GREEN SYNTHESIS OF GOLD NANOPARTICLES FROM MEDICINAL PLANTS

DISSERTATION

Submitted To Lovely Professional University

For the partial fulfillment of the award

Of

Masters of Science in Chemistry (Honors)

By

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CERTIFICATE

This is to certify that dissertation project report on **'Green Synthesis of Gold Nanoparticles From Medicinal Plants'** which is submitted by **Atinderpal Kaur** is in partial fulfillment of the requirement for the award of Master of Science in Chemistry (Honors) is a record of the candidate own work carried out by her under my guidance and supervision. The matter embodied in this report is original and has not been submitted for the award of any other degree.

Date:-

Signature of Supervisor:

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CONTENTS

ABSTRACT

Throughout the work, gold and silver nanoparticles have been synthesized using different plant material to make this synthesis environment friendly. As any type of stabilizing agent is not used, thus this method is green method and does not pose any harm to any living being. Nanoparticles are synthesized and then characterized by UV-Vis Spectroscopy, IR and XRD analysis. Further application of these nanoparticles is seen in their dye reducing activities in which ***Citrus aurantium*** and ***Punica granatum*** plant species give the best results.

1. INTRODUCTION

Nanoparticles are the particles of very small size ranging from 1nm to 100nm and have special characteristics which make them beneficial in almost all the fields like medicine, biological products, cosmetics, aerospace, military, etc. They are of organic or inorganic nature. Size of Nanoparticles plays a vital role. This is because all the electrical, optical, catalytic, magnetic properties vary according to the variation in their size. [1] Nanoparticles can be natural like from volcanic eruptions, ocean spray, etc. They can be incidental like combustion products, frying, mining, etc. They can be engineered like quantum dots, fullerenes, etc. [1] Nanotechnology is a recent science but the use of nanoparticles is very ancient. Artisans of Mesopotamia make glittering pots by the use of gold and silver nanoparticles. However, Michael Faraday gave the first scientific explanation of Nanoparticles in his paper, 'Experimental Relations of Gold with Light' in 1857. Professor Peter Paul was the one who discovered the use of nanoparticles in the fields of drug delivery and vaccines. At present time, nanotechnology is considered as the future to all the technologies. [5]
There are different methods to synthesize nanoparticles which are as follows:-

a. **Chemical Reduction**: - In this method metals are reduced by the use of reducing agents like sodium citrate, etc. and addition of stabilizing agents is required to increase the stability of nanoparticles. [5]

b. **Green Synthesis Method**: - In this method plant extracts are refluxed at high temperature with the metal salt. This method eliminates the need for stabilizing agents and reducing agents.[21]

c. **Sol-Gel Technique**: - In this technique sol act as precursor which is formed by the fabrication of metal oxides and it then can be deposited as a film on the substrate or can be formed in the form of powders. [5]

d. **Laser Ablation**: - This method involves the interaction of solid surface with the laser beam which leads to the removal of the materials. The wavelength of laser beam and the optical properties of the material determine the extent of beam absorbed by the solid and the amount of solid material removed. [5]

There are different applications of nanoparticles which make them an important aspect of research. There are newly discovered nanoparticles which can deliver a large amount of drug in the body and this delivering can be controlled. [3] These nanoparticles are quite useful in treating tumors and cancers and are based on polysilsesquioxane particles which are silicon resin. [3] Nanoparticles are similar to bio-molecules because of same size range from 1 to 100nm and thus quite useful in bio-imaging and bio-sensing. [4] Nanoparticles can modify with different types of moieties like cationic, anionic, neutral, etc. because of their small size and high surface area. Thus, have enormous applications in biological sciences. [1] Nanoparticles of noble metals are very compatible, have good catalytic activity and conductivity. [1] Self cleaning tiles, windows can be made by exploiting the photo catalytic property of nanoparticles. [5]

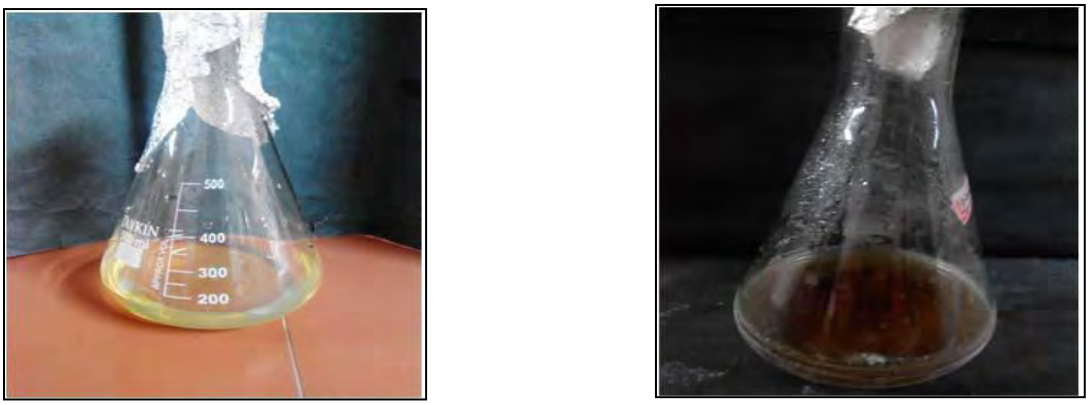
2 LITERATURE REVIEW

2.1 METAL NANOPARTICLES

These nanoparticles are made up of metals like silver, gold, nickel, etc. These particles have greater field in electronics, optics, sensors, biological things, etc. For biological purposes it must be ensured that these metal nanoparticles should not pose any harm to the living system. This is the reason that for medicinal fields noble metals are preferred as they are inert chemically.

a) SILVER NANOPARTICLES

Nanoparticles prepared with chemicals have various disadvantages as they pose problems to environment, hazardous chemicals, and lower output, high energy consumption etc. so there is now increasing need to adopt green methods for developing nanoparticles. Silver nanoparticles are prepared by the interaction of plant extracts with $AgNO_3$. These extracts reduce Ag from +1 oxidation state to 0 oxidation states. This reduction results in the formation of nanoparticles. Any change in color indicates nanoparticles formation as shown in Fig.1. [6] Silver nanoparticles are formed by green method by using plant extracts of a plant having a medicinal importance. The specialty of the green synthesis method is no need of the stabilizing agent to be added. Scheme 1 shows the steps of formation of silver nanoparticles and which are collected by centrifugation and repeated washings of formed nanoparticles is done.



(a) **(b)**

Fig.1 (a) Plant Extract and $AgNO_3$ solution initially (b) Plant Extract and $AgNO_3$ solution after 3hours.

Plant Extract + $AgNO_3$ → Reflux → Change in Color → Centrifugation → Washing and Collection of Newly formed nanoparticles

Scheme 1 Formation of silver nanoparticles by green method

The change in color takes place because surface electrons of metals interact with the nanoparticles. [2]

b) GOLD NANOPARTICLES

Gold nanoparticles are widely used because of their inert nature and many medicinal benefits. Among the different shapes of gold nanoparticles like nanosphere, nanorod, nanostar, etc. the triangular shaped nanoparticles have more usefulness. [1]They have many advantages as they are very stable, easily coordinate to bio-molecules, deliver drugs efficiently, etc. They can be easily prepared from the reduction of $HAuCl_4$ with sodium citrate or sodium borohydride as shown in Scheme 2. Solution of Auric acid and sodium citrate (10ml) is heated up to boiling and change in color indicates formation of gold nanoparticles in which further dialyses takes place for 3 days to purify nanoparticles. [7]. these particles can be coordinated to molecules like octareotide which can be able to enhance the drug delivery capability as well as enhances the phenomenon of targeting of drugs. [8]

$HAuCl_4$ + $Na_3C_6H_5O_7$ ⟶ Raw gold nanoparticles ⟶ gold nanoparticles

(1 M) (1ml 0.1 M) (Color change observed)

Scheme 2 Preparation of gold nanoparticles by chemical method.

2.2 BIMETALLIC NANOPARTICLES

Bimetallic nanoparticles are the nanoparticles in which two elements are combined together and they produce synergistic effect of their individual properties. In this type of formation, one of the elements is present in the core and the other is surrounded by it as a shell as shown in Fig.2. We can enhance the magnetic properties of the nanoparticles in this way. [10] Bimetallic nanoparticles can also lead to the formation of alloys rather than core-shell structures. These are quite useful in the applications of enhancing of electrical, thermal, plasmonic, electromagnetic, electro catalytic properties. [11]

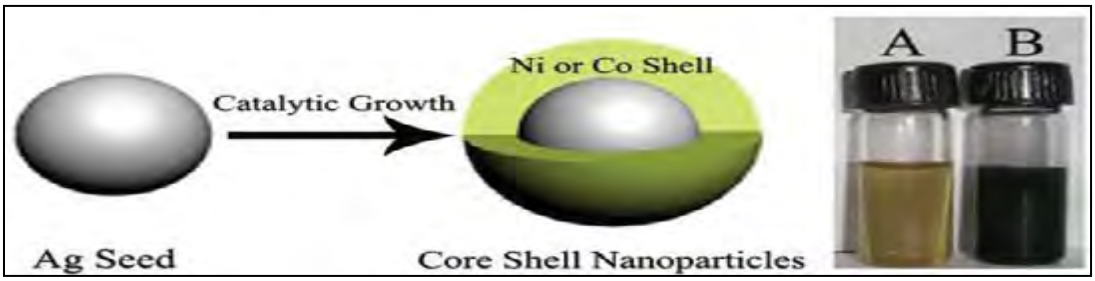


Fig.2 Showing coating of one type of nanoparticles as a shell over the other type of nanoparticles as core.

a) Silver-Nickel Nanoparticles are prepared in which silver metal reduces first because of their low reduction potential and the change in color shows the formation of silver nanoparticles. But when the change in color gets darker like turns black then there is reduction of core shell elements like nickel or cobalt. This combination enhances the magnetic ability of the Nanoparticles. [10]

b) Gold-Silver Nanoparticles are bimetallic nanoparticles in which gold is reduced first to form nanoparticles and then silver is reduced over the gold nanoparticles to form the core-shell structure. There is an equal possibility of formation of alloy of gold and silver. The difference between two possibilities is ensured by the techniques of TEM and SPR. The SPR shows one peak on formation of alloy and shows two continuous peaks which are independent peaks and thus ensures the formation of bimetallic moiety. [12]

2.3 PLANTS USED FOR NANOPARTICLE FORMATION:-

I. ***Camelila sinensis* (Tea plant) :-**

Camelila sinensis plant is known for its medicinal properties as its use helps in controlling the total blood cholesterol level and maintains the ratio of good cholesterol to bad cholesterol. Fluorine and catechin glucosyl transferase present in tea plant destroy carcinogenic bacteria and stops cavities.

(a)

(b)

Fig.3 showing leaves of ***Camelila sinensis*** (a) and tea used at home (b)

II. ***Pisum sativum* (Garden Pea):-**

Pisum sativum is rich in minerals like potassium, iron, magnesium, etc. They have vitamin B complex and niacin in high amounts which ensures the proper functioning of heart and Central Nervous System.

(a)

(b)

Fig.4 showing fresh pods (a) and dried pods (b) of ***Pisum sativum***

III. ***Punica granatum* (Pomegranate)**:-

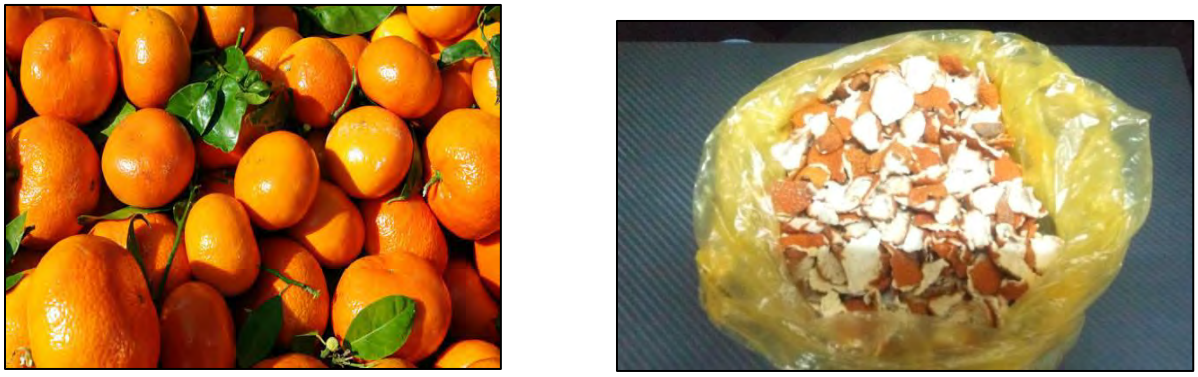
This fruit is commonly available and has various medicinal importances. It is effective against cancer, heart problems, and infertility problems, improves blood pressure, bone health and promotes good cholesterol levels. Shade dried peels of ***Punica granatum*** are used to prepare the extract, which is then used to form silver and gold Nanoparticles.

(a) (b)

Fig.5 showing peels (a) and fruit (b) ***Punica granatum***

IV. ***Citrus aurantium* (Orange):-**

It is well known fruit and has a number of medicinal properties. It consumption helps to prevent birth defects, heals cuts and injuries and manage skin conditions. Orange peels are helpful in clearing, detoxifying and toning the skin. Orange strengthens the nerves and cleanses stomach and intestine for better ingestion.



(a) **(b)**

Fig.6 showing fruit (a) and dried peels (b) of ***Citrus aurantium***

V. ***Cladonia subtenuis* (Lichen):-**

It is an example of symbiotic association. There are number of medicinal uses for which lichen is used. These uses include treatment of wounds, skin disorders, issues related to respiration and digestion etc.

VI. ***Swertia Chirayita* (Chirata):-**

It is an Ayurvedic Plant. It is used to treat various skin related problems like rashes, etc. It can be used to get rid of constipation, for diabetic patients and to treat various lungs related problems.

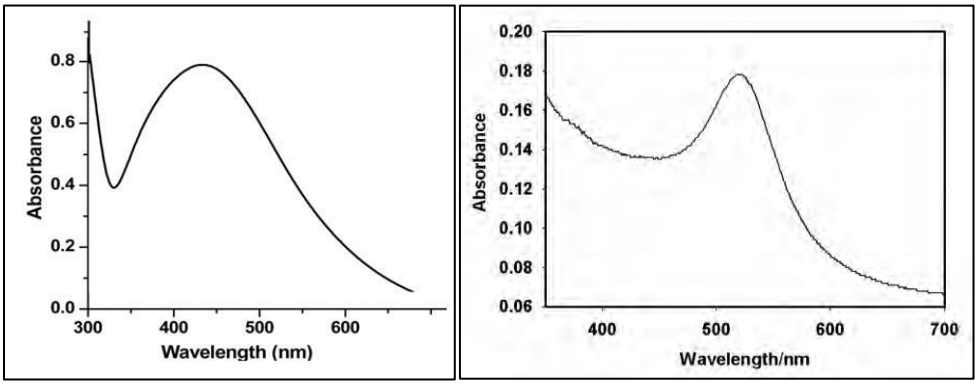
(a) **(b)**

Fig.7 showing leaves (a) and dried leaves and stems (b) of ***Swertia chirayita***

2.4 ANALYTICAL TECHNIQUES AND INSTRUMENTS

There are different types of analytical techniques as well as different types of instruments are present which are quite useful in detection of the formation of nanoparticles.

a) UV-VIS SPECTROSCOPY: - Nanoparticles are characterized by UV-Vis Spectroscopy which gives details about the formation of nanoparticles and its extent of formation. [7]This technique uses light of visible range. The UV-Vis spectra shows absorption maxima peak at 420nm and 520nm as shown in Fig.8.which confirms the formation of silver and gold nanoparticles respectively. [6] More the intensity of the peak more the number of nanoparticles formed.



