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Synthesis of Nanoparticles from Plants and Check the Antimicrobial Activity of That Nanoparticle

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Abstract
Silver nanoparticles (AgNPs) were synthesized using aqueous extract of Asthma plant (Euphorbia hirta) leaves and silver salts. XRD, SEM, FTIR were measured and analyzed. The synthesized AgNPs exhibits lowest energy absorption band at 400nm. The effects of various parameters i.e., extract concentration and interaction time on the synthesis of AgNPs were studied. The AgNPs formed were found to have enhanced antimicrobial properties and showed zone of inhibition against isolated bacteria from garden soil sample. Based on the results obtained, it can be concluded that the resources obtained from plants can be efficiently used in the production of AgNPs and could be utilized in various fields such as biomedical, Nanotechnology. To study the compounds responsible for reduction of silver ions, the functional groups present in plant extract were investigated by FTIR. The silver nanoparticles showed anti-bacterial activities against both gram positive and gram-negative microorganisms. The bio active chemical compound was screened and identified by phytochemical studies.

Keywords
Euphorbia hirta, AgNPs, XRD, SEM, FTIR, Antibacterial activity, and phytochemical compounds.

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INTRODUCTION
The field of nanotechnology has proved to be one of the most active areas of research. Synthesis of nanoparticles is increasing exponentially because of its wide range of application in the field of optoelectronics, biosensors, bio nanotechnology and biomedicine etc.[1,2,3,4].
Various physical and chemical methods have been formulated for the synthesis of nanocrystals of desire shape and size. However, these methods are not economically feasible and environment friendly. Therefore, green synthesis has been considered as one of the promising methods for synthesis of nanoparticle because of their biocompatibility low toxicity and eco-friendly nature.[5]. Various microorganisms and plants have proved to be a source of inspiration nanocrystals synthesis [6]. The plants and plant extracts, which act as reducing and capping agent for nanocrystals synthesis, are more advantageous over other biological processes, because they eliminate the elaborated

process of culturing and maintaining of the cell, and can also be scaled up for large-scale nanocrystal synthesis[7]. Moreover, plant-mediated nanocrystals are preferred because it is cost-effective, environmentally friendly, a single-step method for biosynthesis process and safe for human therapeutic use. Different parts of plants materials such as extracts, fruit, bark, fruit peels, root and callus have been studied so far for the synthesis of silver [8].

The development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. Today, nanometal crystals, especially silver, have drawn the attention of scientists because of their extensive application in the development of new technologies in the areas of electronics, material sciences and medicine at the nanoscale. However, the synthesis of silver nanocrystals using plant constituents has not yet been studied for a large number of natural compounds.

The present study aims to synthesis silver nanocrystals by a green biological route, using an extract derived from Asthma plant*(Euphorbia hirta)* leaf extract and characterization of the synthesis nanoparticle utilizing electron microscope (SEM), and Fourier transform infrared spectroscopy (FT-IR) analysis and to screen the phyto active compounds by phytochemical studies.

MATERIALS AND METHODS

Sample collection

The leaf sample was collected from the plant *Euphorbia hirta.* The leaf was weighed and taken 50gms cutted into fine pieces and washed with distilled water and then washed with 1% mercuric chloride and then again washed with distilled water. The leaf was dried and chocked by grinding the leaf with 100ml of distilled water and the extract was filtered and taken, the filter was then boiled in water bath.

Preparation of mixture of plant extract with silver nitrate solution

The silver nitrate solution was prepared for 1mm concentration for 100ml. The 50ml of silver nitrate solution acts as an control, were as 45ml of silver nitrate solution was mixed with 5ml of plant extract where the mixture was subjected to sunlight for 5min and incubated at room temperature for overnight in dark room, which further checked for the reaction results in the colour change of pink to dark brown the O.D value was taken within half an hour of time interval the results were tabulated.

Confirmation of silver nitrate in the sample extract

The powdered form was analyzed for the presence of silver nitrate particle with SEM (Scanning Electron Microscopy), EDAX test was done for the further confirmation of the presence of silver nitrate particle in the sample extract.

