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In-Vitro Phytosynthesis of Silver (AgNP's) and Gold Nanoparticles (AuNP's) in Peel and Bark of Plant Punica Granatum (Pomegranate)

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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. Punica granatum is rich source in secondary metabolites especially polyphenols such as alkaloids, tannins, flavonoids and also steroids, triterpenes etc. which has lots of medicinal importance. The extract reaction mechanism of the nanoparticles synthesis by using biomaterials is yet to elucidate in detail; the work done proposes the involvement of redox enzymes in the reduction of silver and gold ions.*

Keywords: Nanoparticles, Phytosynthesis, Pomegranate, Silver Nanoparticles, Gold Nanoparticles, NTA, TEM, UV-Vis

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.

In present work, *Punica granatum* plant was taken as an experimental system for Phytosynthesis of AgNP's and AuNP's. Bottom up and 'Top down Approach are considered as two important modes of nanoparticle synthesis. In the bottom up approach, materials and devices are built from molecular components which assemble themselves chemically by principles of molecular recognition. While in the top down approach, nano sized particles are constructed from large entities without atomic level control (Maccuspie et.al. 2011). Nanostructures come in various sizes and shapes and are synthesized by using different metals and other chemicals.

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 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.

Gold plays an important role in pharmaceutical, cosmetic and food industries. Dentists use gold for crowns, and certain medicines, such as sodium aurichloride for rheumatoid arthritis which also contain gold. Small amount of gold sometimes brightens foods such as jelly or liquors, like Goldschlager gold can catalyze, or speed up, certain chemical reactions more efficiently than other toxic catalysts. It plays an important role in reducing pollution. For example, scientists have recently discovered that gold particles energized by the sun can destroy volatile organic chemicals. Recently, gold nanoparticles were used to detect breast cancer. The procedure works by identifying the proteins found on the exteriors of the cancer cells. Different types of cancer have different proteins on their surfaces that serve as unique markers. Nanorods, gold nanoparticles shaped like rods, use specialized antibodies to latch onto the protein markers for breast cancer, or for another cancer type. After the nanorods bind to proteins in a blood sample, scientists examine how they scatter light. Each protein-nanorod combination scatters light in a unique way, allowing for precise diagnose.

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 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.

Silver derives its broad-spectrum antimicrobial activity from the ability of silver ions to bind irreversibly to a nucleophilic group commonly available in cells of bacteria, viruses, yeast, fungi and protozoa. Binding to cellular components disrupts the normal reproduction and growth cycle resulting in death of the cell. Capitalizing on its potent activity, silver and its compounds have been incorporated over the past several decades in a variety of wound care products such as dressings, hydrogels, hydrocolloids, creams, gels, lotions, sutures, and bandages. The preferred form of silver in antimicrobial products has been its compounds or salts as the metallic form of the element itself lacks therapeutically effective oligodynamic action. The compounds or salts upon contact with an aqueous medium ionize to yield silver ions that become available for antimicrobial action. The majority of silver compounds are also photosensitive or heat sensitive making their utilization in stable commercial products challenging. It is well known that the proteins and enzymes present in the plant extract are responsible for the reduction of metal ions. However, the shape, size and the stability of metal nanoparticles is due to the phyto-constituents present in the extract. It is also known that the composition of the enzymes and phyto-constituents varies from plant to plant and thus has the impact on the ability to reduce metal ions as well as the shape, size and stability of the metal nanoparticles.

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

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.

2.7 UV-Vis Spectra Analysis

The bioreduction of Au^{3+} to Au^0 and Ag^{++} to Ag^0 in the aqueous solution was monitored by periodic sampling (0min, 15min, 30min…120min, 24hr) of aliquots of the suspension, if required then by diluting the samples with distilled water and subsequently measuring the UV-Vis Spectra (190nm to 1100nm) of the resulting diluents on the spectrophotometer (Model-Shimazdu UV 1800). Similarly, the spectra of the ions were recorded and compared. Deionized water was used for the baseline correction.

2.8 Transmission Electron Microscopy (TEM)

TEM samples of the silver, gold nanoparticles synthesized by the peel and bark extract of *Punica granatum* were prepared by first sonicating the samples in sonicator (Vibronics VS80) for 15mins. A drop of the nanoparticle's solution was put on carbon coated copper grids of 3mm diameter, blot to remove excess of solution and later was allowed to dry under Infrared light for 40mins. TEM measurements were then performed on instruments operated at an accelerating voltage at 200 Kv (PHILIPS MODEL CM 200).