(a) **(b)**

Fig.8. UV Absorbance spectra for silver nanoparticles at 420nm [21] (a) and for Gold nanoparticles at 520nm [23] (b).

b) **XRD: -**The XRD analysis shows peaks in its spectra. The broader peaks signify the formation of smaller particle size of nanoparticles and vice-a-versa. [6] Monometallic nanoparticles and bimetallic nanoparticles show sharper and broader peaks respectively in diffractograms. Further, this technique is quite useful in calculating the particle size of the nanoparticles formed by the following formula, [11]

$$\mathbf{Dp = 0.9\lambda/\beta\cos\theta}$$

Where,

Dp denotes particle size in Angstroms

λ denotes wavelength of X-Ray

θ denotes Bragg's angle

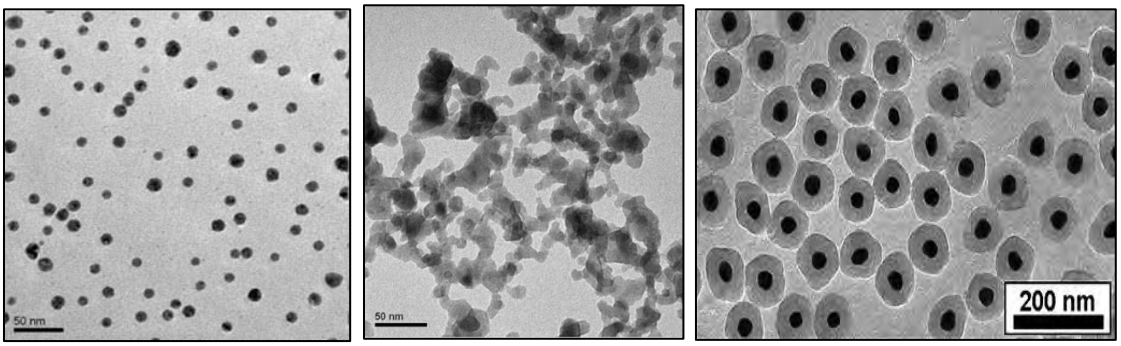
β denotes full width of peak at half maximum in radians

c) ZETA POTENTIAL: - This technique is used to predict the stability of formed Nanoparticles. [6]

ZETA POTENTIAL (mV)	STABILITY OF PARTICLES
0 $\pm$ 5	coagulation or flocculation
10$\pm$30	Incipient
30 $\pm$ 40	Moderate
40 $\pm$ 60	Good
more than 60	Excellent

Table.1 showing stability of nanoparticles according to their zeta potential

d) TEM: - Transmission Electron Microscopy is a technique in which interaction of electron beam with the particles results in the formation of magnified image on a screen takes place as shown in Fig.9. This technique is quite useful for determining the shape and size of formed nanoparticles. [2]



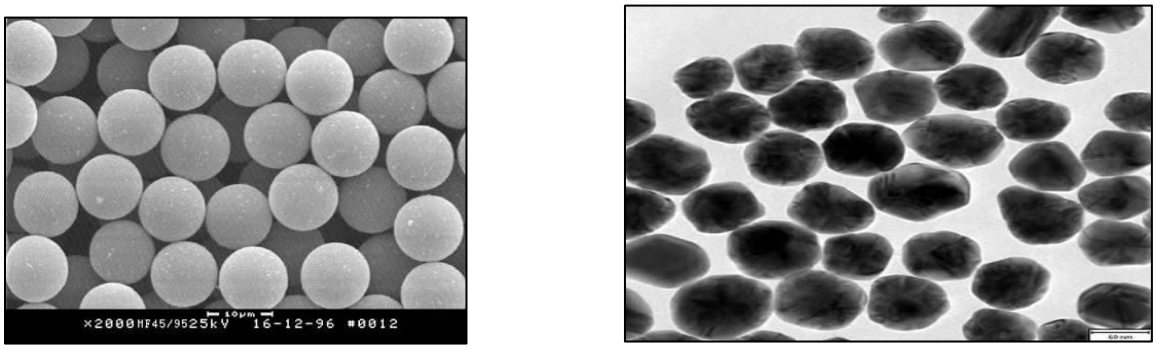
(a) **(b)** **(c)**

Fig.9 (a) Shows the shape and size of nanoparticles without stabilizing agent

(b) Shows the nanoparticles with stabilizing agent

(c) Shows the shape and size of bimetallic nanoparticles.

e) SEM: - Scanning Electron Microscopy is a technique in which focused beam of electrons interact with the atoms in sample and give information about the surface topography and its composition. The backscattered and secondary electrons are detected by this technique [2] and surface composition is detected exactly as shown in Fig.10.



(a) **(b)**

Fig.10. SEM images of nanoparticles are shown in which Fig (a) shows spherical nanoparticles and Fig (b) shows Irregular shaped nanoparticles. [21]

3. OBJECTIVES

The objectives of the work:

1) Standardization of a method for the preparation of nanoparticles using Plant Material.
2) Characterization of nanoparticles using UV Spectroscopy, IR Spectroscopy and XRD analysis.
3) Investigation of chemical properties of synthesized nanoparticles.

4. EXPERIMENTAL WORK

5. METHODOLOGIES:-

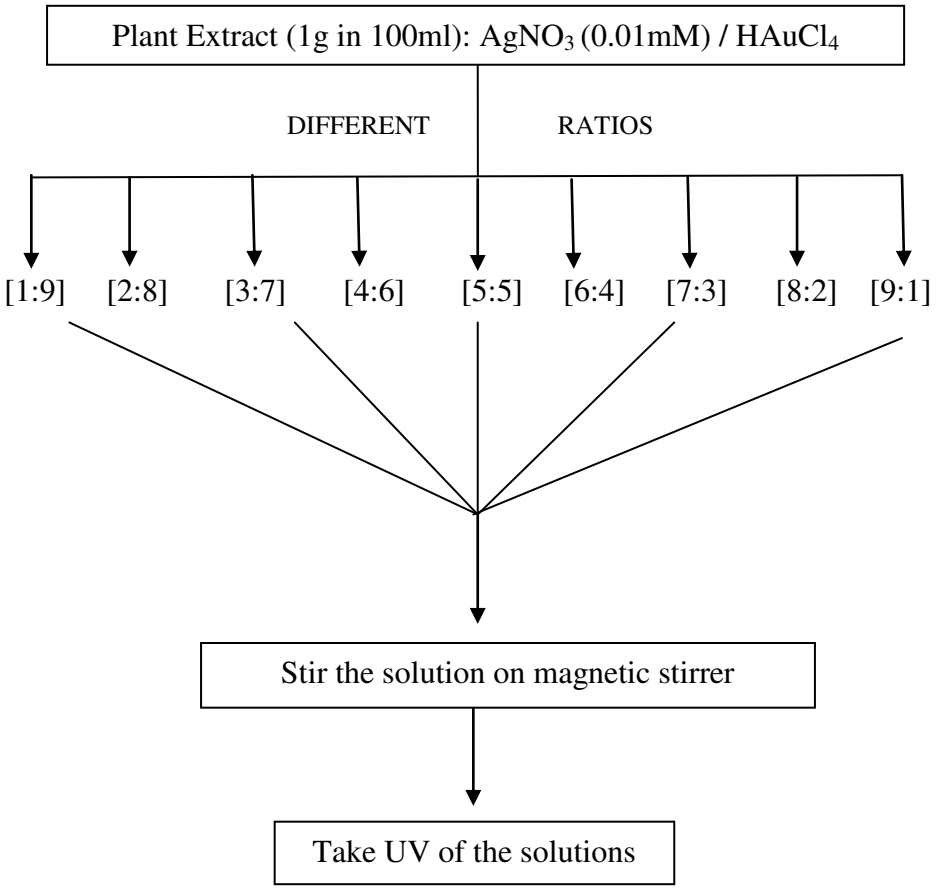
Equipments used: - Soxhlet Apparatus, condenser, conical flasks, round bottom flask, flat bottom flask, beakers, volumetric flasks, magnetic stirrer, magnetic bead, heating mantle, measuring cylinders, test tubes, test tube stand, cotton, weighing machine and glass rod.

Chemicals and solvents used: - Silver Nitrate, Auric Chloride, Ferrous Sulphate, ethanol, methanol, de-ionized water, Congo Red Dye, Sodium borohydride and hexane.

a. Preparation of Plant Extract: - Plant extract is made from waste materials like orange peels, used tea leaves, pea peels and pomegranate peels. The material is washed properly three four times with distilled water and then it is shade dried for a week or two. This dried material is then crushed to break the vesicles. The extract is made in Soxhlet Apparatus using water or ethanol as a solvent. Then after running three to four cycles in Soxhlet, solvent is evaporated on rotatory evaporator and collected pure solvent back into another flask and plant extract is taken out in a petridish and concentrated on water bath and stored.

b. Preparation of nanoparticles

Nanoparticles are synthesized in number of chemical as well as green methods. The method followed in this report is green method. Firstly, optimization of metal nanoparticles is done as shown in Scheme 3**.** Two stock solutions are made, one of plant extract and another of metal salt of which nanoparticles are to be synthesized. 1g of plant extract is taken in 100ml volumetric flask and mixed with 100ml of de-ionized water. 0.1mM $AgNO_3$ or 1mM $HAuCl_4$ is made in 1000 ml of de-ionized water. Different ratios of plant extract to metal salt solution is made as shown in Scheme 3 for optimization of nanoparticles of a particular plant and the best ratio is selected in which it shows better results in UV-Vis Spectroscopy. From observing the results of optimization, a particular ratio is selected and further nanoparticles are synthesized in that ratio only.



Scheme 3 Formation of nanoparticles at different ratios of Plant Extract: Metal Salt.

c. Preparation of Bimetallic nanoparticles:-

Bimetallic nanoparticles are synthesized in two ways:-

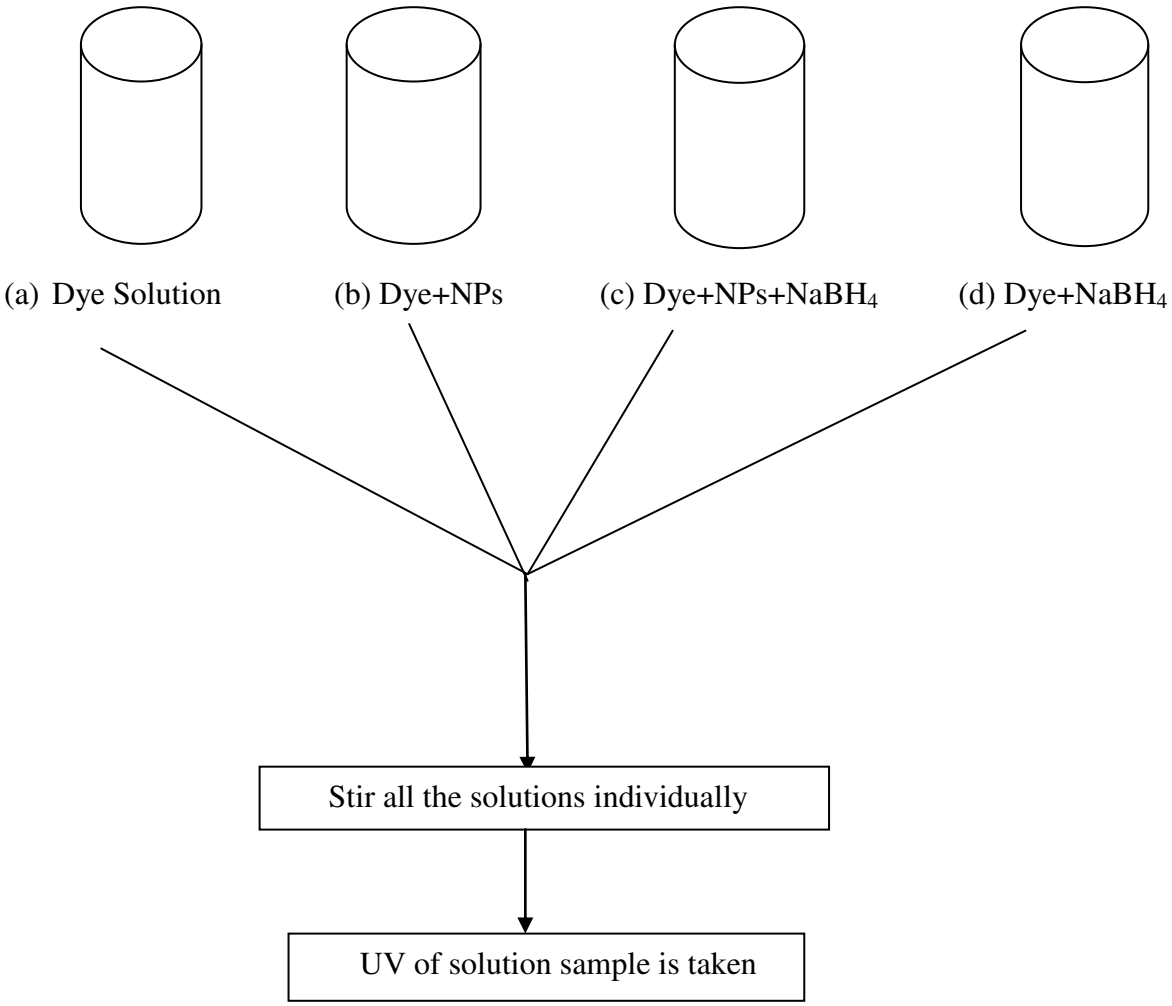
First Method: - In this method two different metal nanoparticles are synthesized simultaneously. Two Metal salts are directly added to the plant extract solution in proper ratio. [11]

Second Method: - In this method firstly, one type of metal nanoparticles is made and then other metal salt solution is added to synthesize other type of metal nanoparticles.

Both these methods eventually form bimetallic nanoparticles either in the form of core-shell or as alloy formation. [10]

d. Reducing Property of nanoparticles[20]

Nanoparticles have shown dye reducing properties. The dye used is Congo Red which is an azo dye and is very toxic and non-biodegradable. This dye is a byproduct of dying industries and it misbalances the aquatic environment by polluting water [20]. This dye is degraded by using nanoparticles of different metals like silver, gold, iron, nickel, etc. Three stock solutions are made. The stock solution I contains 10^{-3} M aqueous solution of Congo Red Dye. The stock solution II contains aqueous solution of metal nanoparticles. The stock solution III contains 10^{-3} M aqueous solution of Sodium borohydride [21]. Four conical flasks are taken as shown in Scheme 4. The first conical flask contains aqueous solution of Congo Red Dye. The second conical flask contains Dye and metal nanoparticles. The third flask contains Dye, metal nanoparticles and aqueous solution of Sodium borohydride. The fourth flask is a control as it contains Dye and Sodium borohydride. These flasks are continuously stirred and UV is taken after 15 minutes till the discoloration of dye takes place.



Scheme 4 Reduction of dye by the use of nanoparticles of different metals

6. RESULTS AND DISCUSSIONS

The nanoparticles of various plants having medicinal values were synthesized from their waste material as per the method described earlier.

6.1 GOLD NANOPARTICLES OF *Camelilia sinesnis*:-

Gold nanoparticles of ***Camelilia sinensis*** are optimized using the method described above and it is monitored by UV-Vis Spectroscopy as shown in Fig 11. The used tea bags are used for the formation of gold nanoparticles. UV Spectra shows the characteristic peak of AuNPs in the range 500-560nm due to surface plasmon resonance of gold and 3:7 ratio is best as it shows higher absorbance which ensures the higher yield. [22]

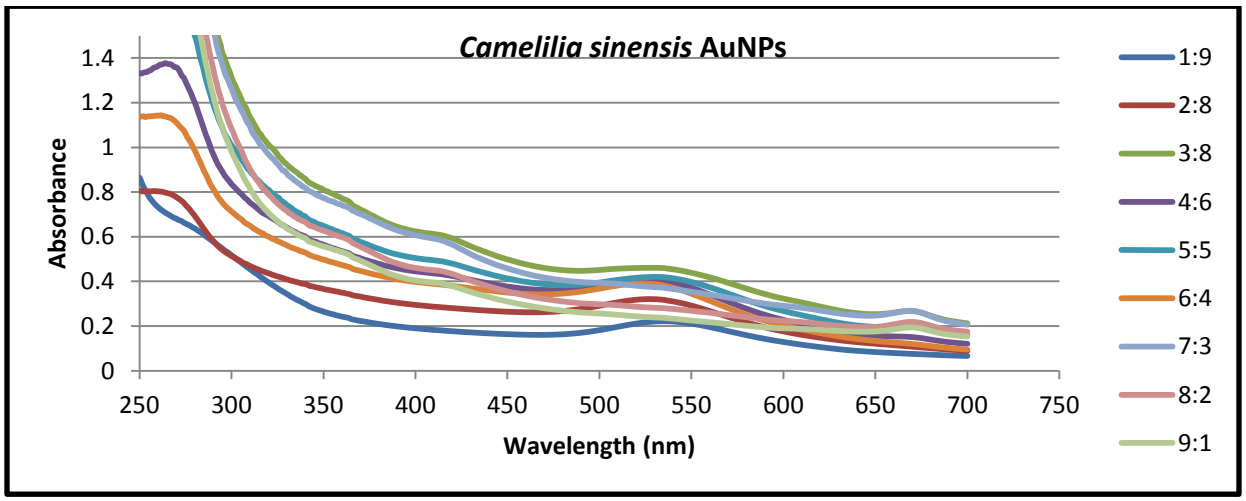


Fig.11 UV Spectra of AuNPs of ***Camelilia sinensis*** at different concentrations