FTIR studies

In FT-IR spectroscopy, Radiation is passed through a sample some of the IR radiation is absorbed by the sample and some of it is passed through the resulting spectrum represent molecular absorption and transmission creating molecular fingerprint of the sample. Synthesized nanocrystals were subjected to this study to ensure stretching and bending vibration of molecules during synthesize process. Nanoparticles synthesized has been confirmed based on the peaks observed in the cm wavelength this facility acquired at Karunya university.

Phytochemical activity of plant extract

Chemical tests for the screening and identification of bioactive chemical compounds like alkaloids, carbohydrates, glycosides, Saponins, phenolic compounds, sterols, proteins, amino acids, flavonoids and tannins. This study were carried out in extracts by using standard procedure [9].

Antibacterial activity

Mullar-Hinton agar (MHA) were inoculated with overnight culture of each bacterial suspension. The plates with the inoculated organisms were evenly spread out with sterile cotton swabs. The following bacterial strains are used: *Staphylococcus aureus, E.coli, Streptococcus pyogenes, Pseudomonas aeurogenesa.* The wells cups were filled with different concentration of plant extract, $AgNO_3$, Plant extract with $AgNO_3$. Concentration of well cups is 25µl, 50µl, 75µl, 100µl. The plates were then incubated at 37ºC 24-48 hours. Anti-bacterial activity was determined as growth inhibition of the target organism around agar cup as appearance of clear zone.

RESULT AND DISCUSSION

Sample collection

The plant *Euphorbia hirta* was collected from farm of Hindusthan College of Arts and Science, Coimbatore. *Euphorbia hirta* the plant also called Asthma plant (English), Ambalarisi (Tamil).

Preparation of Plant Extract

The leaf was weighed and taken 50gms cutted into fine pieces and washed with distilled water and then washed with 1% mercuric chloride and then again washed with distilled water. The leaf was dried, and

chocked with 100ml of distilled water, the filter was then boiled in water bath. The extract was filtered with Whatman No.1 filter paper.

The effect of Silver nitrate (AgNO3) formation in various parameters (Temperature, pH, Reactants ratio)

In the first day the temperature of control is 25 and sample is 26. Whereas, the pH for sample is 6.8 and the control is 7.

In the second day the temperature for sample is 34 and the control is 30. Whereas, the pH for sample is 7.5 and the control is 7. So, when the silver nitrate has been found to synthesis, the temperature and pH of the sample is increased day by day.

TABLE: 1 OD Value for plant extract with $AgNo_3$

S.NO	MINUTE	PLANT EXTRACT WITH $AgNo_3$
1.	30	0.381
2.	60	0.419
3.	90	0.452
4.	120	0.485
5.	150	0.510
6.	180	0.520

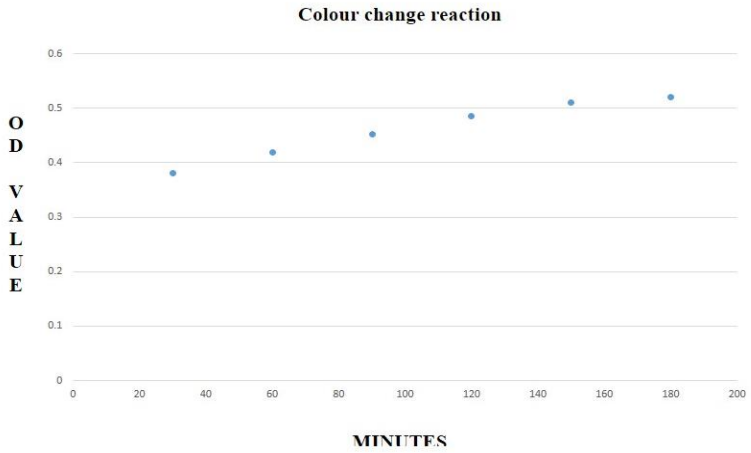


Figure: 1. Graph for OD Value of silver nitrate

OD value for 30 minutes explore along with Plant Extract + $AgNO_3$ complex shows 0.381 absorbance. Whereas in 180 minutes it is found to be 0.520. Hence Optical density of the silver nanoparticles has found to be increased with increase in time which is shown in Table:1 and Figure:1.

