a fine powder with the help of mortar and pestle.

2.2 Chemicals

1. Silver nitrate ($AgNo_3$)
2. Chloroauric acid ($HAuCl_4$)
3. Deionized water

2.3 Preparation of Bark and Fruit Peel Extract

The 15 gm. of bark powder and 15gm. of peel powder was mixed with sterile D/W in 500ml Erlenmeyer flask. This mixture was then boiled for 25min on a heating plate. After boiling, the mixture was filtered with Whatman filter paper separately. The supernatant was used as a plant extract for the experiment.

2.4 Preparation of 1mM aqueous AgNo3 and HAuCl4 Solution

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76.441mg standard AgNo3 powder and 177.223mg standard HAuCl4 powder was separately diluted with 450ml de-ionized d/w. 1mM aqueous AgNo3 solution and HAuCl4 solution was used for the treatment of the plant extract.

2.5 Synthesis of Silver Nanoparticles in Pomegranate Bark and Peel Extract

Accurately measured bark and peel extract of 25ml and 50ml was separately added to the 75ml and 50ml in 1mM aqueous AgNo3 solution respectively in a jar. The jar was agitated for a few minutes and then incubated at room temperature.

2.6 Synthesis of Gold Nanoparticles in Pomegranate Bark and Peel Extract

Accurately measured as prepared bark extract and peel extract of 25ml and 50ml was separately added to the 75ml and 50ml in 1mM aqueous $HAuCl_4$ solution respectively in a jar. The jar was agitated for a few minutes and then incubated at room temperature.

III. OBSERVATIONS

The data obtained on analysis of characters of Phytosynthesized nano scale particles in Peel and Bark extract of *Punica granatum* is tabulated in this chapter.

Table 1: Observation table for average particle size, shape and distribution frequency of Phytosynthesized silver nanoparticles in *Punica granatum* bark & peel extract.

Sr. No.	Salt Used	Plant Materials	NTA analysis		TEM analysis	UV Spectra (nm)
			Mean size (nm)	Particles per frame	Shape of the particles	
1	Silver Nitrate	Peel extract	59	26.99	Oval, Spherical	463
2	Silver Nitrate	Bark extract	61	37.66	Circular	400

Sr. No.	Salt Used	Plant materials	NTA analysis		TEM analysis	UV Spectra (nm)
			Mean size (nm)	Particles per frame	Shape of the particles	
1	Gold Chloride	Peel extract	54	25.51	Spherical	548
2	Gold Chloride	Bark extract	58	10.91	Circular	520

Table 2: - Observation table for average particle size, shape and distribution frequency of Phytosynthesized gold nanoparticles in *Punica granatum* bark & peel extract.

IV. RESULT AND DISCUSSION

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The present work deals with the aspect of Phytosynthesis of Silver nanoparticles (AgNPs) and Gold nanoparticles (AuNPs) in peel and bark extract of *Punica granatum*. The finely grinded powder of pomegranate peels and bark (**Photoplate-1**) was used to prepare the extract. Au and Ag NPs have a wide range of application in areas such as catalysis, medical diagnostics, and biological imaging. Various physiochemical method of metal nanoparticles synthesis has been reported, all having their inherent limitations.

PHOTO PLATE 1	
Peel powder of *Punica granatum*	**Bark powder of *Punica granatum***
	

Fig. 1: UV-Vis Spectra recorded as a function of time for the solutions prepared using silver nitrate (1mM), peel and bark extract of *Punica granatum*.

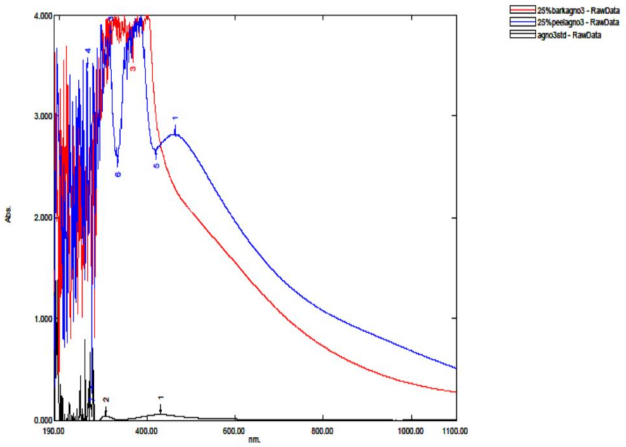

Fig. 2: UV-Vis spectra recorded as a function of time for the solutions prepared using gold chloride (1mM), peel and bark extract of *Punica granatum*.