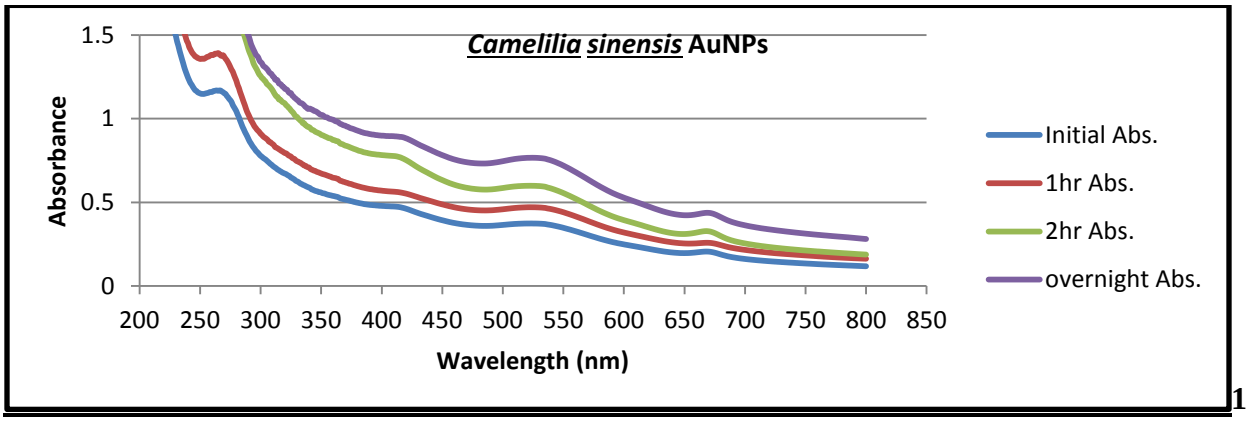


Fig 12. UV Spectra of AuNPs of ***Camelilia sinensis*** at ratio 3:7

CHARACTERIZATION:-

FTIR ANALYSIS:- The surface interaction of gold nanoparticles with the biomolecules which are polyphenolic in nature is confirmed by FTIR Spectra. The IR bands at 2924, 2853, 1646, 1575, 1366, 1098 and 724 cm^{-1} as shown in Fig.13 corresponds to C-H stretching, C=C stretching, C-O stretching and C-C stretching and thus indicates the presence of phenolic compounds. [23]

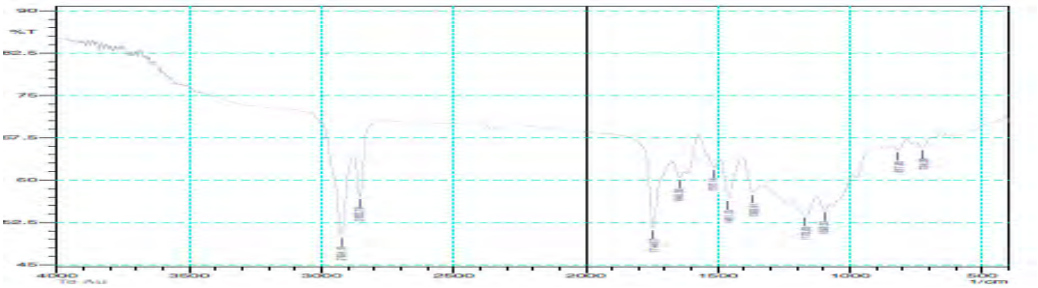


Fig.13 FTIR Spectra of ***Camelilia sinensis***

XRD ANALYSIS:- Gold nanoparticles are synthesized by the reduction of their oxidation state from Au^{+3} to Au^{0} by ***Camelilia sinensis***. The XRD is analyzed using 2 theta values which range from 20 to 80 degrees as shown in Fig.14. The crystalline size and lattice strain of AuNPs is calculated using Scherrer Equation as shown in Table 2. [24] From the Table it is clear that gold nanoparticles of ***Camelilia sinensis*** are formed as the average crystalline size is 19.318 nm. There are four major peaks at 33.58, 35.79, 66.97 and 77.16 which corresponds to (111), (200), (220) and (311) which is in agreement with the planes of AuNPs available in literature [25]

Pos. [°2Th.]	FWHM [°2Th.]	Crystallite Size(nm)	Lattice Strain
23.32	0.6034	14.05	0.0128
33.58	0.4127	21.009	0.0060
35.79	0.6532	13.35	0.0088
50.23	0.3815	24.03	0.0036
53.27	0.3465	26.8	0.0030
66.97	0.7927	12.55	0.0052
77.16	0.4529	23.44	0.0025
	Average	19.318	

Table 2 XRD results of AuNPs of ***Camelilia sinensis***.

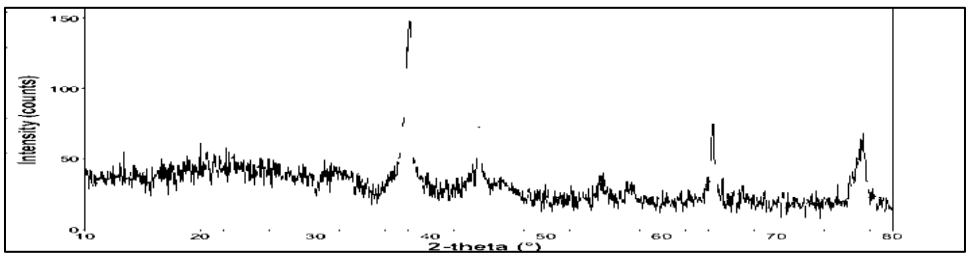


Fig.14 XRD Spectra of gold nanoparticles of ***Camelilia sinensis***

SILVER NANOPARTICLES OF *Camelilia sinensis*:-

Silver nanoparticles of ***Camelilia sinensis*** are optimized by taking different concentration of extract and $AgNO_3$ using the method as discussed above. This optimization is monitored by UV-Vis Spectroscopy as shown in Fig.15.which shows the characteristic peak in the wavelength range of 380-460nm due to surface plasmon resonance of silver and best ratio is in the concentration range of 3(Extract):7($AgNO_3$ solution) as the absorbance is higher in this ratio and eventually leads to the higher yield. [25]

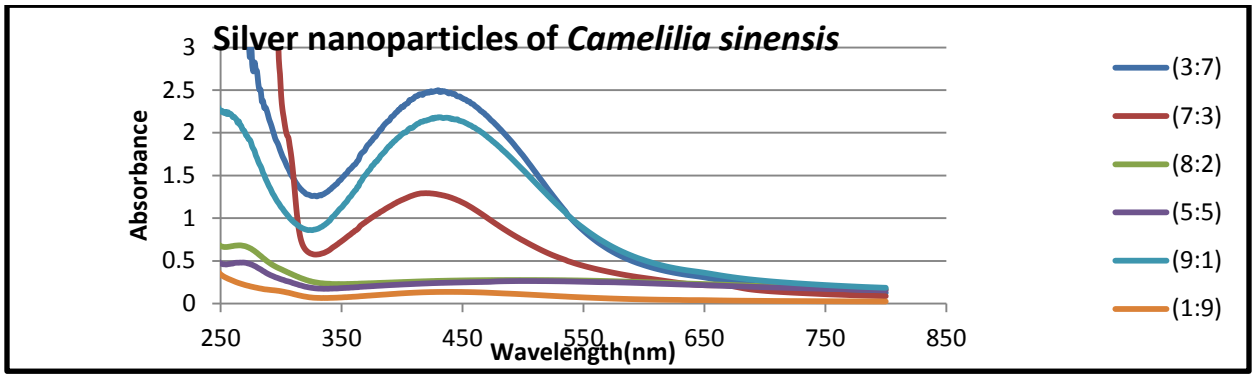


Fig.15 UV Spectra showing optimization of silver nanoparticles of ***Camelilia sinensis*** at different ratios. [25]

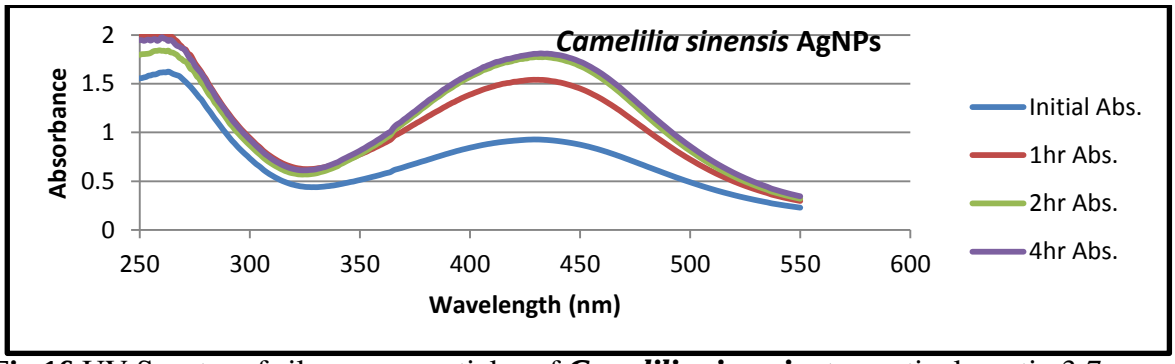


Fig.16 UV Spectra of silver nanoparticles of ***Camelilia sinensis*** at a particular ratio 3:7

CHARACTERIZATION:-

FTIR ANALYSIS:- The FTIR Spectra of ***Camelilia sinensis*** shown in Fig.17 shows bands at 3404, 2916, 2369, 1620, 1045 and 669cm^{-1} which are characteristic of O-H, C-H, C=C and C=O stretching modes. These bands show the presence of polyphenolic compounds. [25]

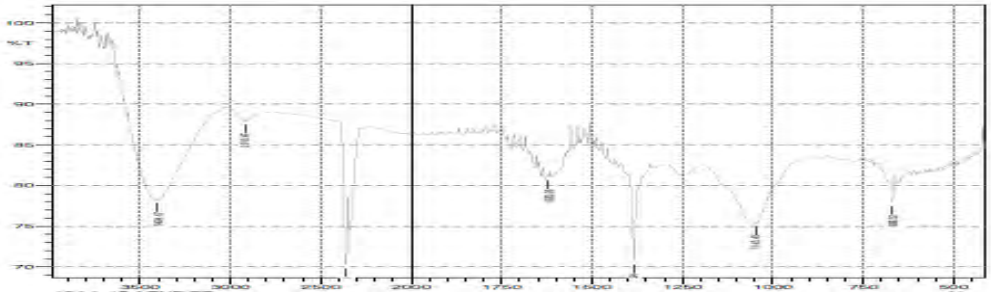


Fig.17 FTIR Spectra of silver nanoparticles of ***Camelilia sinensis***.

XRD ANALYSIS:- The particle size of silver nanoparticles of ***Camelilia sinensis*** is determined by X-Ray Diffraction. The crystalline size of nanoparticles is calculated using the Scherrer Formula as mentioned above from the values of 2 theta and full width at half maximum as shown in Table 3. The average size of silver nanoparticles is 13.59nm which ensures the formation of silver nanoparticles of ***Camelilia sinensis.*** 2 theta values of 41.978, 53.235 and 73.042 correspond to peak indexes (200), (311) and (222) are available in the literature as shown in Fig.18. [25]

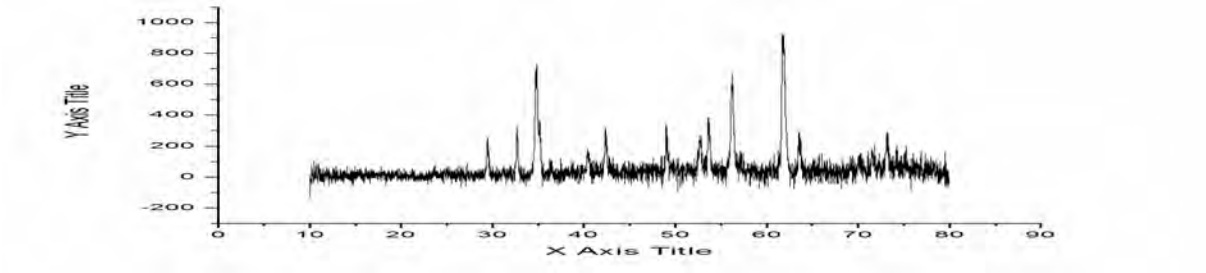


Fig.18 XRD Spectra of silver nanoparticles of ***Camelilia sinensis***.

Pos. [°2Th.]	FWHM [°2Th.]	Crystalline Size (nm)	Lattice Strain
34.471	1.9021	4.57	0.0268
34.803	0.25006	34.79	0.0035
41.978	2.5145	3.54	0.0286
49.321	1.7929	5.09	0.0170
53.235	1.3356	6.95	0.0116
56.221	0.35124	26.79	0.0029
61.822	0.39052	24.77	0.0028
73.042	4.6049	2.239	0.0271
	AVERAGE	13.592	

Table 3 XRD results of AgNPs of ***Camelilia sinensis***

6.2 GOLD NANOPARTICLES OF *Pisum sativum*:-

Gold nanoparticles of ***Pisum sativum*** are optimized using the same method as discussed above and the results are monitored by UV-Vis Spectroscopy as shown in Fig.19 it is clear that the best ratio for the formation of gold nanoparticles of ***Pisum sativum*** is 3:7 as is shows characteristic

peak of gold nanoparticles in the wavelength range of 510-560nm due to surface plasmon resonance of gold.

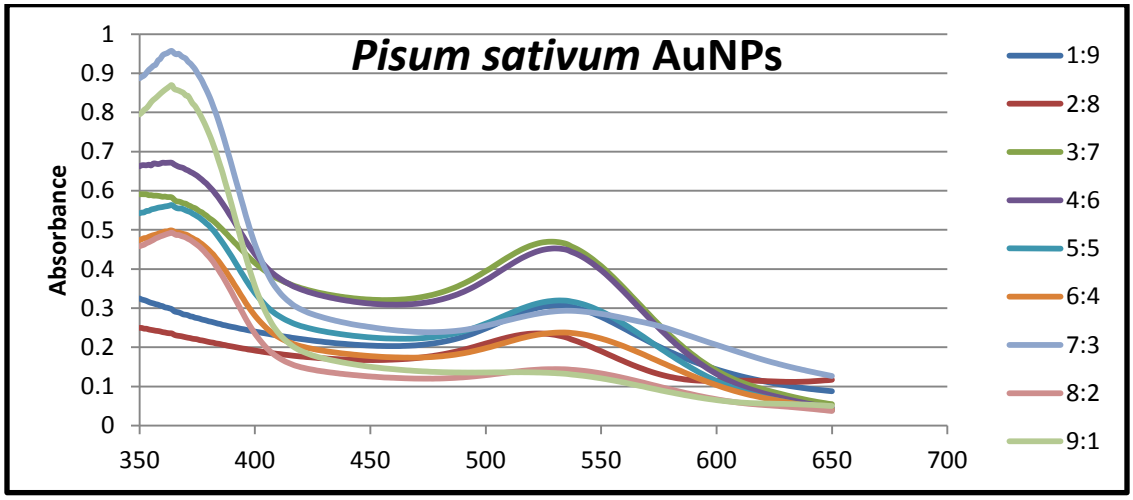


Fig.19 UV Spectra showing formation of gold nanoparticles at different ratios.