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.

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IV. RESULT AND DISCUSSION

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. Development of easy, reliable and ecofriendly biological methods helps in endorsing extra interest in the synthesis and application of nanoparticles which are good for mankind (Bhattacharya and Gupta, 2005). In this context the utilization of biological systems for nanoparticles synthesis provide move towards this multifaceted approach. Biological systems have shown ability to interact with metal ions and reduce them to form metallic nanoparticles (Beveridge et.al.1997).

Phytosynthesized Silver NP's in Peel and Bark extract of *Punica granatum*:

UV-Vis Studies

A UV-Vis measurement gives the precise report of the absorbance of the light on the basis of shape of the nanoparticles. Absorption spectra show the production of SNP's within an hour on the reduction of Ag^{++} ions. The UV-Vis spectra of reaction mixture of 1mM aqueous $AgNo_3$ solution and 25% peel and 25% bark extract of plant *Punica granatum* is shown in the **Fig.-1**. The spectra clearly shows the absorption band at around 463nm in the peel extract and at around 406nm in the bark extract of silver nanoparticles. There is a close relationship between the UV-Vis and light absorbance characteristics and size and shape of the absorbate (Ankavmar et.al, 2005). The absorption band is a characteristic feature of anisotropic nanoparticle and occurs in structures such as triangular, spherical and hexagonal particles (Ankavmar et.al, 2005).

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

Fig. 1: UV-Vis Spectra re… ver nitrate (1mM), peel and bark extract of *Punica gra…*

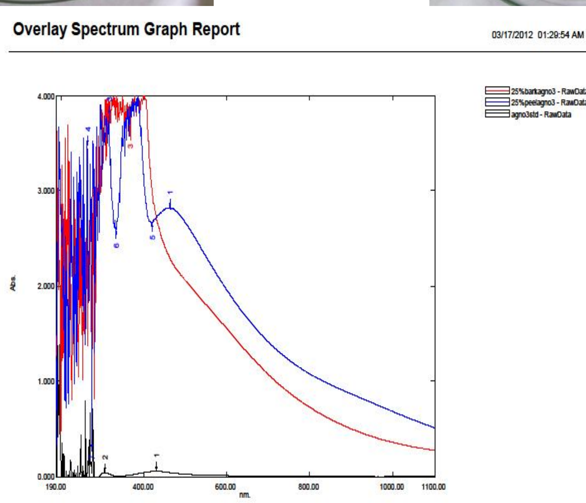
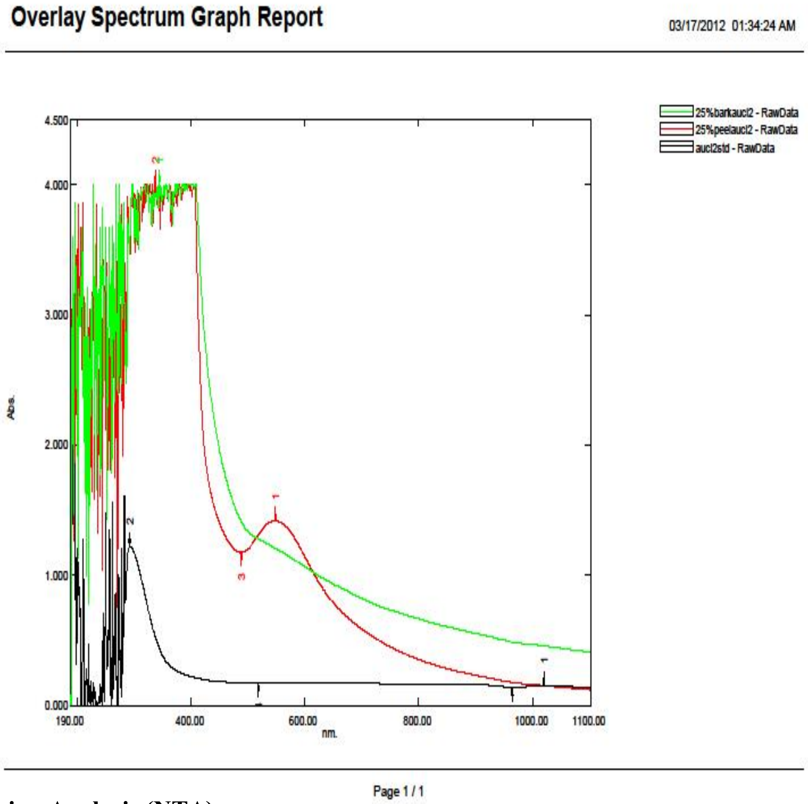


Fig. 2: UV-Vis spectra recorded as a function of time for the solutions prepared using gold chloride (1mM), peel and bark extract of *Punica granatum*.