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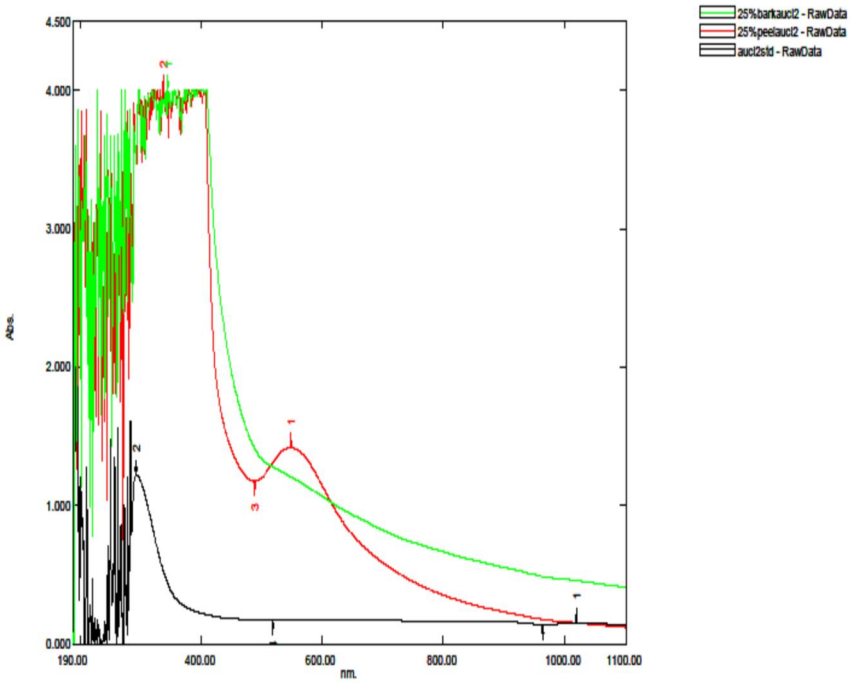

Fig. 3 NTA analyses of 25% Peel extract of Silver nanoparticles

Photo plate 2
Photo plate 2: a, b and c-TEM images of Ag Nanoparticles showing spherical and oval shape nanoparticles in *Punica granatum* peel extract

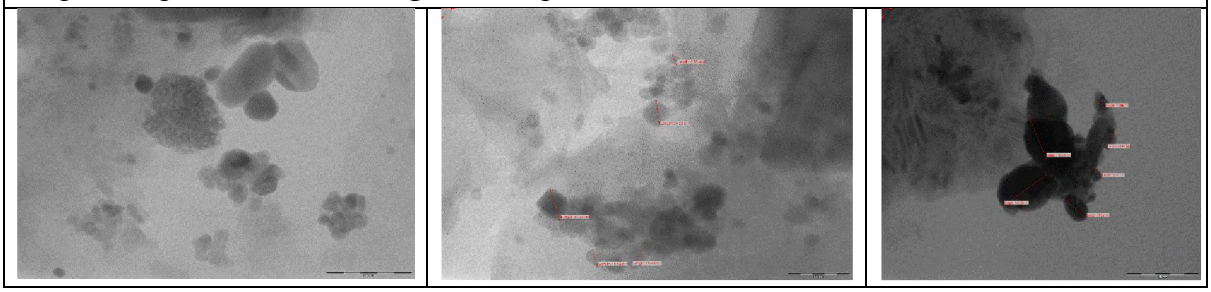

Photo plate 3
Photo plate 3: a and b- TEM images of Ag Nanoparticles showing circular shape nanoparticles in *Punica granatum* bark extract