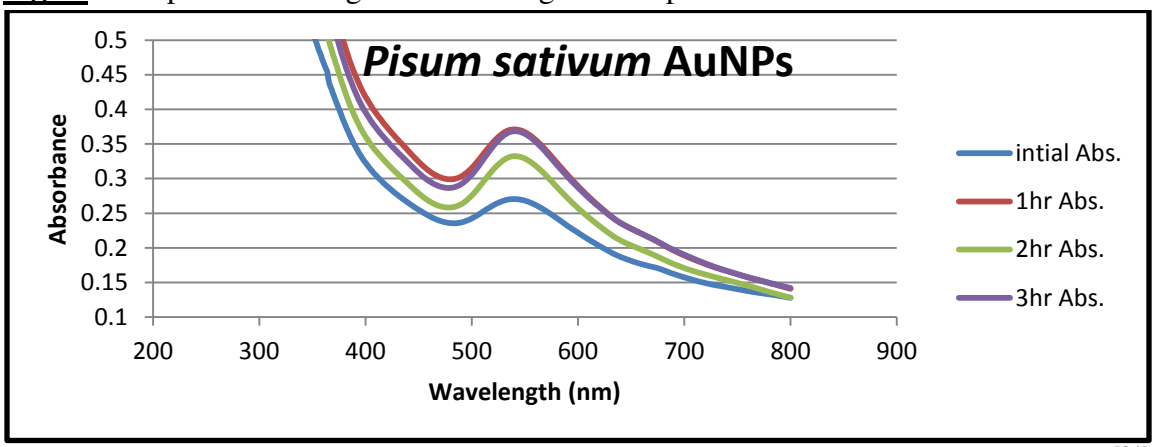


Fig.20 UV Spectra showing AuNPs of ***Pisum sativum*** at a concentration range of 3:7. [24]

CHARACTERIZATION:-

FTIR ANALYSIS:- The FTIR Spectra in Fig.21 shows peaks at 3422, 2923, 2873, 1641, 1519, 1357, 1068 and 808 cm^{-1} which corresponds to O-H, C-H, C=O and C=C stretching. This predicts the interaction of surface of gold nanoparticles with the phenolic biomolecules present in the plant species. [23]

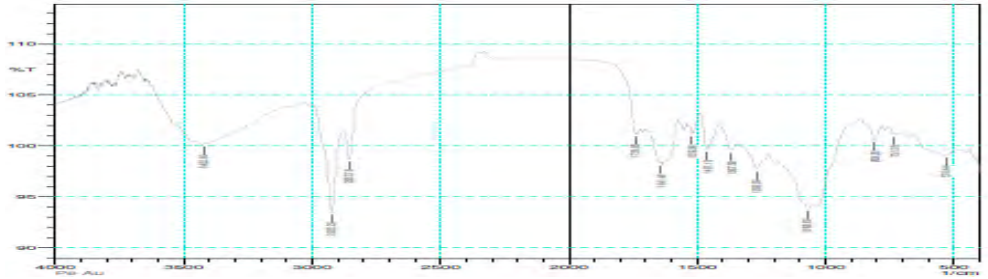


Fig.21 FTIR Spectra of gold nanoparticles of ***Pisum sativum***

XRD ANALYSIS:- The crystallite size of gold nanoparticles is determined by XRD Analysis using the Scherrer Equation and the average size of particles is 20.80nm as shown in Table 4 which ensures the formation of gold nanoparticles of ***Pisum sativum***. The XRD Spectra of gold of gold nanoparticles is shown in Fig.22. The major peaks at 36.20, 47.55, 62.80 and 69.03 correspond to peaks (111). (200), (220) and (311) are present in literature. [21]

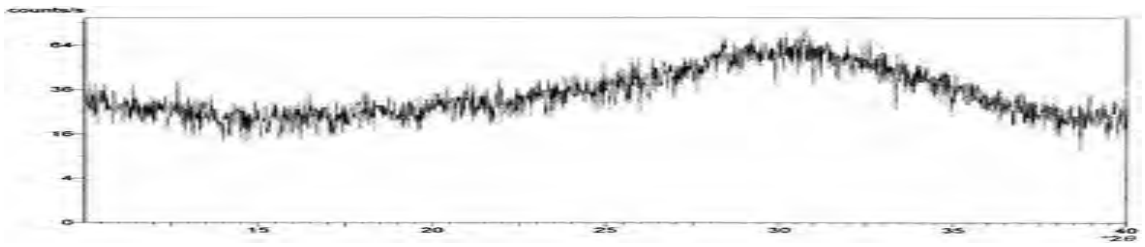


Fig.22 XRD Spectra of gold nanoparticles of ***Pisum sativum***

Pos. [°2Th.]	FWHM [°2Th.]	Crystallite Size(nm)	Lattice Strain
31.74	0.3931	21.95	0.0060
34.37	0.1723	50.43	0.0024
36.20	0.4634	18.85	0.0062
47.45	0.4041	22.44	0.0040
56.55	1.4683	6.42	0.0119
62.80	1.3267	6.62	0.0105
67.96	1.5021	6.659	0.0097
69.03	0.3043	33.1	0.0019
	Average	20.80	

Table 4 XRD Results of gold nanoparticles of ***Pisum sativum***

SILVER NANOPARTICLES of *Pisum sativum*:-

Silver nanoparticles of ***Pisum sativum*** are optimized using the method discussed above and their results are monitored by UV-Vis Spectroscopy as shown in Fig.23. The UV Spectra shows characteristic peak of silver nanoparticles in the wavelength range 390-450nm due to surface plasmon resonance. From the UV Spectra a particular ratio 3:7 is selected which gives higher absorbance and eventually higher yield. [21]

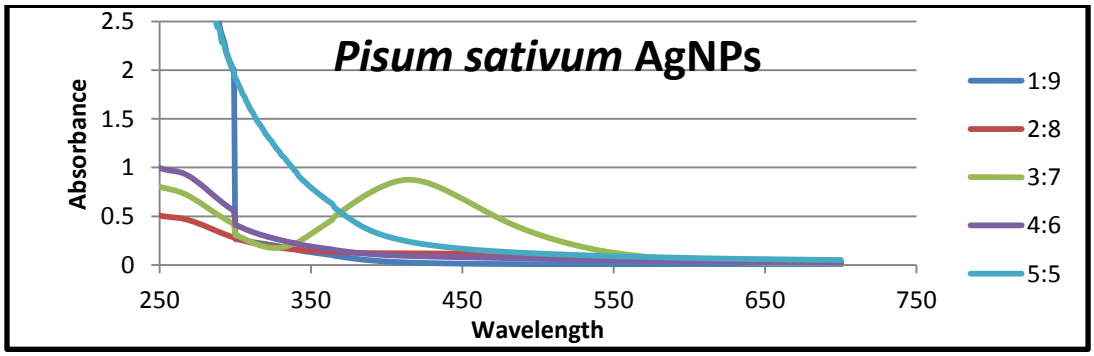


Fig.23 UV Spectra shows formation of silver nanoparticles of ***Pisum sativum*** at different concentrations.

CHARACTERIZATION:-

FTIR ANALYSIS:- FTIR Spectra of silver nanoparticles of ***Pisum sativum*** shows characteristic peaks at 3473, 2360, 2332, 1613 and 1076 cm^{-1} as shown in Fig.24 which corresponds to O-H stretching which is responsible for the reducing activities, C-H,C=O and C-C stretching and shows the presence of some phenolic compounds responsible for the reducing activities . [21]

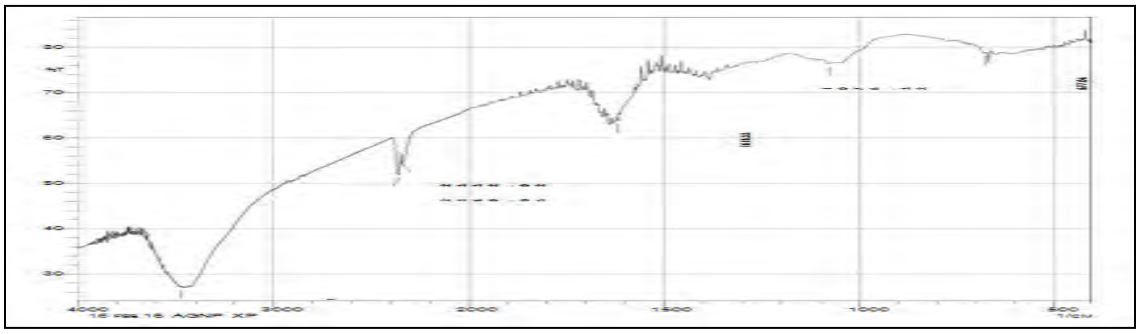


Fig.24 IR Spectra showing characteristic peaks of silver nanoparticles of ***Pisum sativum***

6.3 GOLD NANOPARTICLES OF *Citrus aurantium*:- Gold nanoparticles of ***Citrus aurantium*** are optimized using the same method as discussed above and monitored by UV Spectroscopy as shown in Fig.25. The UV Spectra shows characteristic peak in the range 510-560nm due to surface plasmon resonance and the appropriate ratio for the formation of gold nanoparticles of ***Citrus aurantium*** is 3:7

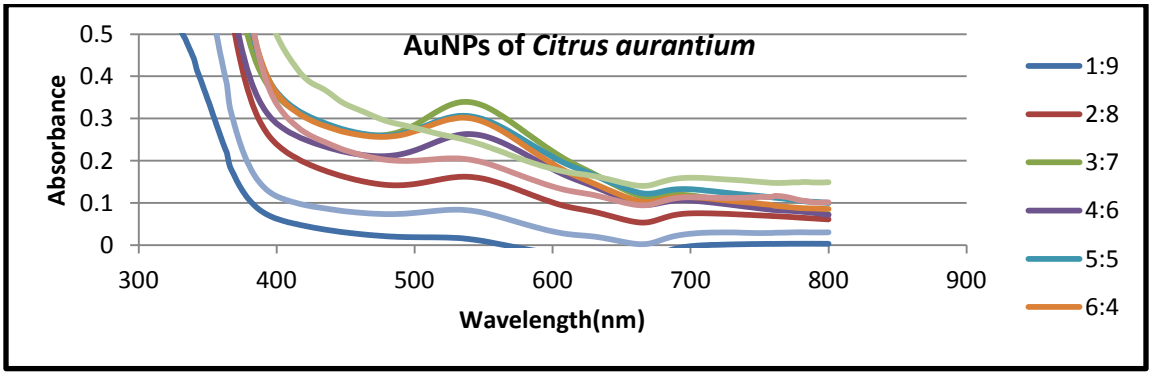


Fig.25 UV Spectra shows gold nanoparticles at different concentration ratios.

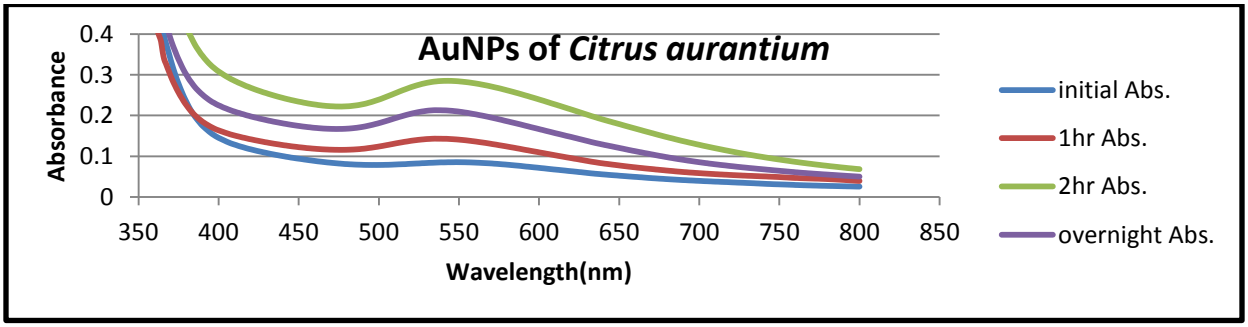


Fig.26 UV Spectra showing formation of AuNPs of ***Citrus aurantium*** at particular ratio of 3:7

CHARACTERIZATION:-

FTIR ANALYSIS:- The FTIR Spectra of gold nanoparticles of ***Citrus aurantium*** showed peaks at 3427, 2925, 2855, 1646, 1604, 1516, 1362 and 1067 cm^{-1} as shown in Fig.27 corresponds to O-H, C=C, C=O, and C-C stretching. These bands shows the presence of polyphenolic biomolecules which interact with gold nanoparticles for the reducing activities.

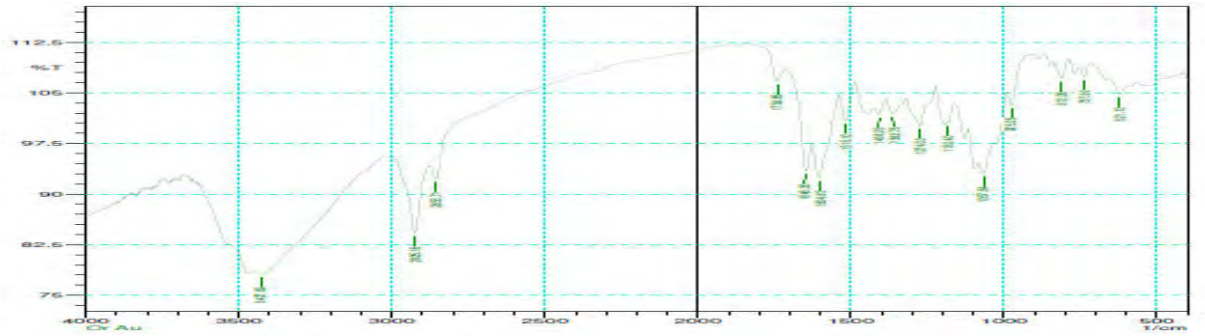


Fig.27 FTIR Spectra of gold nanoparticles of ***Citrus aurantium***

XRD ANALYSIS:- The particle size of gold nanoparticles of ***Citrus aurantium*** is estimated by X-Ray Diffraction using 2 Theta values and Full width at Half Maximum values as shown in Fig.28. The particle size is calculated using Scherrer Equation and is shown in Table 5. The Average Crystallite size of Gold Nanoparticles of ***Citrus aurantium*** is 27.96nm. Thus Gold Nanoparticles of ***Citrus aurantium*** have been synthesized. The peaks at 38.0648, 42.30, 63.4 and 77.05 corresponds to planes (111), (200), (220) and (311) as given in literature [25]

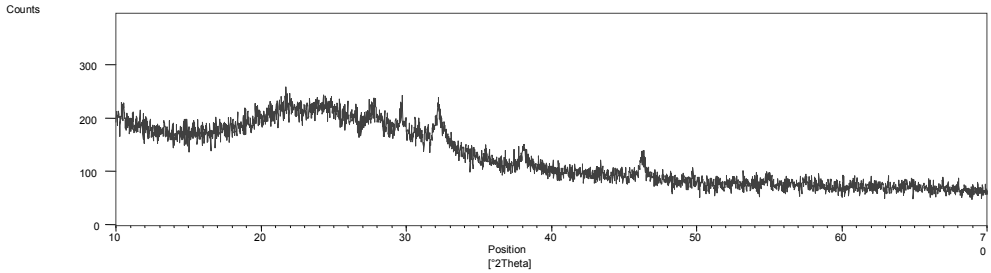


Fig.28 XRD Spectra of gold nanoparticles of ***Citrus aurantium***

Pos. [°2Th.]	FWHM [°2Th.]	Crystallite size (nm)	Lattice Strain
38.0648	0.3442	25.51	0.0044
42.3086	0.4753	18.73	0.0054
45.2610	0.1615	55.678	0.0017
54.8576	0.1698	55.08	0.0014
56.235	1.3356	7.05	0.0109
63.4006	0.4181	23.33	0.0030
75.041	0.6049	17.3	0.0034
77.042	0.5049	21.009	0.0028
	Average	27.96088	

Table 5 shows XRD results of ***Citrus aurantium***

SILVER NANOPARTICLES OF *Citrus aurantium*:- Silver nanoparticles of ***Citrus aurantium*** are made using the ratio 3:7 and their formation is monitored by UV Spectroscopy as shown in Fig.29. A characteristic peak of silver nanoparticles is seen in the UV Spectra in the range 410-480nm which marks the formation of silver nanoparticles of ***Citrus aurantium***.