Synthesis of Silver nanoparticles

After the extract preparation, 5ml of plant extract is mixed with 45ml of silver nitrate. Reddish brown colour is formed. Solution and 50 ml of silver nitrate without plant extract, kept as control. The two conical flasks are kept in a dark room overnight. Next day the conical flask containing the sample would appear dark brown in colour. Measured using UV Spectroscopy which is found to be in Table 1.

Figure: 2, Silver nitrate formation

Control **Silver nitrate(sample)**

4.4 FTIR Analysis results

FTIR peaks were derived for plant extract *(Euphorbia hirta)*. The absorbance of peaks falls on nearby 3500 cm^{-1} for raw extract. The same peak was identified after the treatment with $AgNO_3$. These two peaks were interpreted an impact of plant extract leads the synthesis of silver nanoparticles or nanocrystals, which is shown in figure 3 and 4.

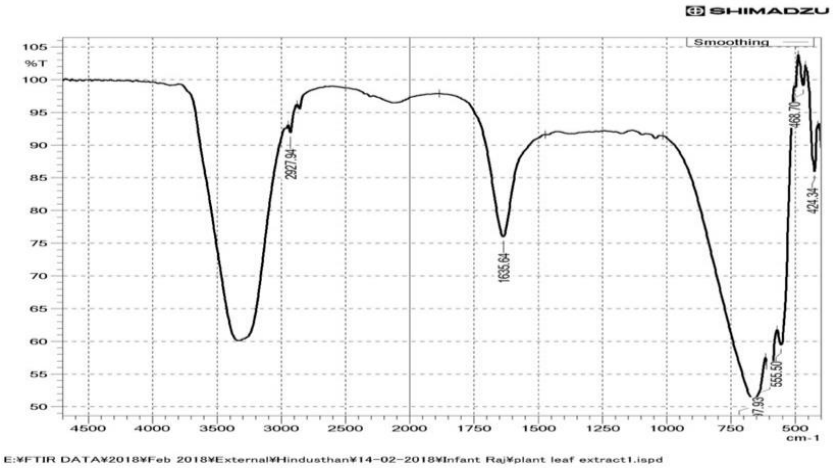
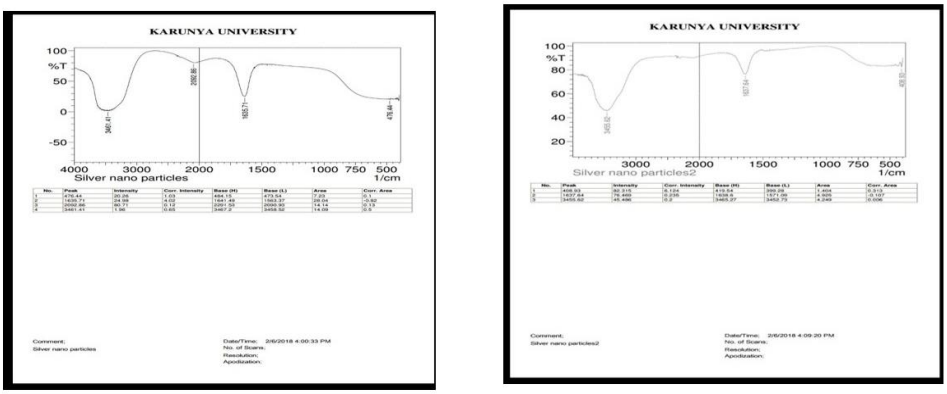


Figure: 3. FTIR Result for Plant Extract



CONTROL **SAMPLE**

Figure: 4. FTIR Result Plant Extract with $AgNo_3$ (AgNPs)