Nanoparticle Tracking Analysis (NTA)

NTA analysis for silver nanoparticles was done using the Nano sight Ver.2.1 Instrument in the Institute of Science. NTA studies indicated the average size of SNP's synthesized in the peel extract as 59 nm with the frequency of 26.99 particles per frame (**Fig.-3**). The nanoparticles synthesized using bark extract showed the average particle size of 61nm with the average frequency of 37.66 particles per frame (**Fig.-4**). These results indicated the high frequency of SNP' synthesis in bark extracts compared to peel extract. It is to be noted that the average size of Phytosynthesized SNP's was also more in bark extract (61nm) against peel extract (59nm).

TEM Analysis for Silver Nanoparticles

The shape of the Phytosynthesized nanoparticles can be conspicuously observed by TEM analysis. TEM samples of the aqueous suspension of silver nanoparticles after sonication for 15 minutes were produced by placing a drop of the suspension on Carbon coated copper grids and allowing water to evaporate in vacuum. TEM observations were performed on Philips Electron Microscope operated at an accelerating voltage of 200Kv with the resolution of 0.22. The shape of the AgNP's in pomegranate peel extract was spherical and Oval (**Photoplate-2**). This morphology of silver ions is obtained by reduction of Ag++ to Ag^0. A large density of silver NP's was observed under low magnification. Thus, silver nanoparticles are quite polydispersed and range in size from 9-70nm. The SNP's in bark extract showed spherical shape (**Photoplate-3**). The Silver nanoparticles are quite polydispersed and range in size from 9-22nm. These observations indicated the variations in the shape of nanoscale particles in peel and bark extract.