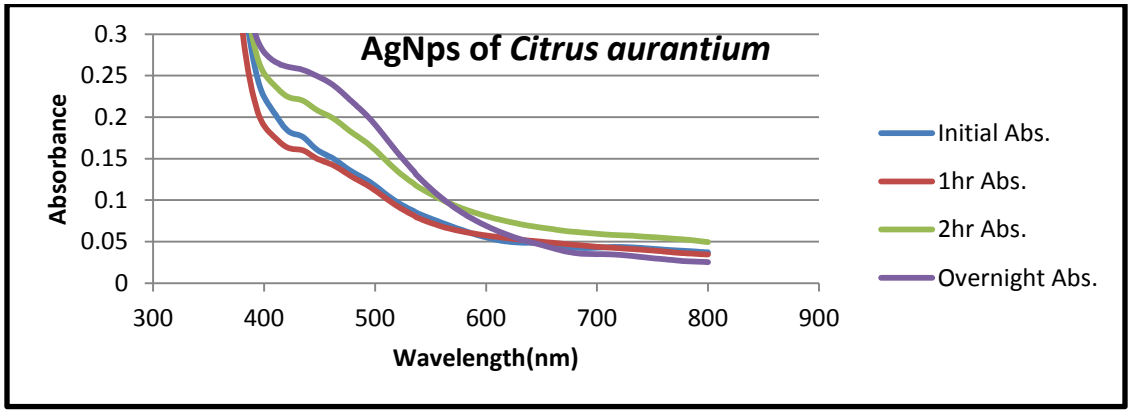


Fig.29 UV Spectra showing formation of silver nanoparticles of ***Citrus aurantium***

CHARACTERIZATION:-

FTIR ANALYSIS:-

The Characterization of silver nanoparticles of ***Citrus aurantium*** is done by using Fourier Transform Infra Red Spectroscopy as shown in Fig.30. The IR spectra shows the peaks at 1024cm^{-1}, 1359cm^{-1}, 1599cm^{-1} and 1683cm^{-1} which corresponds to C-C, C=C and C=O which shows the presence of phenolic compounds responsible for reduction.[25]

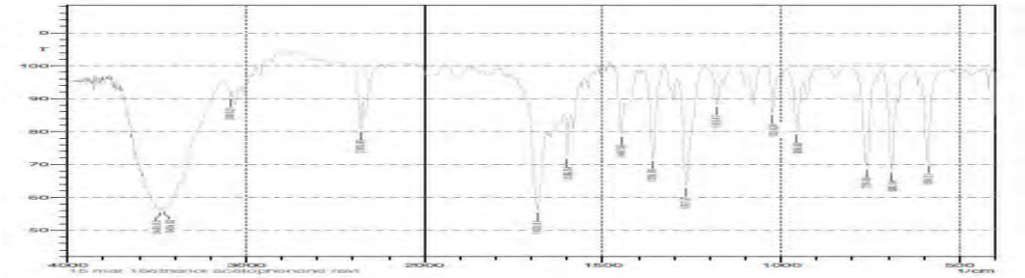


Fig.30 FTIR Spectra of silver nanoparticles of ***Citrus aurantium***

6.4 GOLD NANOPARTICLES OF *Punica granatum*-: Gold nanoparticles of ***Punica granatum*** are optimized using the method as discussed above and their formation is monitored by UV Spectroscopy as shown in Fig.31. Different ratios of plant extract and gold salt is made. From the UV Spectra it is clearly seen that the ratio 3:7 gives higher absorbance and eventually higher yield in the characteristic wavelength region of 510- 560nm due to surface plasmon resonance of gold. [24]

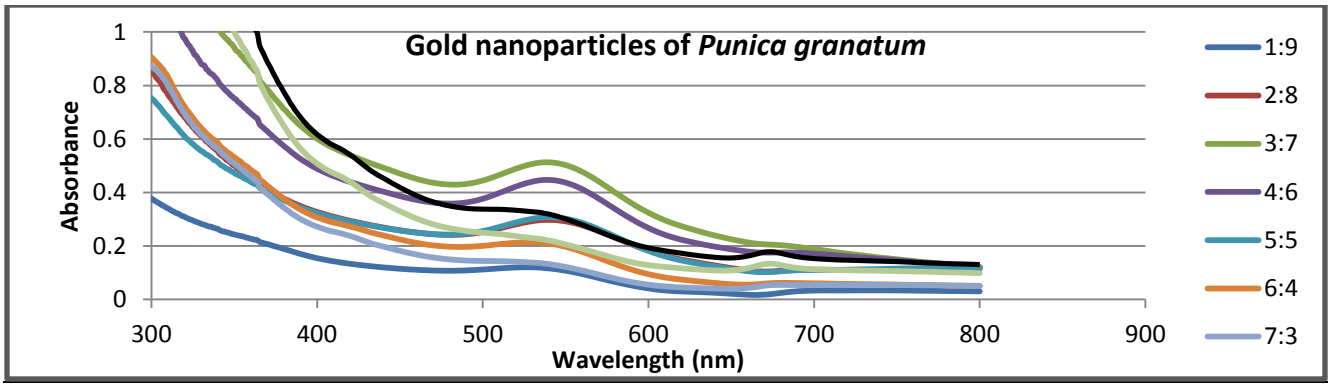


Fig.31 UV Spectra of gold nanoparticles of ***Punica granatum*** at different concentrations

CHARACTERIZATION:-

FTIR ANALYSIS:- The FTIR Spectra of gold nanoparticles of ***Punica granatum*** shows peaks at 3279, 2922, 2854, 1715, 1645, 1513, 1451 and 1067 cm^{-1} as shown in Fig.32 which corresponds to O-H, C-H, C=O, C=C and C-C stretching which shows the interaction of gold nanaoparticles with the phenolic compounds present in the plant material. [24]

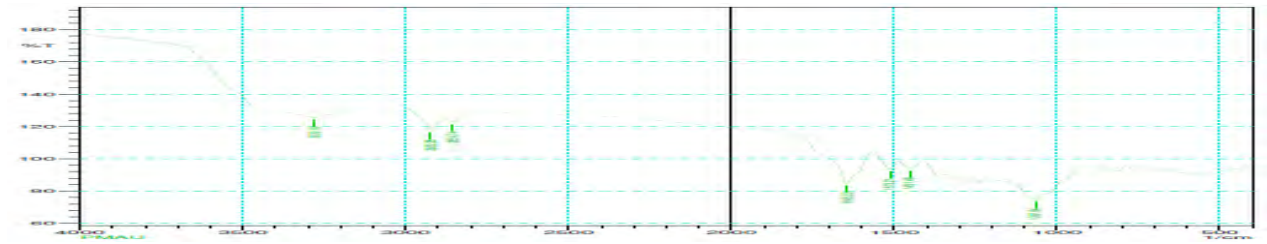


Fig.32FTIR Spectra of gold nanoparticles of ***Punica granatum***

XRD ANALYSIS:- XRD spectra of gold nanoparticles is shown in Fig.33. The particle size of gold nanoparticles of ***Punica granatum*** is characterized by X-Ray Diffraction using the Scherrer Equation as shown in Table 6. The average crystallite size of gold nanoparaticles of ***Punica granatum*** is 35.143 nm. The peaks at 34.50, 48.31, 72.50 and 77.32 corresponds to planes (111), (200), (220) and (311) as given in literature [23]

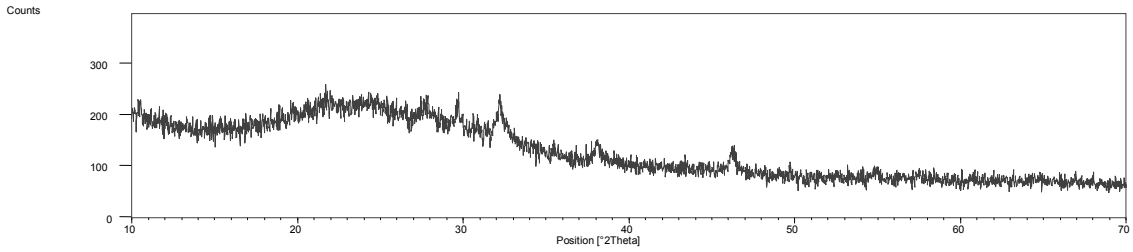


Fig.33 XRD Spectra of gold nanoparticles of ***Punica granatum***

Pos. [°2Th.]	FWHM [°2Th.]	Crystalline size (nm)	Lattice Strain
31.72	0.4321	19.97	0.0066
34.50	0.2364	36.769	0.0033
38.32	0.4625	19	0.0058
48.31	0.4532	20.07	0.0044
57.43	0.5431	17.43	0.0043
68.06	0.1978	50.63	0.0013
72.58	0.1432	71.9	0.0009
77.32	0.2342	45.38	0.0013
	Average	35.143	

Table 6 XRD results of gold nanoparticles of ***Punica granatum***

SILVER NANOPARTICLES OF *Punica granatum*:- Silver nanoparticles of ***Punica granatum*** are synthesized using the ratio 3:7 and their formation is monitored by UV-Vis Spectroscopy over a period of one day as shown in Fig.34. The UV Spectra shows characteristic peak in the wavelength range of 410-480nm which marks the formation of silver nanoparticles of ***Punica granatum***. [25]

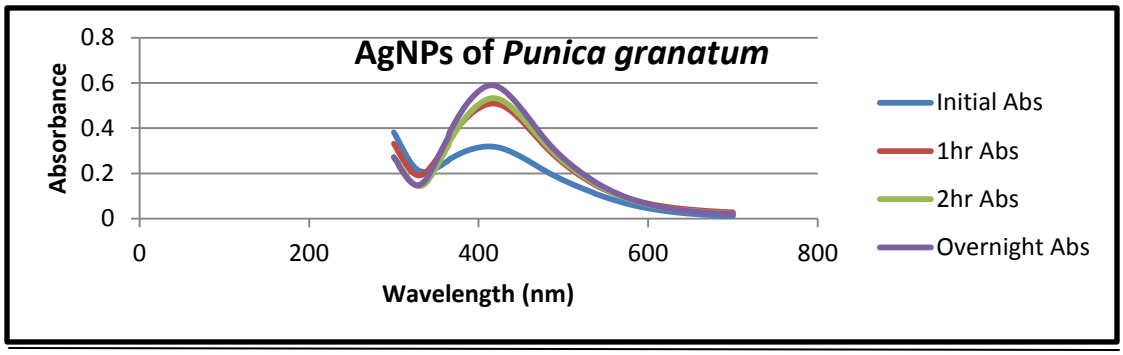


Fig.34 UV Spectra showing formation of silver nanoparticles of ***Punica granatum***

CHARACTERIZATION:-

FTIR ANALYSIS:- Silver nanoparticles of ***Punica granatum*** are characterized using the Fourier Transform Infra Red Spectroscopy as shown in Fig.35. The IR Spectra shows characteristic peaks at 1384cm^{-1}, 1649cm^{-1} and 2364cm^{-1} which corresponds to C-C, C=C, C=O and C-H stretching and marks the presence of some phenolic compounds.[21]

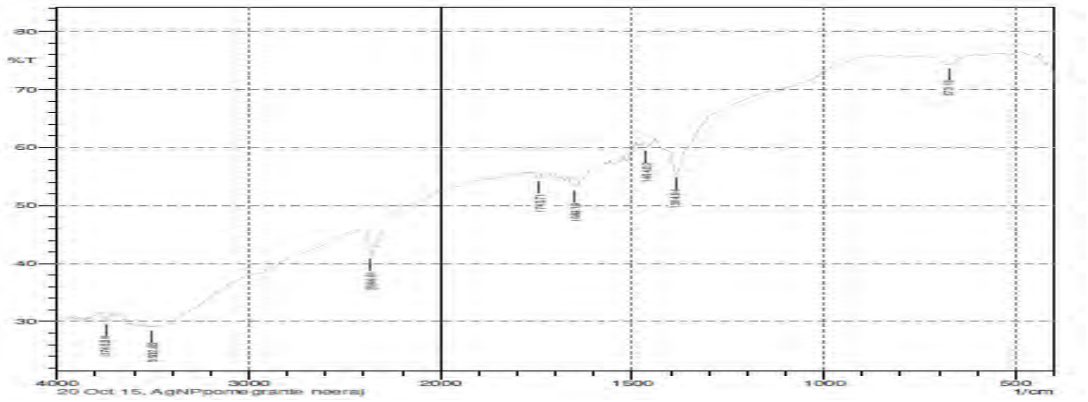


Fig.35 FTIR Spectra of silver nanoparticles of ***Punica granatum***

6.5 SILVER NANOPARTICLES OF *Cladonia subtenis*:-

Nanoparticles of this species were made for the first time and their formation is monitored by UV-Spectroscopy as shown in Fig.36**.** From the UV Spectra of ***Cladonia subtenis,*** it can be seen that the characteristic peak of silver nanoparticles appears in the wavelength range of 410-480nm which marks the formation of silver nanoparticles. [21]

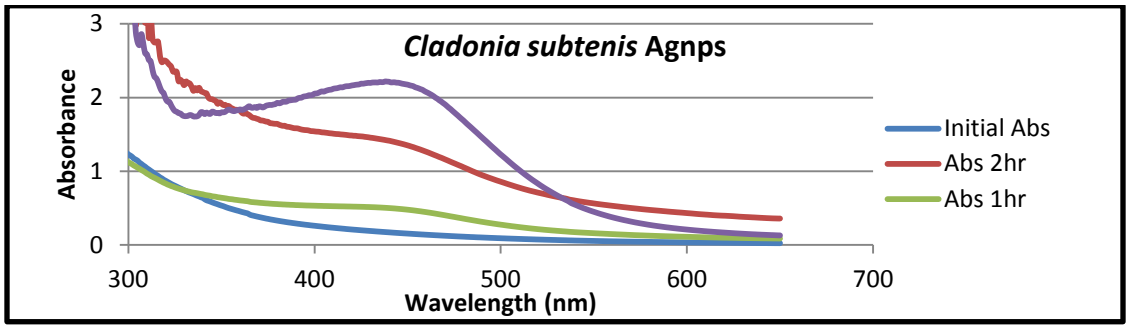


Fig.36 UV Spectra showing formation of silver nanoparticles of ***Cladonia subtenis***

6.6 SILVER NANOPARTICLES OF *Swertia chirayata*:- Silver nanoparticles of ***Swertia chirayata*** are optimized using the same method as discussed above and their formation is reported for the first time. Their formation is monitored by UV Spectroscopy as shown in Fig.37. From the UV Spectra, it is seen that the characteristic peak of silver nanoparticles came in the wavelength range of 410-480nm and thus marks the formation of silver nanoparticles. [23]

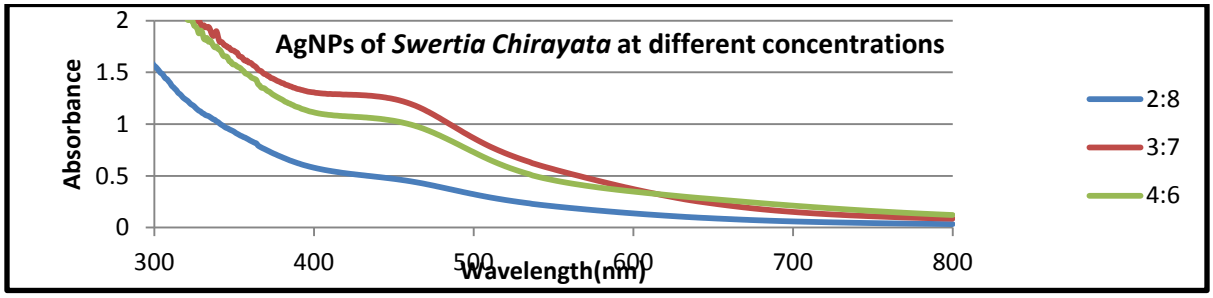


Fig.37 UV Spectra of silver nanoparticles of ***Swertia chirayata*** at different concentrations

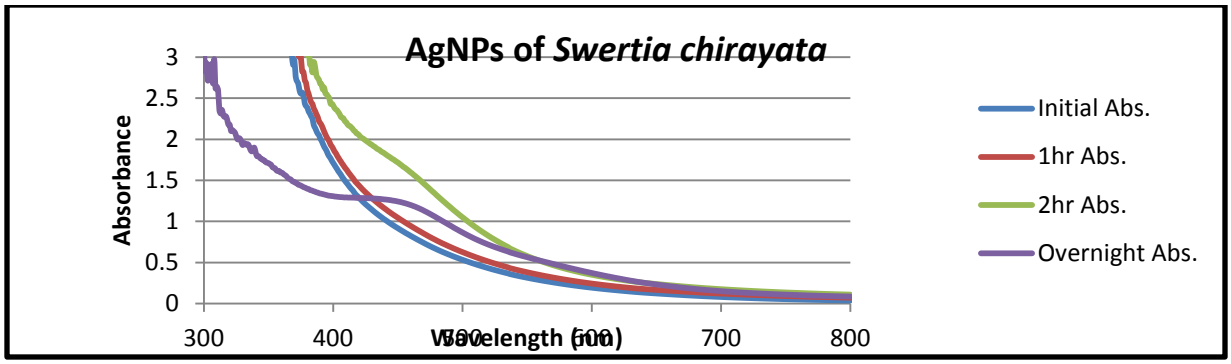


Fig.38 UV Spectra showing silver nanoparticles of ***Swertia Chirayata*** at a particular concentration of 3:7

6.7 BIMETALLIC NANOPARTICLES:-

Bimetallic Ag-Ni Nanoparticles :-

These metals have comparable size and their bimetallic nanoparticles formation is monitored by UV-Spectroscopy Technique. These nanoparticles are formed by using the extract of ***Pisum sativum*** plant. Fig.39 shows the UV Spectra of bimetallic nanoparticles in which already synthesized silver nanoparticles are used and then nickel nanoparticles are formed and two peaks at 280nm and 340nm appeared. Fig.40 shows the UV Spectra of bimetallic nanoparticles in which simultaneous formation of both metal nanoparticles takes place and only one peak at 280nm appeared. Appearance of two peaks shows the formation of bimetallic nanoparticles by first method whereas second method gave only one peak so there is possibility of formation of alloy of two metals. [19]