In alkanes:
C – H Stretch form = 3000 – 2850 cm^{-1}
C – H bend form =1470 – 1450 cm^{-1}
C – H methyl form = 1370 – 1350 cm^{-1}
Methyl and Alkane = 725 – 720 cm^{-1}
In alkenes:
C = C Stretch form = 1680 – 1640 cm^{-1}
C – H Stretch form = 3100 – 3000 cm^{-1}
C – H bend form = 1000 – 650 cm^{-1}
In Alkynes:
-C – C- stretch form = 2260 – 2100 cm^{-1}
-C – C – H: C- stretch form = 3330 – 3270 cm^{-1}
-C – C – H: C – H- bend form = 700 – 610 cm^{-1}

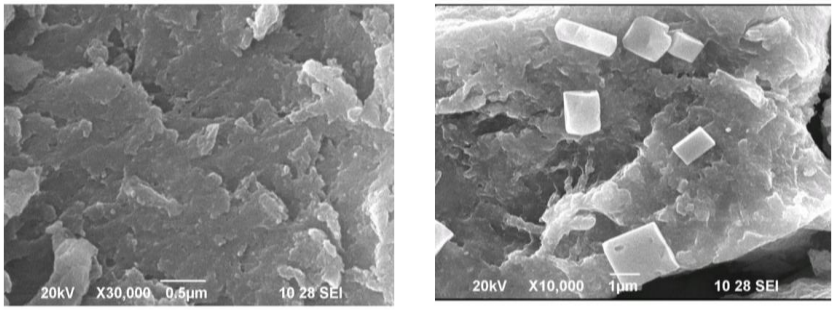
The FT-IR spectrum of the prepared sample was recorded in a PerkinElmer FT-IR in the range of 4000–450 cm^{-1} at a resolution of 4 cm^{-1}. The aqueous suspension of the synthesized silver nanoparticles was filtered through a 0.22µm syringe driven filter unit.

The FTIR measurements of the freeze-dried lyophilized samples were carried out to identify the possible interactions between silver and bioactive molecules, which may be responsible for the synthesis and stabilization of silver nanoparticles with the capping agent available in the plant filtrate.

FTIR has become an important tool in understanding the involvement of functional groups in relation between metal particles and biomolecules which is used to search the chemical composition of the silver nanoparticles and identify the biomolecules for capping and efficient stabilizing of the metal nanoparticles. There were many functional groups present which may have been responsible for the bio reduction of Ag ions.

FTIR spectrum of silver nanocrystals shows characteristic peaks at anyone the peaks 1625 and 1516, 1384 and 1047 cm^{-1}.

SEM ANALYSIS



CONTROL SAMPLE

Figure: 5. SEM analysis of synthesized nanocrystals

The shape of the synthesized silver nanoparticles was analyzed by SEM, representative SEM micrographs of control and treated BPE magnified at 750x and 1500x are shown in Fig: 5, respectively. Mono-dispersed square shaped silver nanocrystals were formed on the surface of BPE derived biological materials as indicated in Fig: 5. The image obtained by the FESEM also showed square nanocrystals Fig: 5, confirming the result obtained by SEM.

EDAX ANALYSIS

Table: 2. EDAX analysis for silver nitrate

Table
EDAX analysis result

Elements	App conc.	Intensity corn.	Weight %	Weight % Sigma	Atomic %
O K	25.29	0.5388	52.74	1.76	74.47
Na K	1.53	0.6884	2.50	0.31	2.46
Mg K	1.96	0.6407	3.44	0.28	3.20
Al K	0.45	0.7270	0.70	0.17	0.59
Si K	2.29	0.8324	3.09	0.23	2.49
S K	1.75	0.9438	2.09	0.25	1.47
Cl K	5.00	0.8311	6.76	0.36	4.31
K K	5.49	1.0645	5.80	0.35	3.35
Ca K	6.60	0.9110	8.14	0.41	4.59
Ag L	10.57	0.8058	14.74	0.85	3.09