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

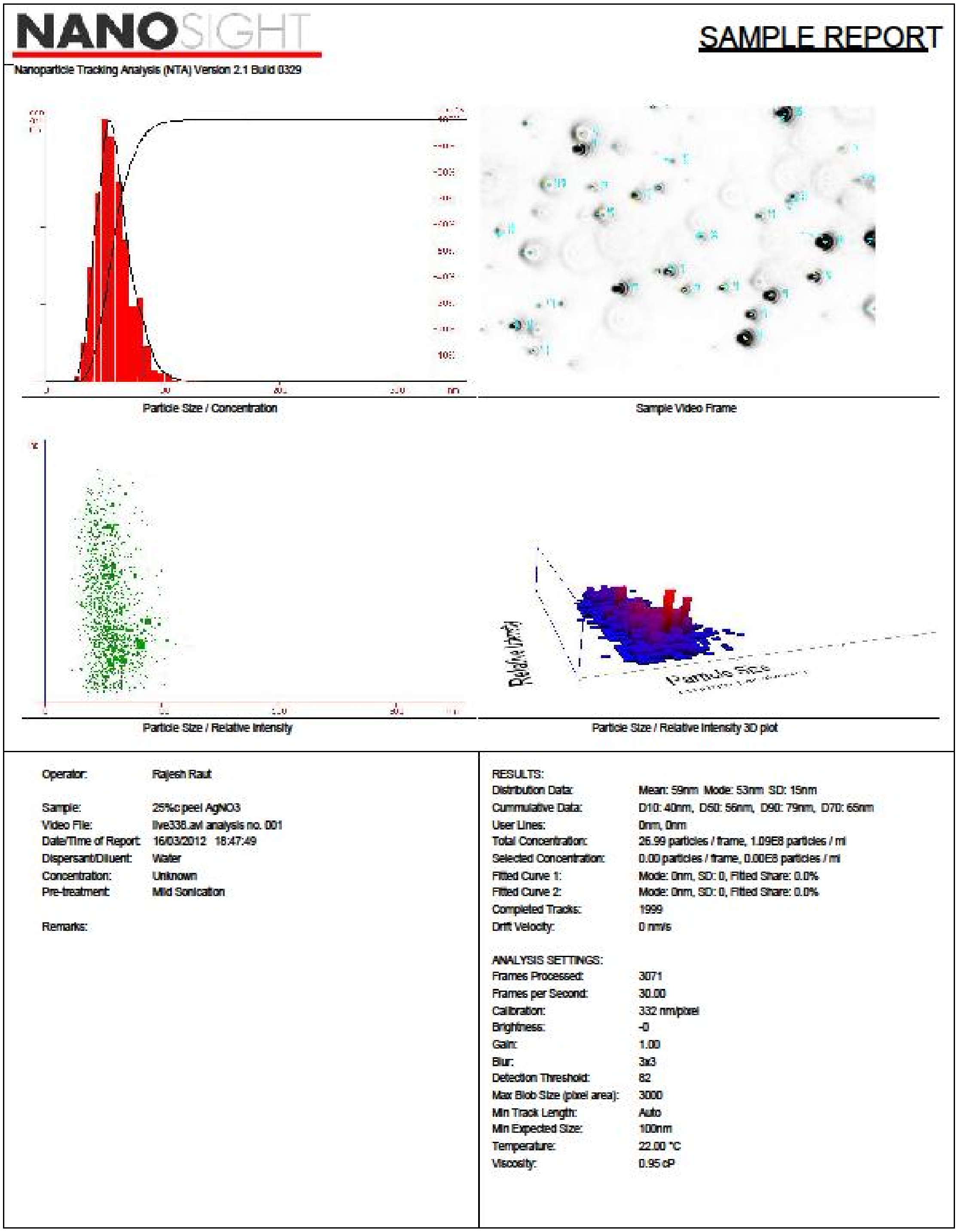

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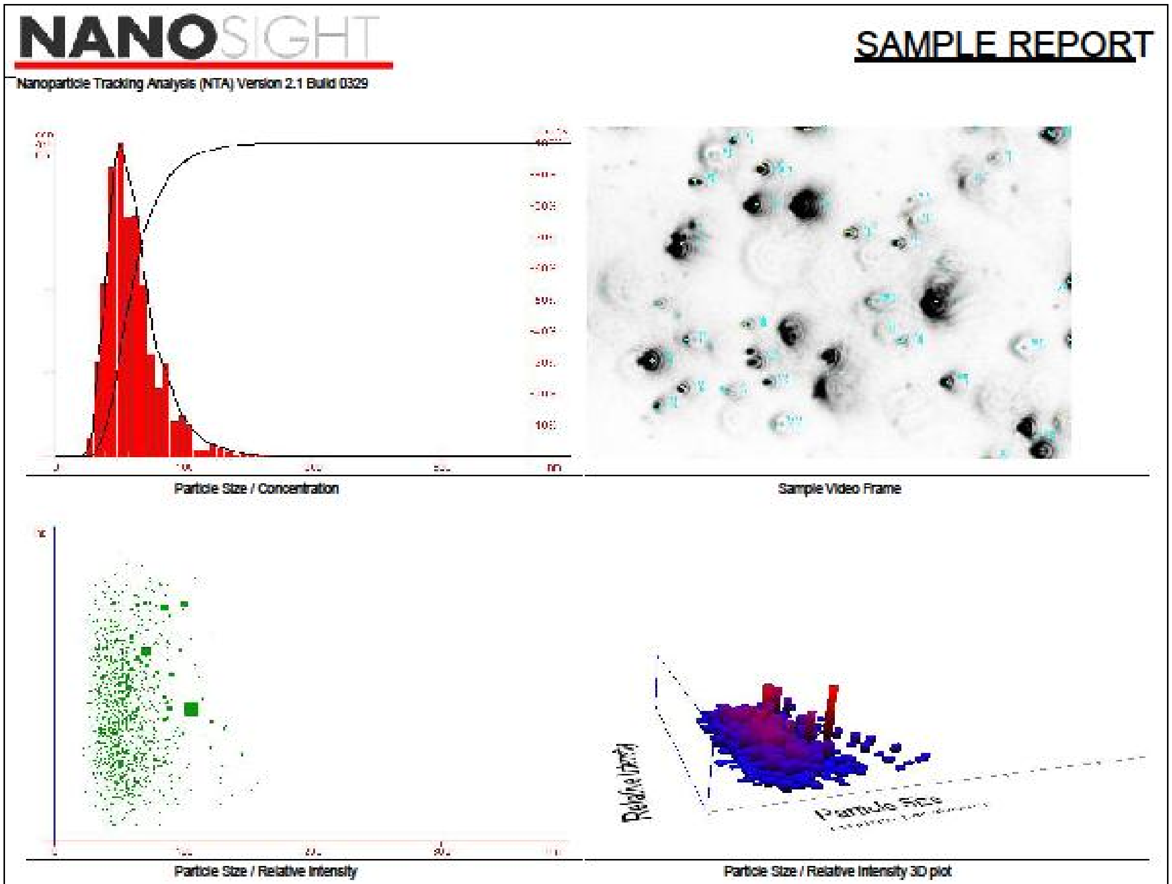
Fig. 4: NTA analyses of 25% bark extract of silver nanoparticles