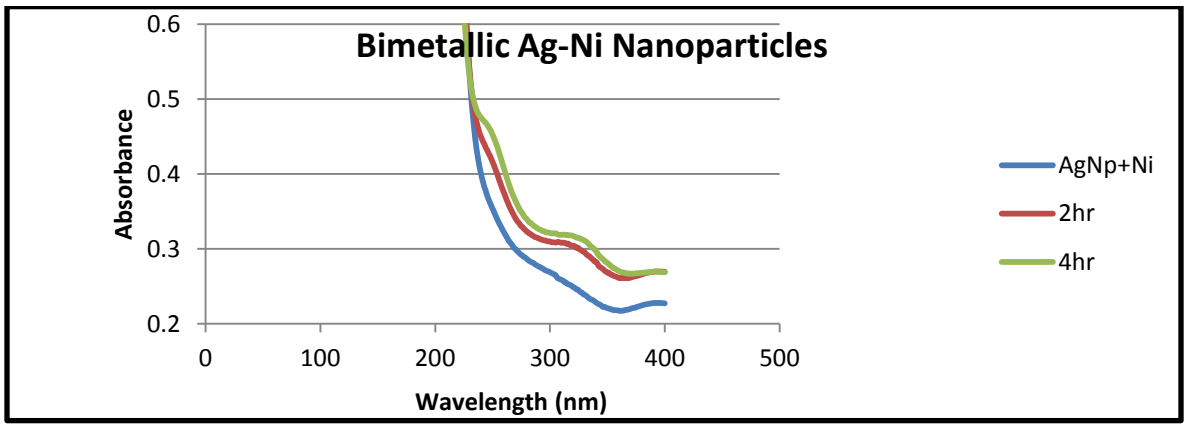


Fig.39. UV spectra of formation of bimetallic silver and nickel nanoparticles by first method [11]

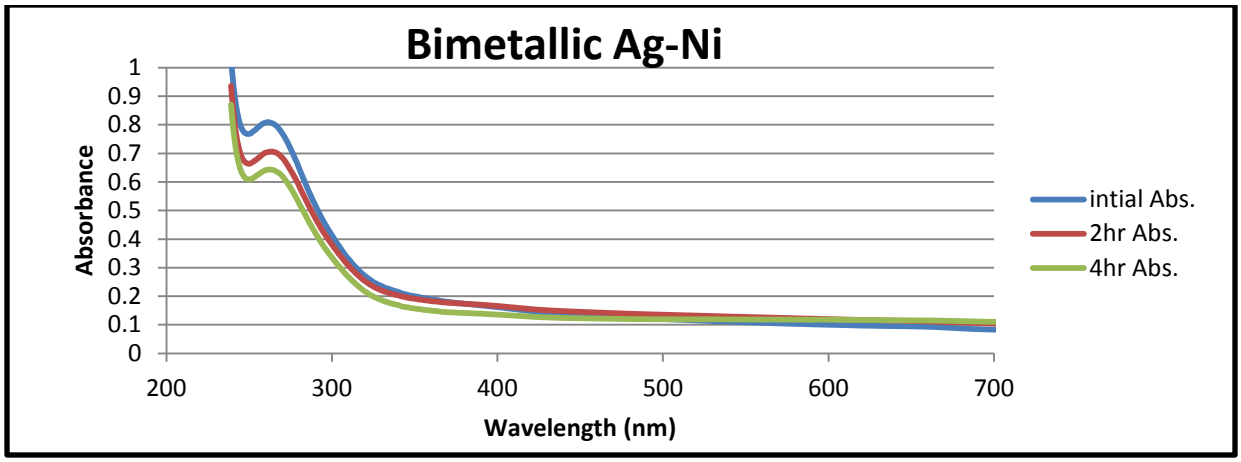


Fig.40 UV Spectra showing formation of bimetallic nanoparticles of silver and nickel by second method. [10]

Bimetallic Ag-Au Nanoparticles:-

Silver and gold metals have comparable size and thus they can form bimetallic nanoparticles in an efficient manner. Fig.41 shows the formation of bimetallic nanoparticles by first method in which silver nanoparticles are made first and then gold solution is added to the same solution to form gold nanoparticles. The appearance of 2 peaks at 420nm and 660nm shows the formation of bimetallic Ag-Au nanoparticles. Fig.44 shows the formation of bimetallic nanoparticles by second method in which both type of metal nanoparticles are made simultaneously and appearance of two peak at 410nm and 640nm marks the formation of bimetallic nanoparticles. In this case both the methods are efficient to form bimetallic nanoparticles as two peaks are observed in both cases due to the comparable masses of two metals. [10]

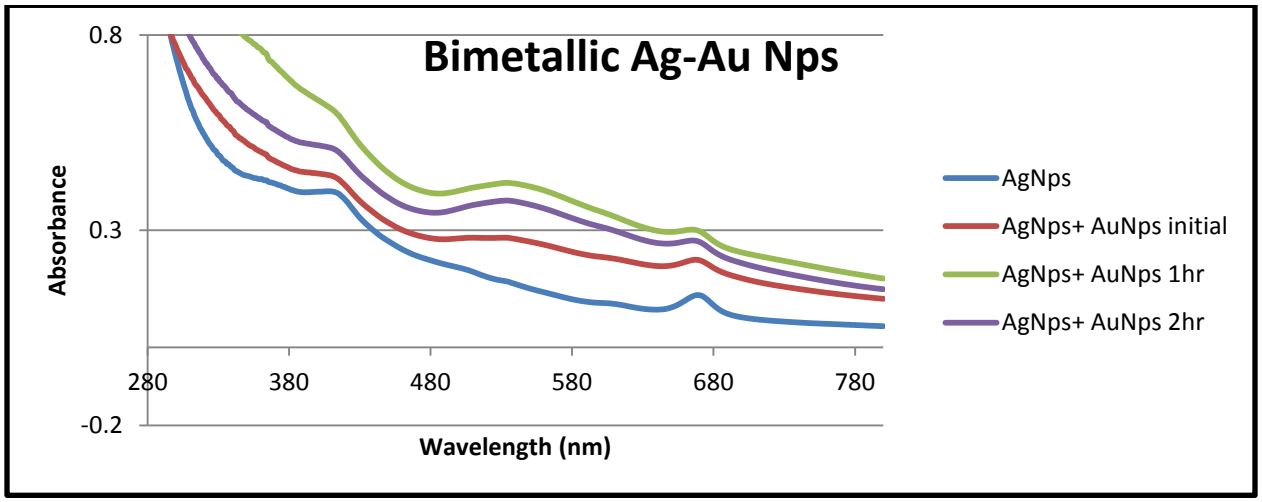


Fig.41 UV Spectra of formation of bimetallic nanoparticles by first method.

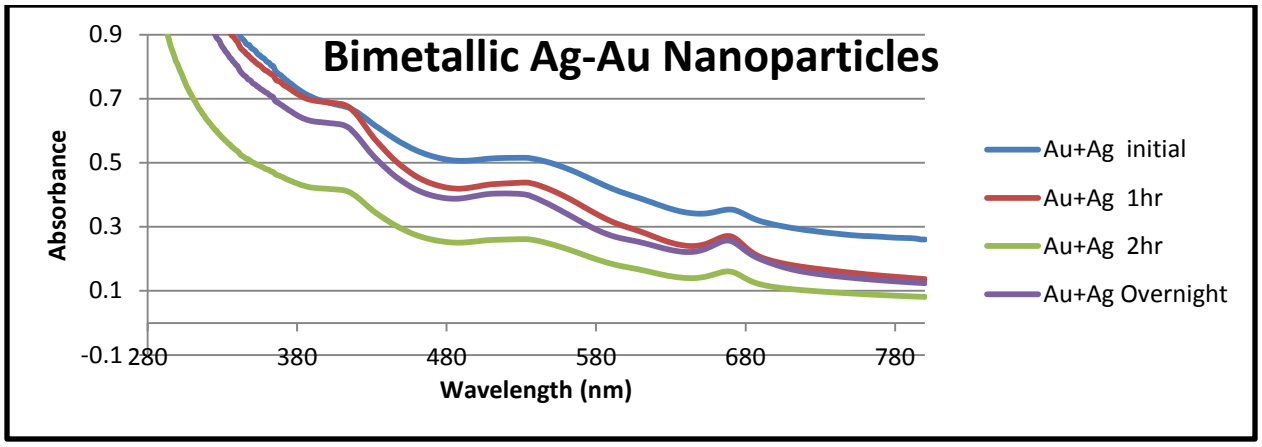


Fig.42 UV Spectra of bimetallic formation of Ag-Au nanoparticles by second method.

The mechanism which can be predicted for the reduction of gold in plant materials can be represented in Fig.43. All the plant materials have some carbonyl groups or phenolic groups which are reduced by gold nanoparticles and are helpful in causing capping of gold nanoparticles.

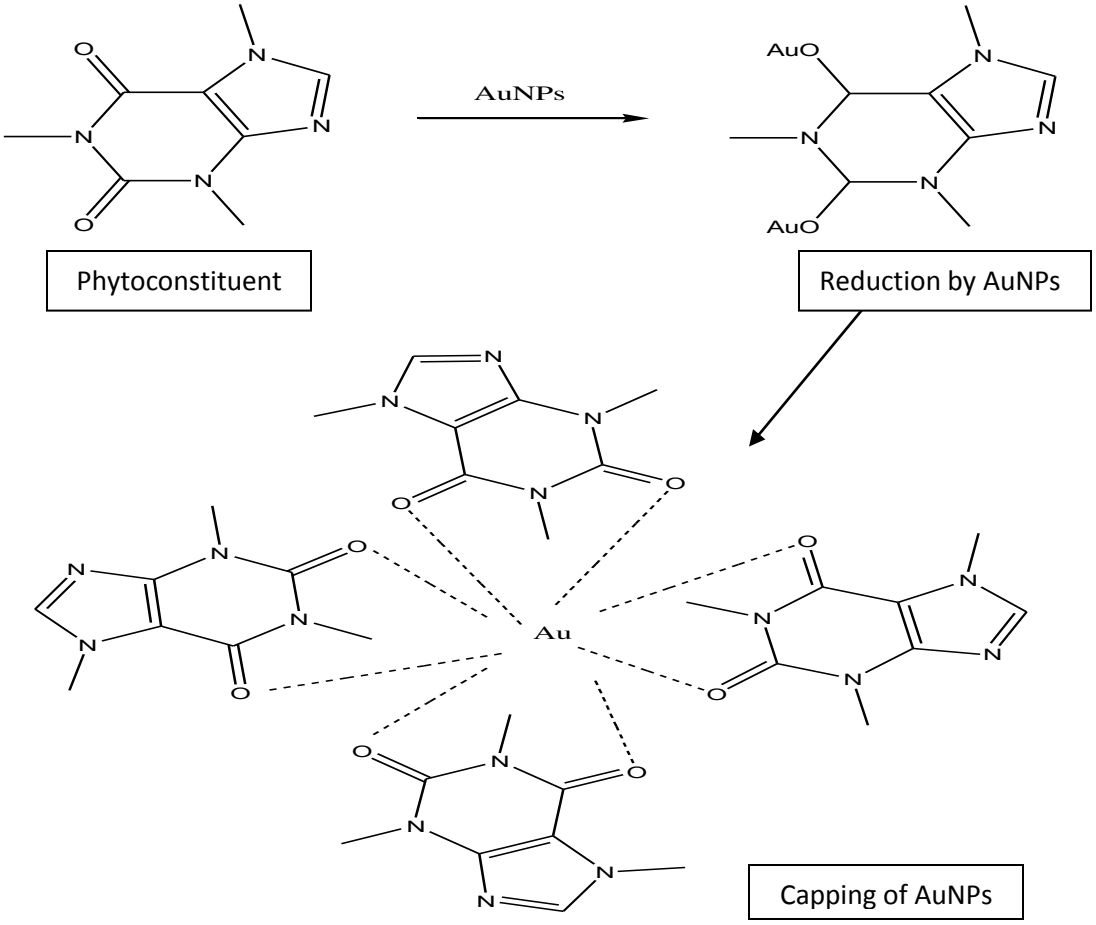


Fig.43 Mechanism of action of AuNPs on the phytoconstituents present in plant materials

APPLICATION: - REDUCING ACTIVITY

Camelilia sinensis AuNPs:-

Gold nanoparticles of ***Camelilia sinensis*** showed reducing activity as these reduce the Congo Red Dye and their reduction is monitored by UV Spectroscopy as shown in Fig.44. The reduction in the absorbance on addition of these nanoparticles ensures the reducing activity over a time period of 120 minutes as shown in Fig.46. When different concentration of gold nanoparticles of ***Camelilia sinensis*** is taken as shown in Fig.45, it is seen that as the concentration of these nanoparticles increases their extent of reduction also increases. [21]

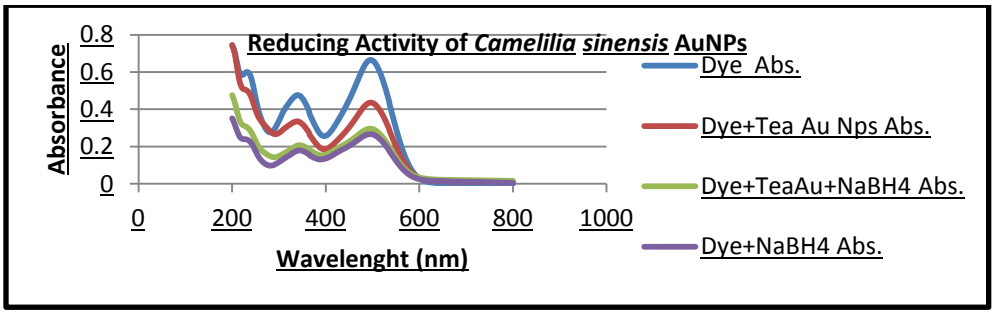


Fig.44 UV Spectra shows reducing property of gold nanoparticles of ***Camelilia sinensis***.

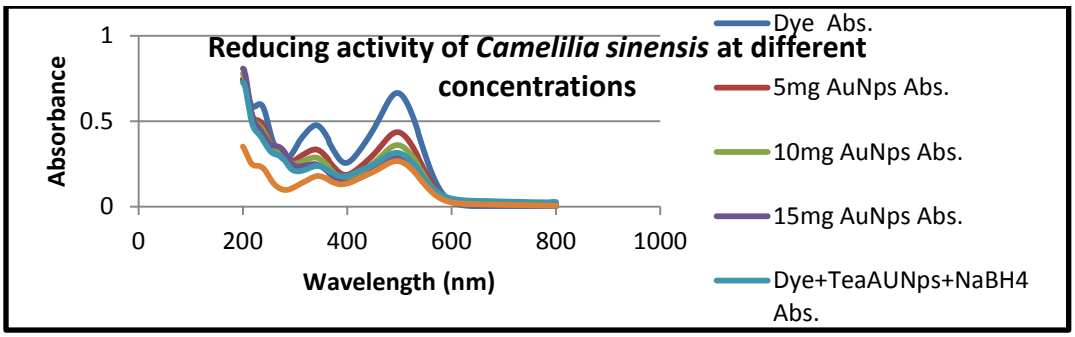


Fig.45 UV Spectra shows reducing activity of gold nanoparticles of ***Camelilia sinensis*** at different concentrations.

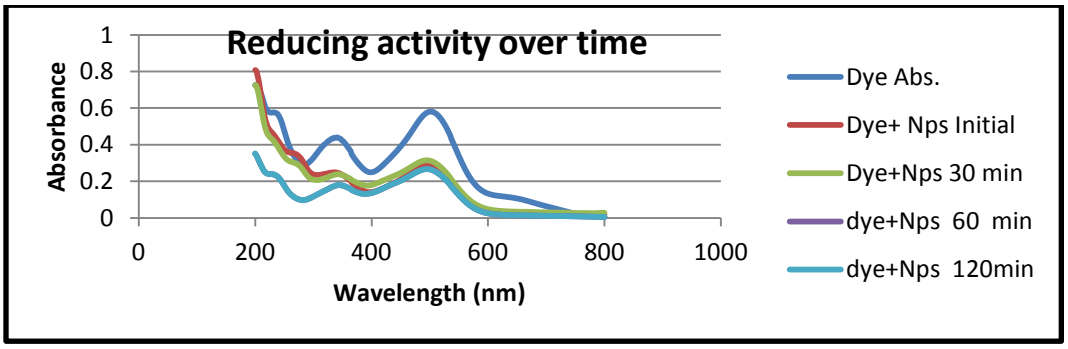


Fig.46 UV Spectra showing reducting activity of gold nanoparticles of ***Camellia sinensis*** over a time period of 120 minutes.

Camelilia sinensis AgNps:-Silver nanoparticles of Camelilia sinensis do not showed any dye reducing activity with Congo Red Dye.