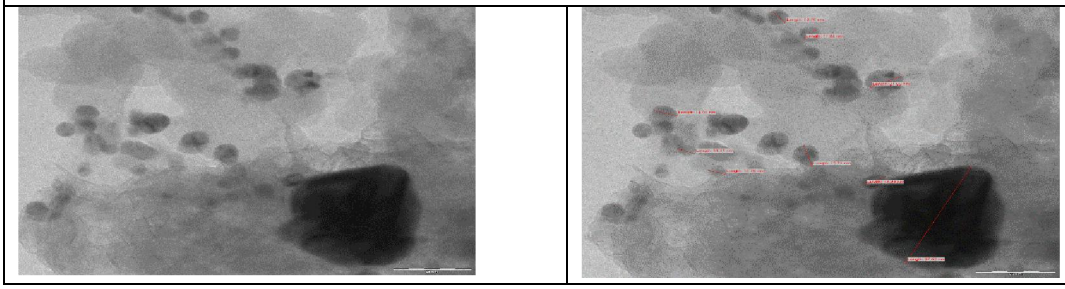

Fig. 4: NTA analyses of 25% Peel extract of Gold nanoparticles

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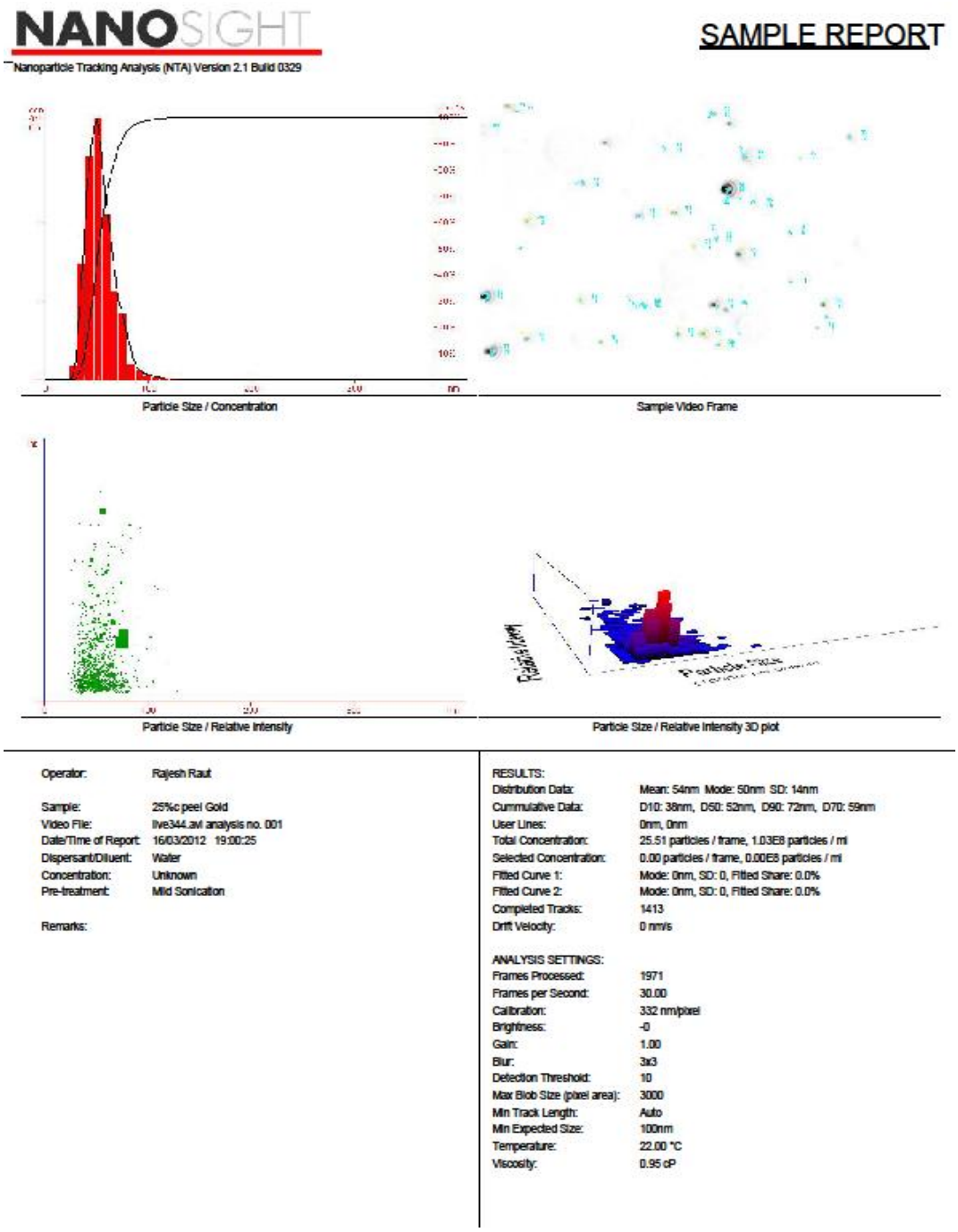