Operator:	Rajesh Raut
Sample:	25% Bark AgNO3
Video File:	live328.avi analysis no. 001
Date/Time of Report:	21/02/2012 17:34:44
Dispersant/Diluent:	Water
Concentration:	Unknown
Pre-treatment:	Nil
Remarks:	

RESULTS:	
Distribution Data:	Mean: 61nm Mode: 51nm SD: 20nm
Cummulative Data:	D10: 39nm, D50: 56nm, D90: 87nm, D70: 66nm
User Lines:	0nm, 0nm
Total Concentration:	37.66 particles / frame, 1.52E8 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:	1868
Drift Velocity:	0 nm/s
ANALYSIS SETTINGS:	
Frames Processed:	2277
Frames per Second:	30.00
Calibration:	332 nm/pixel
Brightness:	-0
Gain:	1.00
Blur:	3x3
Detection Threshold:	78
Max Blob Size (pixel area):	3000
Min Track Length:	Auto
Min Expected Size:	100nm
Temperature:	22.00 °C
Viscosity:	0.95 cP

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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

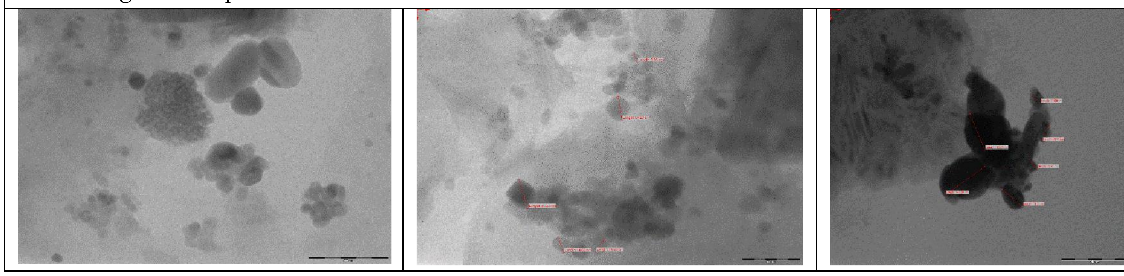
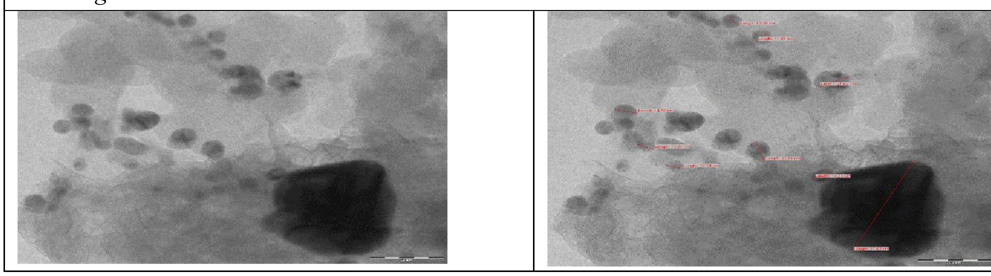


Photo plate 3

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



Phytosynthesized Gold NP's in Peel and Bark extract of *Punica granatum*: -

UV-Vis Studies:

The absorption spectra show the production of NP's within an hour on the reduction of Au^{+++} ions in to Au^{0}. The UV-Vis Spectra of reaction mixture of 1mM aqueous AuCl4 solution and 25% peel and 25% bark extract of plant *Punica granatum* is shown in the Fig.**-2.** The spectra clearly show the absorption band at around 548 nm in the peel extract and at around 520 nm in the bark extract of gold nanoparticles.

Nanoparticle Tracking Analysis (NTA):

NTA analysis for gold nanoparticles indicated the average size of gold NP's synthesized in the peel extract as 54 nm with the frequency of 25.51 particles per frame (**Fig.-5**). The nanoparticles synthesized using bark extract showed the average particle size of 58 nm with the average frequency of 10.91 particles per frame (**Fig.-6**). These results indicated the high frequency (25.51) of gold NP's synthesis in peel extracts compared to bark extract (10.91). It is to be noted that the average size of Phytosynthesized gold NP's was also more in bark extract (58 nm) against peel extract (54 nm).