Pisum sativum AuNPs:- Gold nanoparticles of ***Pisum sativum*** showed Congo Red Dye reducing property due to the interaction of surface of gold nanoparticles with the phenolic biomolecules present in these plant species as shown in Fig.47 over a time period of 45 minutes as shown in Fig.49. On using different concentrations of gold nanoparticles of ***Pisum sativum*** as shown in Fig.48, it is seen that there is not so much difference in the reducing activities.

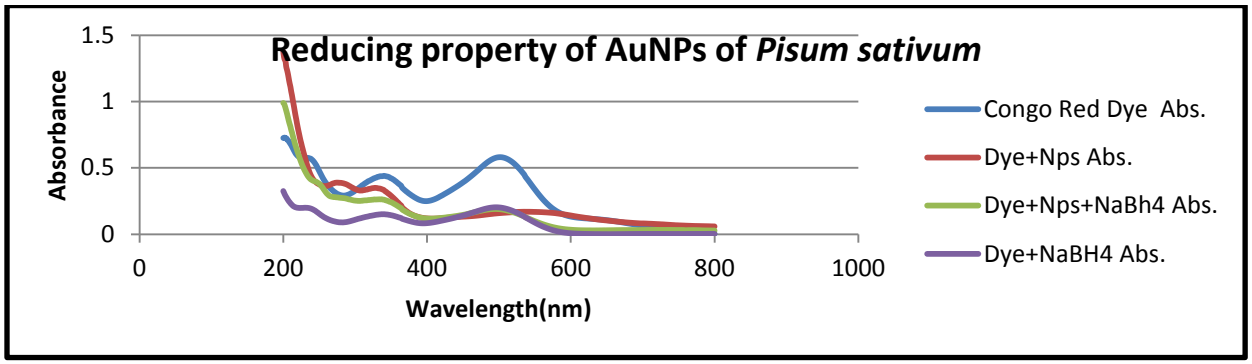


Fig.47 UV Spectra showing reducing activity of AuNPs of ***Pisum sativum***

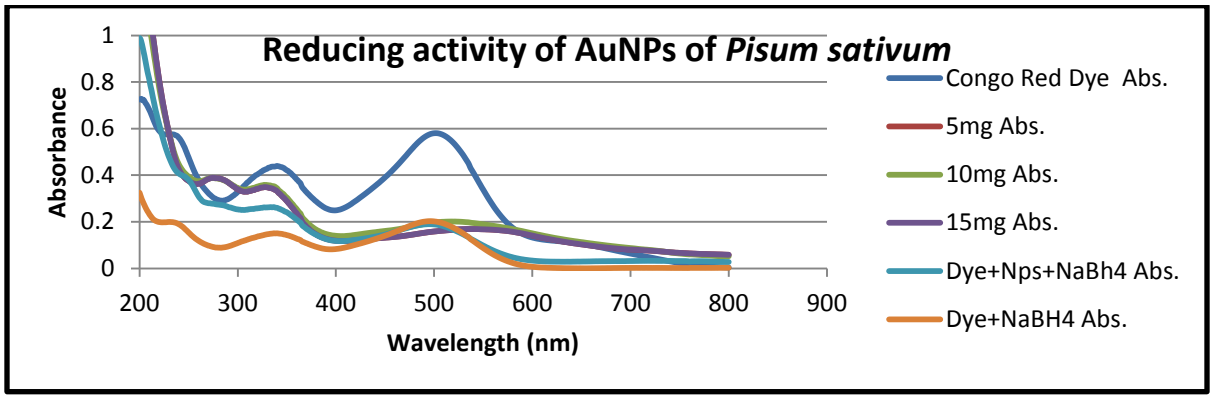


Fig.48 UV Spectra showing reducing activity of gold nanoparticles at different concentration ranges.

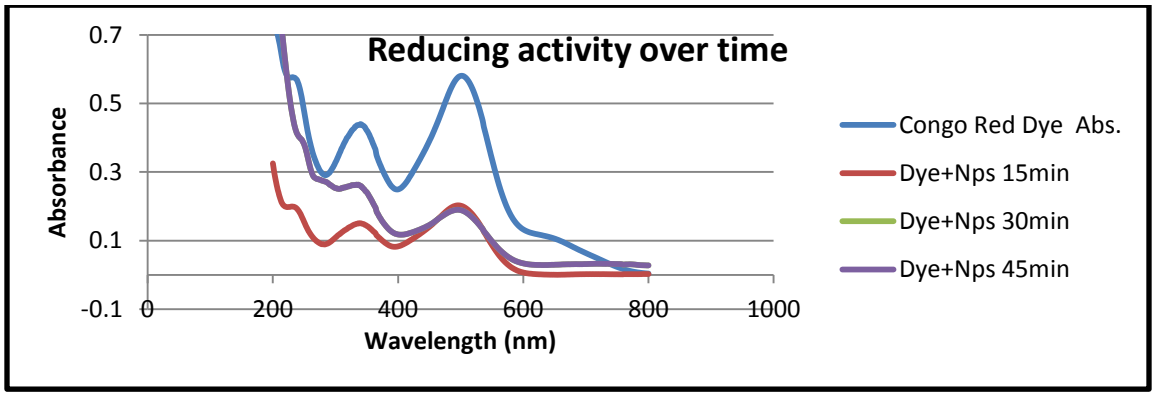


Fig.49 UV Spectra showing reducing activity over a time period of 45 minutes.

Pisum sativum AgNps*: -** Silver nanoparticles of ***Pisum sativum showed Congo Red Dye reducing activity by reducing the azo group dye as shown in Fig.50. From the UV Spectra it can be predicted that silver nanoparticles of ***Pisum sativum*** showed good dye reducing ativity over a time period of 60 minutes as shown in Fig.52. When different concentrations of silver nanoparticles are used as shown in Fig.51, it is seen that reducing activity increases as concentration increases.

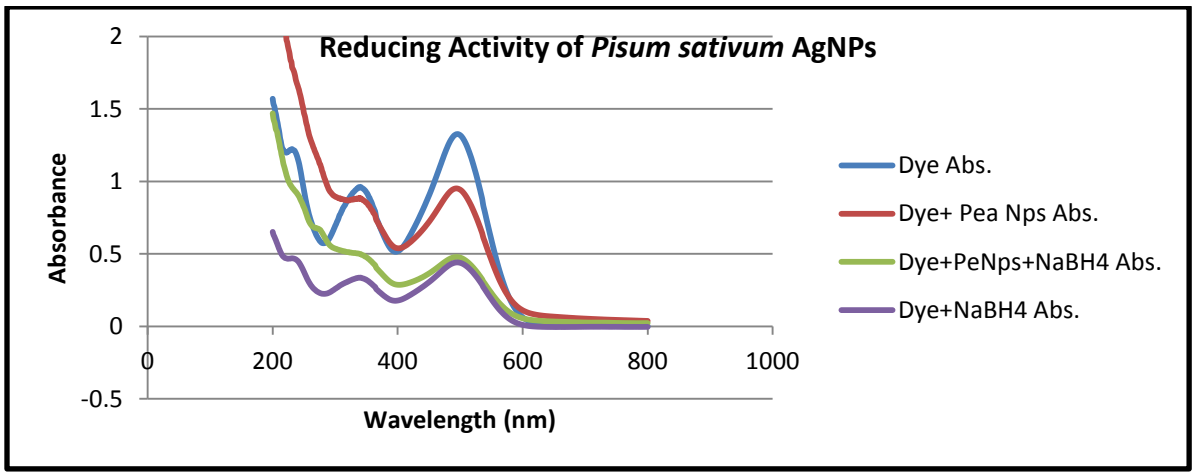


Fig.50 UV Spectra showing reducing activity of silver nanoparticles of ***Pisum sativum***.

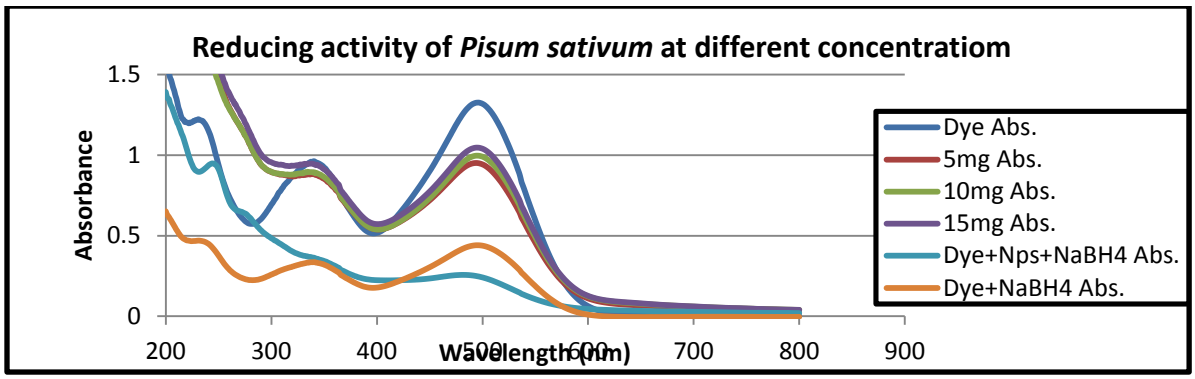


Fig.51 UV Spectra shows reducing property of silver nanoparticles of ***Pisum sativum*** at different concentration

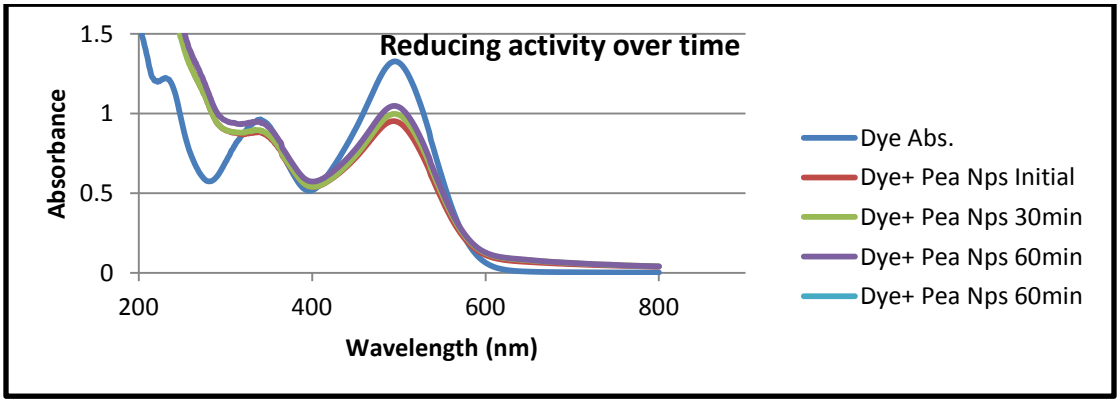


Fig.52 UV Spectra showing reducing activity of silver nanoparticles of ***Pisum sativum***

Citrus aurantium* AuNPs-** Out of all the four plants, ***Citrus aurantium is the one which showed best reducing activity as the reducing action of gold nanoparticles of ***Citrus aurantium*** is more as compare to the gold nanoparticles of other plants. On addition of these nanoparticles to the solution of dye and Sodium borohydride, the reducing activity is more as compare to the solution containing only dye and Sodium borohydride as shown in Fig.53. The UV Spectra in Fig.54 shows the reducing activity over a time period of 120 minutes. [10]

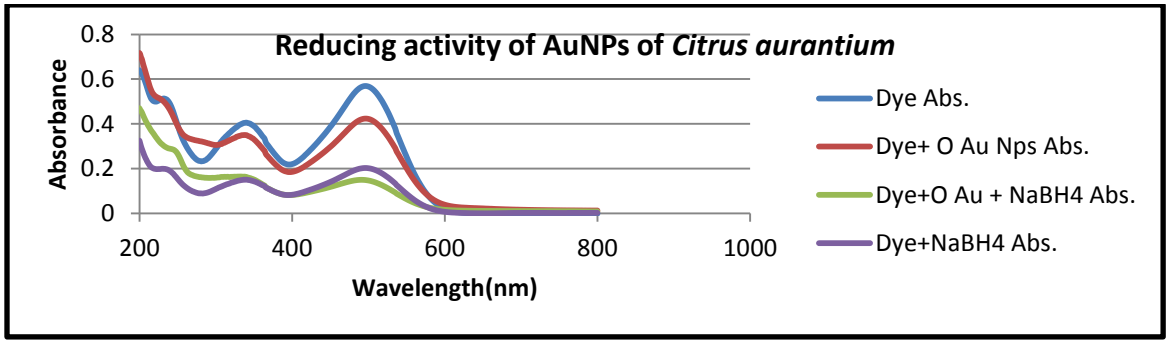


Fig.53 UV Spectra showing reducing activity of gold nanoparticles of ***Citrus aurantium***

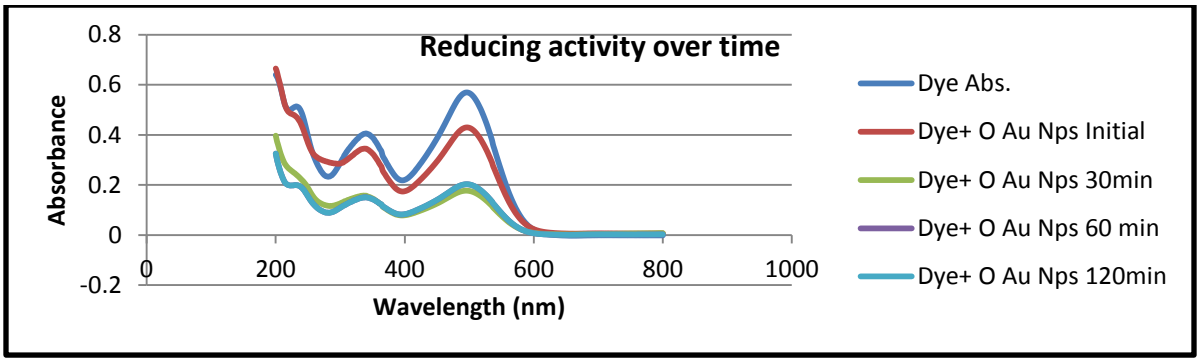


Fig.54 UV Spectra showing reducing activity of AuNPs over a time period of 120 minutes.

***Citrus aurantium* AgNPs**:-

Silver nanoparticles of ***Citrus aurantium*** showed good reducing activity which is monitored by UV-Vis Spectroscopy as shown in Fig.55. It can be predicted that the reducing action of silver nanoparticles of ***Citrus aurantium*** is higher than using Sodium borohydride. The reducing activity of these nanoparticles is observed for a time period of 120 minutes as shown in Fig.56 after which no further reduction takes place. [21]

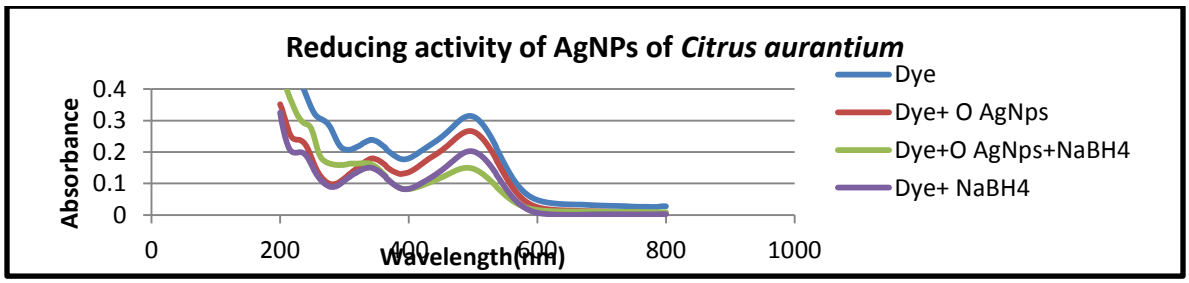


Fig.55 UV Spectra of silver nanoparticles of ***Citrus aurantium*** showing reducing activity

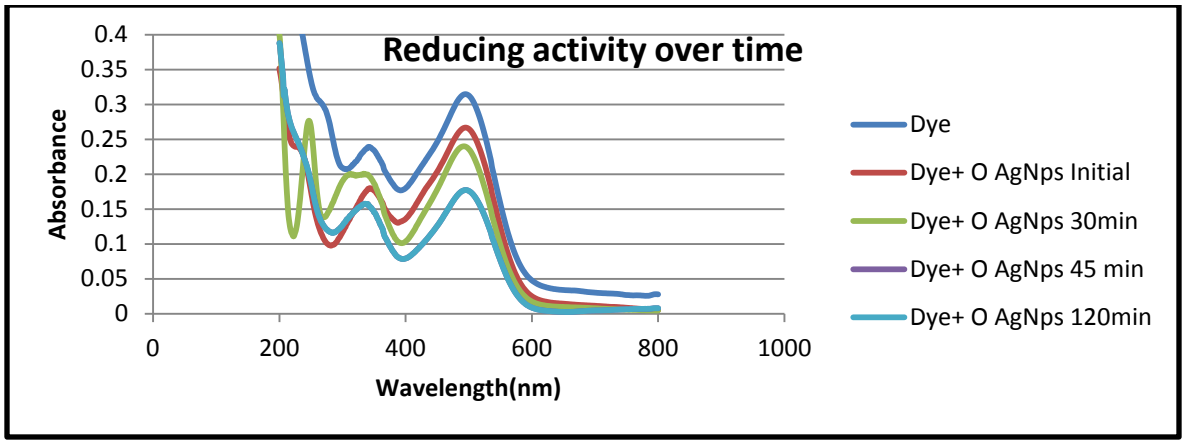


Fig.56 UV Spectra showing reducing activity of silver nanoparticles over a time period of 120 minutes.