Fig. 5 NTA analyses of 25% bark extract of Gold nanoparticles

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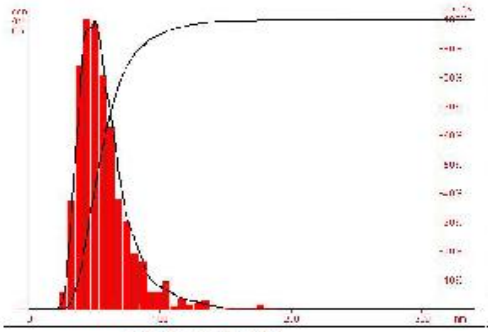
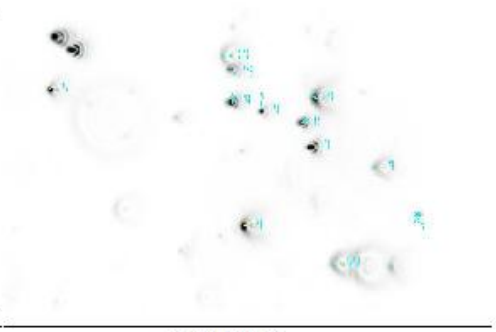
NANOSIGHT

Nanoparticle Tracking Analysis (NTA) Version 2.1 Build 0329

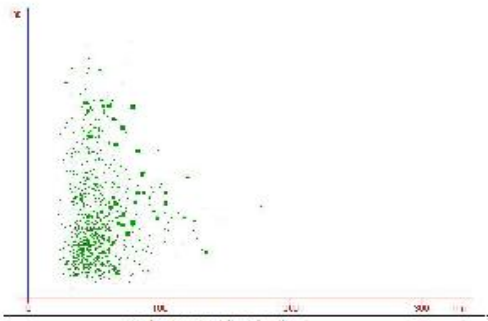
SAMPLE REPORT



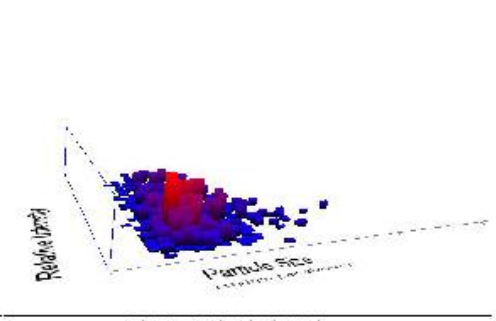
Particle Size / Concentration



Sample Video Frame



Particle Size / Relative Intensity



Particle Size / Relative Intensity 3D plot

Operator:	Rajesh Raut
Sample:	25% Bark AuCl4
Video File:	live331.avi analysis no. 001
Date/Time of Report:	21/02/2012 17:49:43
Dispersant/Diluent:	Water
Concentration:	Unknown
Pre-treatment:	Nil
Remarks:	

RESULTS:	
Distribution Data:	Mean: 58nm Mode: 50nm SD: 20nm
Cummulative Data:	D10: 37nm, D50: 53nm, D90: 82nm, D70: 62nm
User Lines:	0nm, 0nm
Total Concentration:	10.91 particles / frame, 0.44E8 particles / ml
Selected Concentration:	0.00 particles / frame, 0.00E8 particles / ml
Fitted Curve 1:	Mode: 0nm, SD: 0, Fitted Share: 0.0%
Fitted Curve 2:	Mode: 0nm, SD: 0, Fitted Share: 0.0%
Completed Tracks:	1079
Drift Velocity:	0 nm/s