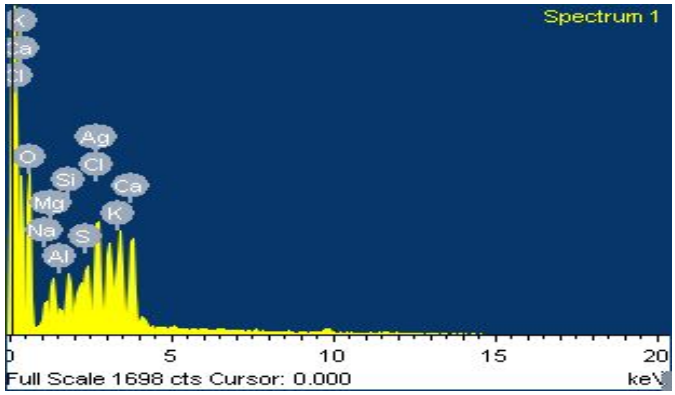


Figure: 6. EDAX spectra for silver nanocrystals

In the analysis of the silver nanoparticles by Energy Dispersive Spectroscopy (EDS), the presence of elemental silver signal was confirmed in the sample (Figure: 6). The Ag Nano crystallites display an optical absorption band peaking at 3 keV which is typical of the absorption of metallic silver Nano crystallites.

The EDS spectra recorded from the silver nanoparticles are shown in figure. The EDS profile shows a strong silver signal along with weak oxygen and carbon peaks, which may have originated from the biomolecules bound to the surface of the silver nanoparticles. Carbon and copper peaks may be due to the same being present in the grids. It has been reported that nanoparticles synthesized using plant extracts are surrounded by a thin layer of some capping organic material from the plant leaf broth and are, thus, stable in solution up to 4 weeks after synthesis. This is another advantage of nanoparticles synthesized using plant extracts over those synthesized using chemical methods.

Phytochemical analysis

Screening of bioactive compounds were done using standard procedure, which is shown in table: 3 and figure: 7.

Figure: 7. Phytochemical test results

Table: 3. Presence of Phytochemicals

S.NO	Phytochemicals	Result
1	Tannis	Negative
2	Alkaloids	Positive
3	Glycosides	Positive
4	Saponins	Negative
5	Flavonoids	Positive
6	Proteins	Negative
7	Triterpenoids	Positive
8	Carbohydrates	Positive
9	Steroids	Negative

4.8 Antibacterial activity

The antibacterial activity of the AgNPs, silver nitrate and plant extract against the four bacterial strains. There are; *E. coli, Streptococcus pyogenes, Staphylococcus aureus* and *Pseudomonas sp.* The zone of bacterial inhibition by AgNPs prepared from *Euphorbia hirta* plant leaf extract show inhibition for gram negative *E. coli* and *Pseudomonas sp.*The plant leaf extract show the inhibition against all the bacterial strains, which all are used.The $AgNO_3$ (Silver nitrate) will be inhibition only against the *E. coli* culture. Results obtained are shown in Table: 4.

Table: 4. Antibacterial activity result

Extracts	Bacteria	Zone of inhibition (mm) in various concentration			
		25µl	50µl	75µl	100µl
Plant extract	*E.Coli*	1	3	5	8
	S. Pyogenes	2	5	7	9
	S. Aureus	No zone	No zone	No zone	No zone
	Pseudomonas	No zone	1	3	5
AgNPs	*E.Coli*	3	5	8	10
	S. Pyogenes	No zone	No zone	No zone	No zone
	S. Aureus	No zone	No zone	No zone	No zone
	Pseudomonas	2	5	7	8
$AgNO_3$	*E.Coli*	1	3	3	5
	S. Pyogenes	No zone	No zone	No zone	No zone
	S. Aureus	No zone	No zone	No zone	No zone
	Pseudomonas	No zone	1	3	4

The plant extract, nanocrystals and silver nitrate are mostly active against the *E.coli* and *Pseudomonas sp.* The plant extract will also active against *Streptococcus pyogenes.* Silver nitrate and Nanocrystals couldn't active against *Streptococcus pyogenes* and *Staphylococcus aureus.* Plant extract also couldn't active against *Staphylococcus aureus.*

CONCLUSION

Silver nanocrystals synthesized with help of Plant Extract. Silver nanocrystalsshould higher amount of active against *E.coli* and *Pseudomonas sp.,* only.

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