Punica granatum* AuNPs:-** Gold nanoparticles of ***Punica granatum showed reducing activity by reducing azo group of Congo Red Dye as shown in Fig.57. The absorbance decreases when gold nanoparticles are added to the solution of dye. This decrease is up to the time period of 120 minutes as after that there is no further decrease as shown in Fig.58. [10]

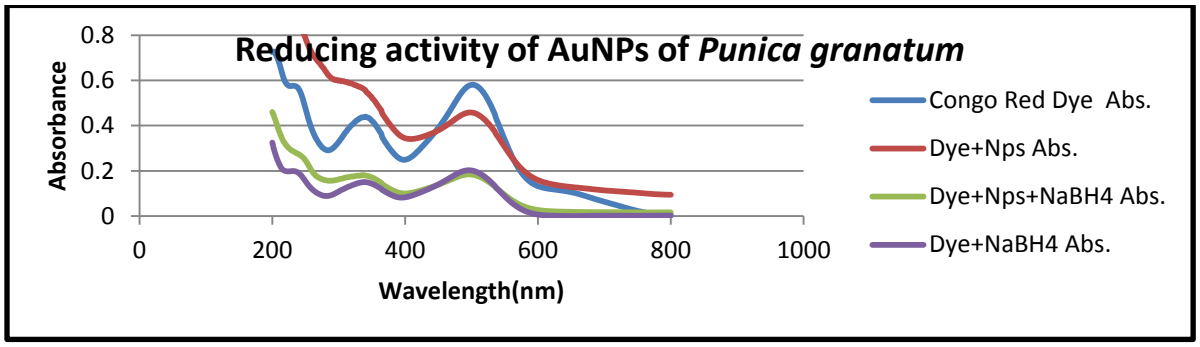


Fig.57 UV Spectra showing reducing activity of gold nanoparticles of ***Punica granatum***

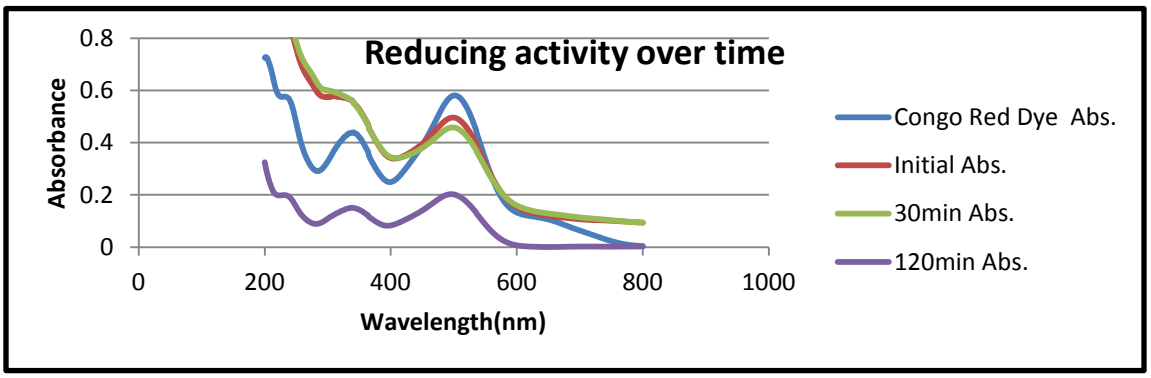


Fig.58 UV Spectra showing reducing activity of gold nanoparticles over a time period of 120 minutes.

Punica granatum* AgNPs:-** Silver nanoparticles of ***Punica grantum showed good reducing activity as the reducing extent is enhanced when these nanoparticles are added to the mixture of dye and sodium borohydride as compare to the solution containing only dye and sodium borohydride as shown in Fig.59. This activity is monitored for a time period of 60 minutes as after this no further reduction takes place as shown in Fig.60. [21]

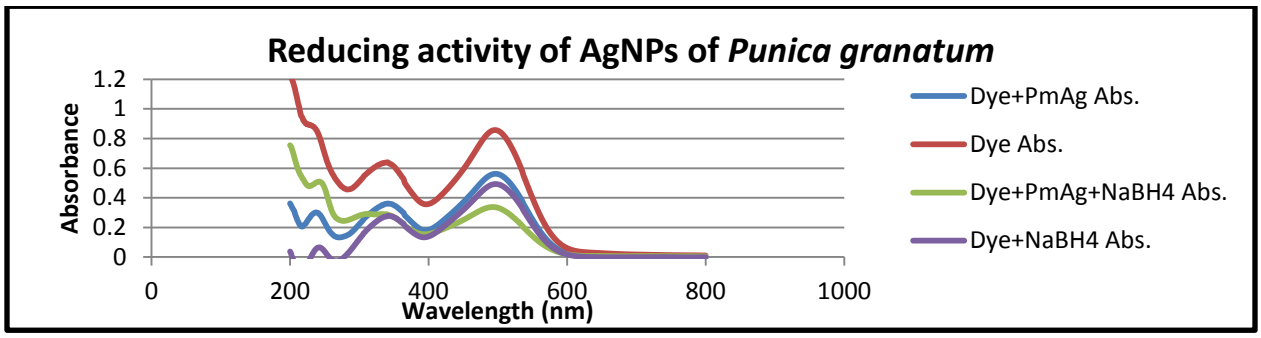


Fig.59 UV Spectra showing reducing activity of silver nanoparticles of ***Punica granatum***

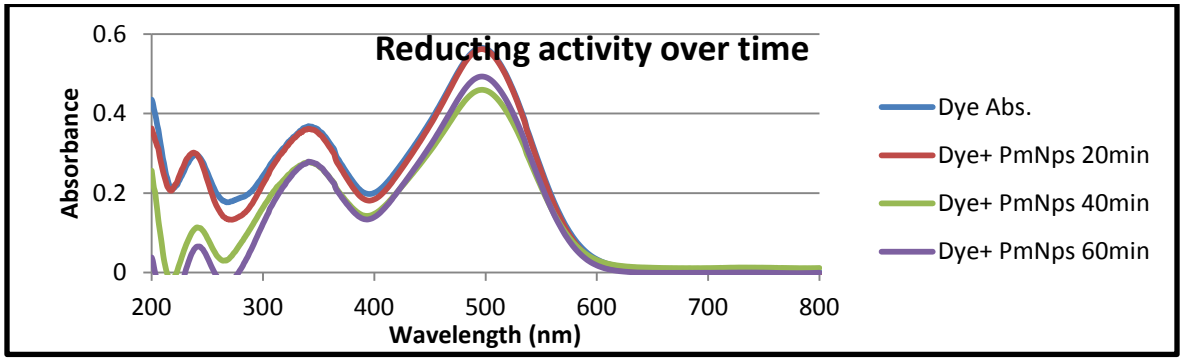


Fig.60 UV Spectra showing reducing activity of AgNPs over a time period of 60 minutes

Cladonia subtenuis* AgNPs**:- Silver nanoparticles of ***Cladonia subtenis showed reducing activity as these nanoparticles reduces the azo group of Congo Red Dye as shown in Fig.61. This activity is monitored by UV Spectroscopy over a time period of 60 minutes as shown in Fig.62. The action is similar to that of $NaBH_4$ but these nanoparticles are environment friendly and do not pose any threat to living beings. [25]

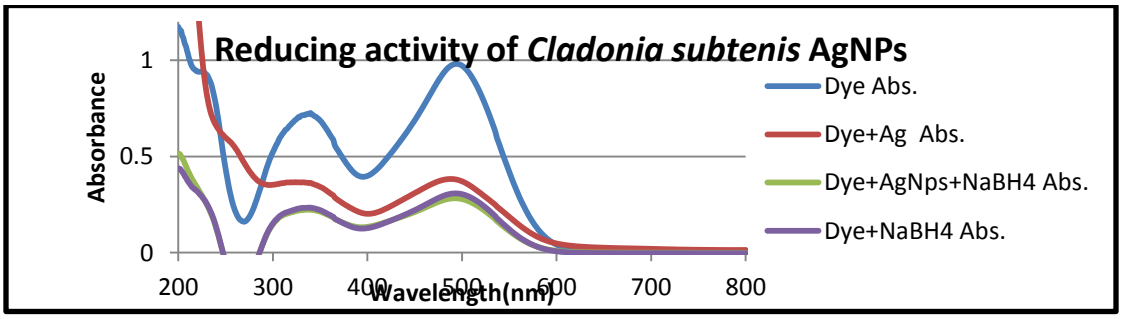


Fig.61 UV Spectra showing reducing activity of silver nanoparticles of ***Cladonia subtenis***

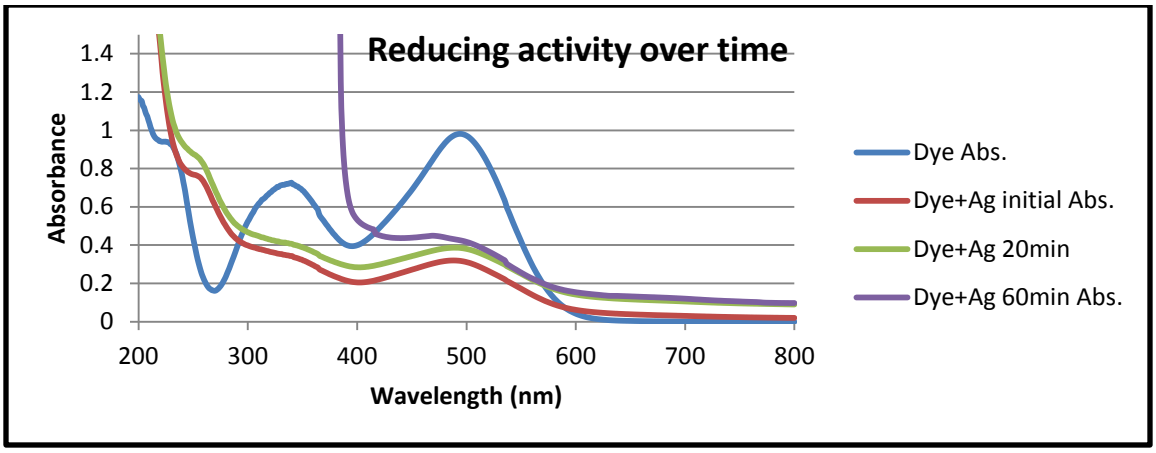


Fig.62 UV Spectra showing reducing activity of AgNPs over a time period of 60 minutes

Plant Material	Nanoparticles	Reducing activity	Concentration (mg)	Catalytic activity	Time (minutes)
Camelilia sinensis	Gold	Yes	10	No	120
Camelilia sinensis	Silver	No	-----	No	-----
Pisum sativum	Gold	Yes	5	No	45
Pisum sativum	Silver	Yes	5	No	60
Citrus aurantium	Gold	Yes	5	Yes	120
Citrus aurantium	Silver	Yes	5	Yes	120
Punica granatum	Gold	Yes	3	Yes	120
Punica granatum	Silver	Yes	5	Yes	60
Cladonia subtenuis	Silver	Yes	5	No	60

Table 7 showing plant materials and their time and concentration of reduction

The mechanism which can be possible for the reducing activity of gold nanoparticles on the Congo Red Dye is shown in Fig.63. The azo groups which are present in dye are broken together to give corresponding amine groups. The reason can be the larger size of gold nanoparticles which is responsible for the catalytic activity of these nanoparticles.

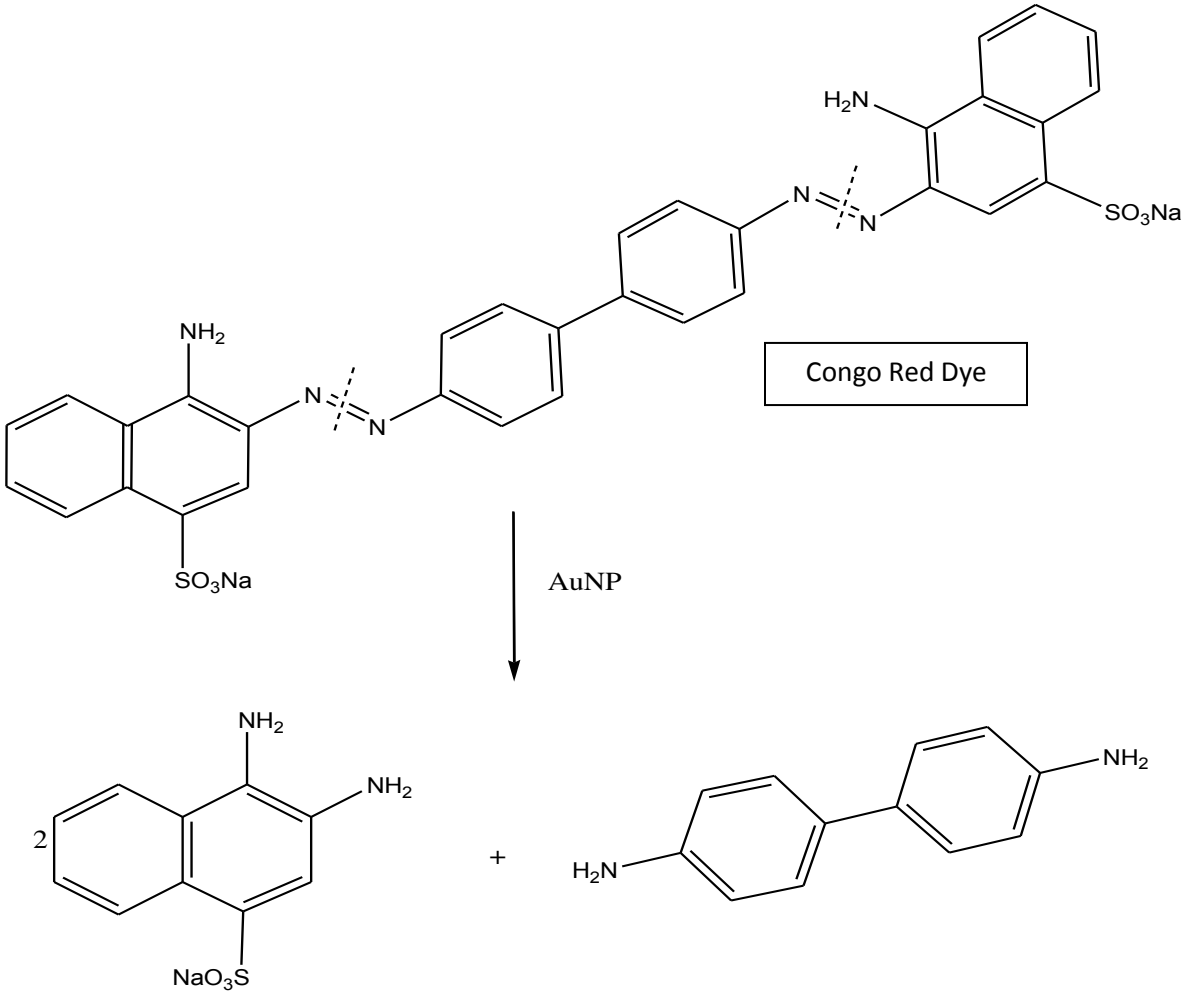


Fig.63 Proposed Scheme of catalytic activity for reduction of Congo red by AuNP

CONCLUSION:-

Green method for the synthesis of gold and silver nanoparticles is devised. These nanoparticles are made from the waste plant materials like used tea bags, peels of orange, pea and pomegranate. This method is beneficial as there is no requirement of any type of stabilizing agent and thus poses no threat to living beings as well as to environment. The formation of nanoparticles is confirmed by UV Spectroscopy, FTIR and XRD analysis. From these studies we came to know the reducing as well as catalytic action of gold nanoparticles and their proposed mechanisms are represented which are predicted through the characterization techniques.

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