TEM Analysis for Gold Nanoparticles:

TEM samples of the aqueous suspension of gold nanoparticles after sonication for 15 minutes were produced by placing a drop of the suspension on Carbon coated copper grids and allowing water to evaporate in vacuum. TEM observations were performed on Philips Electron Microscope operated at an accelerating voltage of 200Kv with the resolution of 0.22. The shape of the Au NP's in pomegranate peel extract was spherical (**Photoplate-4**). This morphology of gold ions is obtained by reduction of Au+++ to Au^{0}. In TEM analysis, the particle range observed was 8-20nm. The Au NP's in bark extract showed circular shape (**Photoplate-5**). These observations indicated the variations in the shape of nanoscale particles i.e. spherical in peel and circular bark extract.

Fig. 5: NTA analyses of 25% Peel extract of Gold nanoparticles

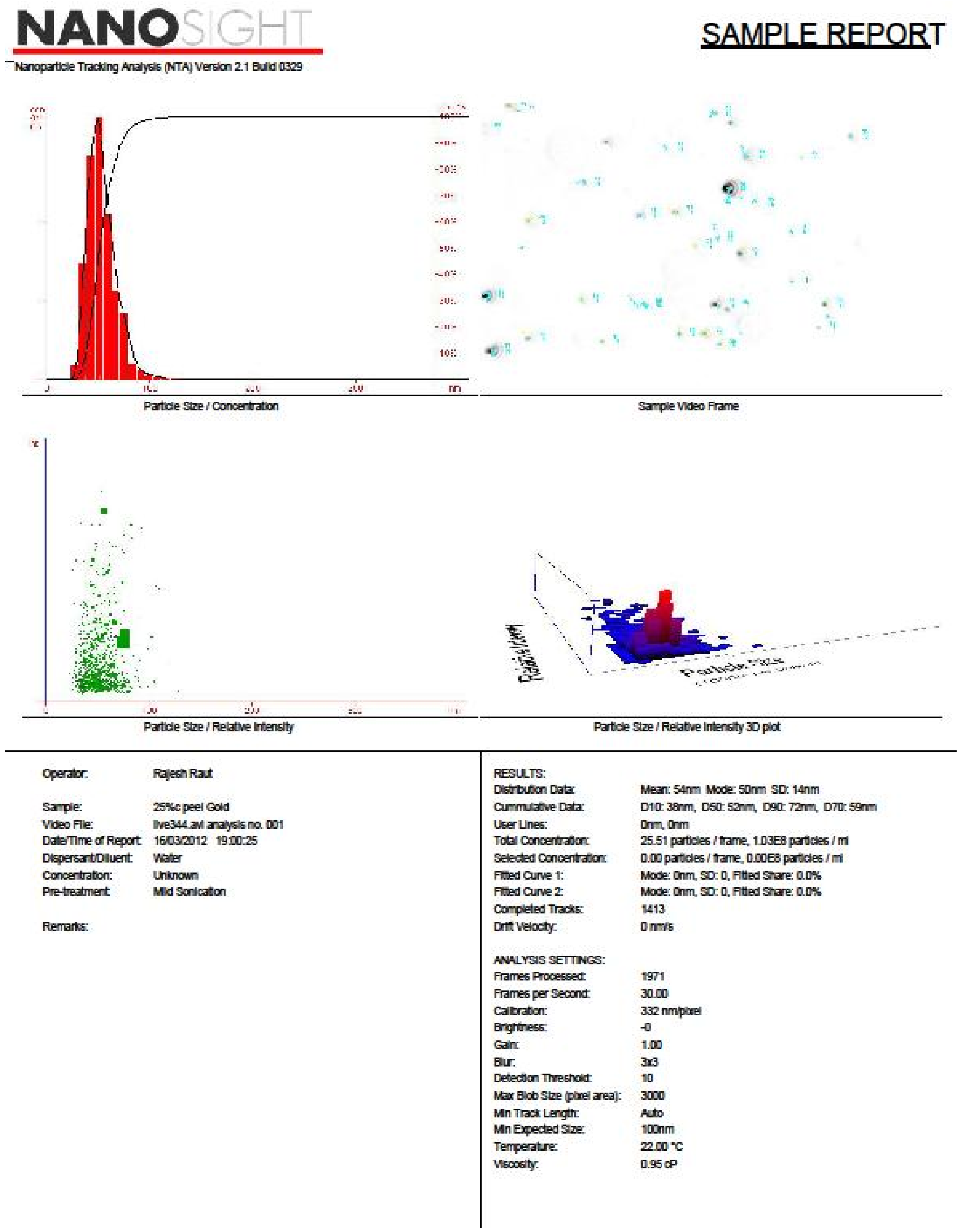


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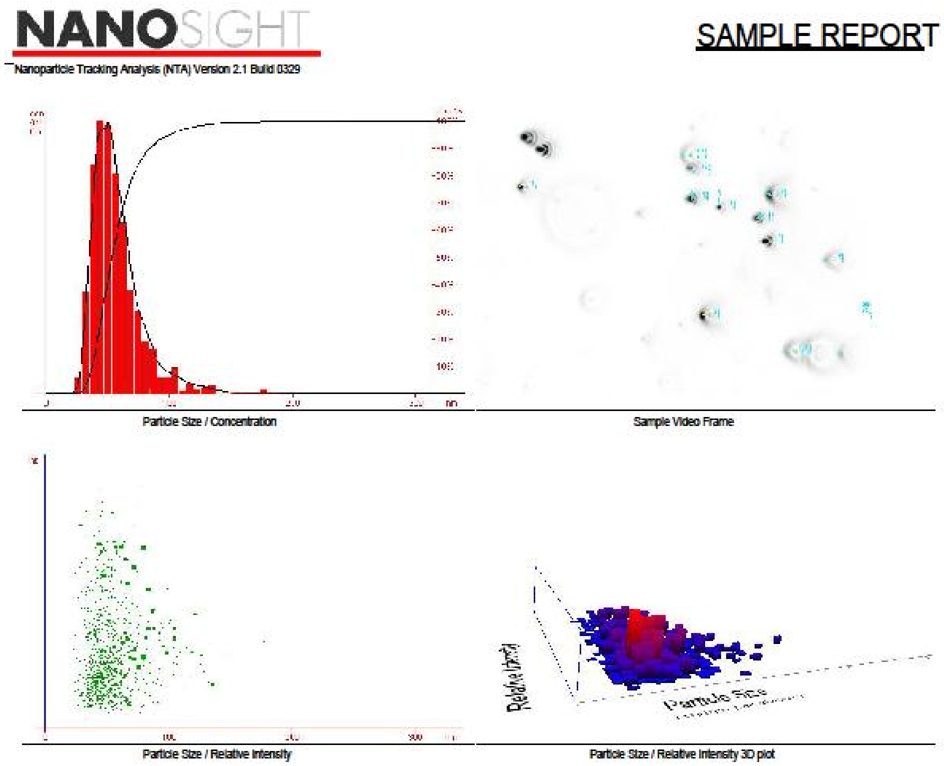
Fig. 6: NTA analyses of 25% bark extract of Gold nanoparticles



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

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

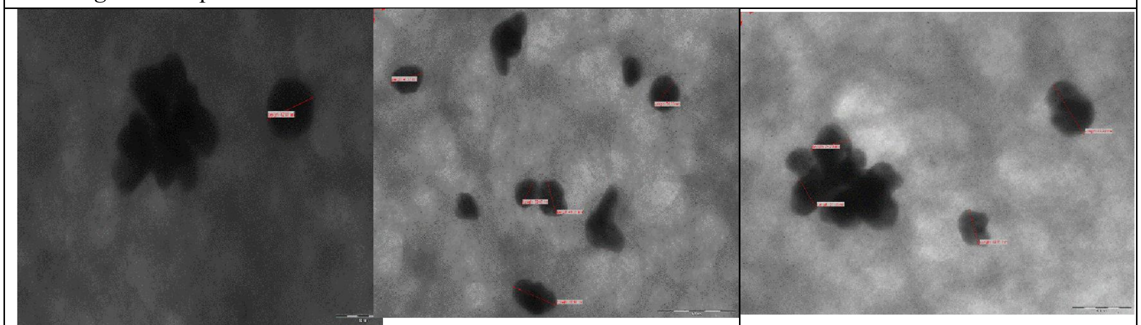
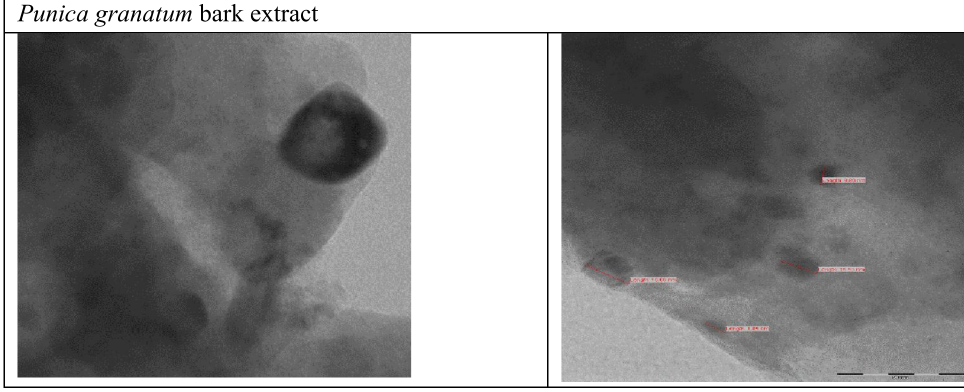