ANALYSIS SETTINGS:	
Frames Processed:	3880
Frames per Second:	30.00
Calibration:	332 nm/pixel
Brightness:	-0
Gain:	1.00
Blur:	3x3
Detection Threshold:	56
Max Blob Size (pixel area):	3000
Min Track Length:	Auto
Min Expected Size:	100nm
Temperature:	22.00 °C
Viscosity:	0.95 cP

Photo plate 4

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Photo plate 4: a, b and c- TEM images of Au Nanoparticles showing spherical shape nanoparticles in *Punica granatum* peel extract

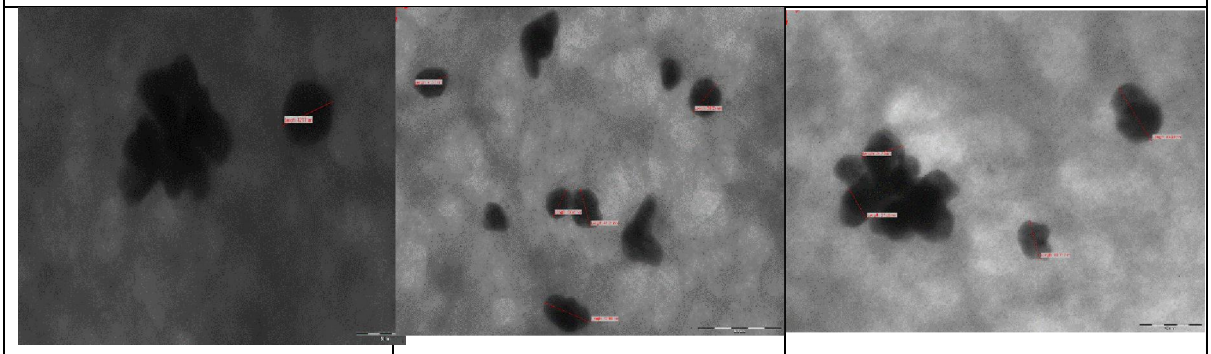
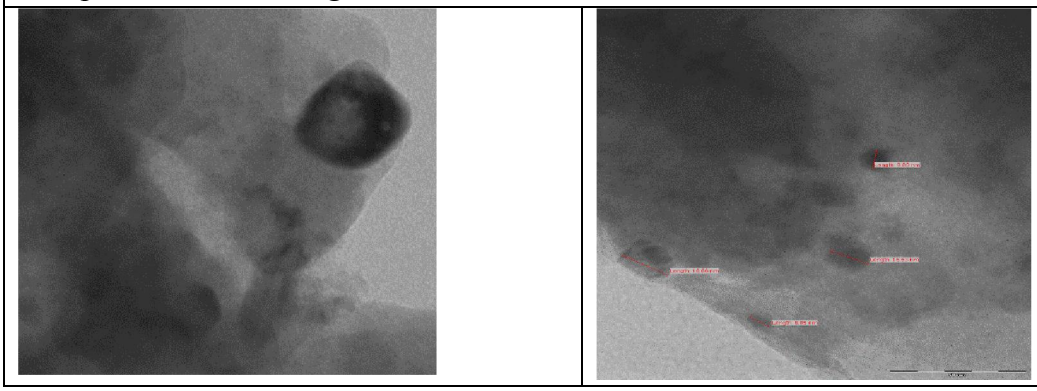


Photo plate 5

Photo plate 5: a and b- TEM images of Au Nanoparticles showing circular shape nanoparticles in *Punica granatum* bark extract



V. CONCLUSION

The present work was conducted in the bark and peel aqueous extract of Punica granatum to synthesized silver and gold nanoparticles by treating with 1mM concentration of silver nitrate and gold chloride. The characterization of Phytosynthesized nanoparticles in pomegranate peel and barks was done with the help of UV-Vis spectrometry, nanoparticle tracking analysis and transmission electron microscopy.

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