Photo plate 5

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



Green synthesis of nanoparticles is a coming up experimental exercise where the biological agents like Bacteria, Fungi, and Higher plants are used (Mukherjee *et. al.,* 2001a, Singaravelu *et. al.,* 2007) have reported the use of plant materials for the synthesis of nanoparticles. The use of inactivated biomass to recover metal ions from solution has been studied extensively. The possibility of using live bacteria for the remediation of metal-contaminated waters has shown the bacterial production of silver-carbon composite materials. Many reports on synthesis of metal and semiconductor nanoparticles using fungi or bacteria have appeared. In our quest for new eco-friendly "green" methods for the synthesis of noble metal nanoparticles, many workers have identified fungi (Mukherjee *et. al*., 2001), Actinomycetes **(**Ahmad *et. al*., 2003), and plant extracts (Shivshankar*et. al*., 2003), for the synthesis of silver and gold nanoparticles (Shivshankar*et. al*., 2003).

The synthesis of stable silver, gold and bi-metallic Ag/Au core shell nanoparticles using 20 g of leaf biomass have been reported by Shivshankar *et.al,* (2004) using *Azadirachta indica*. In vivo synthesis of nanoparticles of gold-silver-copper alloy has been reported by R.G. Haverkamp*et.* al.2007. Most of the above research involves the synthesis of colloidal silver or gold nanoparticles employing plant broths resulting from boiling fresh or dried plant leaves (Shivshankar*et. al*., 2003, 2004, 2005 and 2006).

In the present work, the attempt was made to synthesize Silver and Gold nanoparticles employing the fruit peel and bark extracts of *Punica granatum* Linn. *Punica granatum* is a rich source in secondary metabolites especially polyphenols such as alkaloids, tannins, flavonoids and also steroids, triterpenes etc. which has lots of medicinal importance. The extract reaction mechanism of the nanoparticles synthesis by using biomaterials is yet to elucidate in detail; the work done proposes the involvement of redox enzymes in the reduction of silver and gold ions. Different chemical components present in the plant contribute to the stability, shape and size of the phytonanoparticles.

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. The results obtained in the present work are concluded as follows:-

- In both the experimental samples (peel and bark extract of *Punica granatum*, it is concluded that the biomass used yields good response for the The Phytosynthesis of silver and gold nanoscale particles.
- The UV-Vis Spectrophotometry analysis revealed the spectrum status for both the samples in accordance with the nature of salt used.
- Nano tracking analysis for silver nanoparticles indicates high frequency of nano sized particles in bark extract compared to peel extract of the experimented plant.
- The average size of the silver nanoparticle was more i.e. 61nm for a bark extract when compared to a peel extract sample.
- The gold nanoparticles appeared with high frequency in peel extract however the size of nanoparticles was more (58 nm) in the bark extract sample compared to peel extract.
- The TEM analysis indicates circular, spherical, and oval shaped phytonanoparticles.
- Silver nanoparticles in peel extract were spherical as well as oval in shape whereas in bark extract only spherical nano sized particles were observed.
- The gold nanoparticles were circular in shape in bark extract whereas in the peel extract sample the shape of nano scale particles was spherical.

The work conducted was useful to understand the process of nanoparticle synthesis using plant material. The work also gave an insight to understand the characters of phyto nanoparticles using analytical techniques. This mode of synthesis is a part of nano biotechnology using the green synthesis approach. These aspects generate more interest in plant science researchers because the phytonanoparticles are capped with biological molecules which are either enzymes or the phytoconstituent's derivatives of different bioactive groups. Therefore, the use of these nano size particles in modern medicine and drug delivery technology deserves high importance and generates more scope for research in future.

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