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Synthesis and characterization of bio-active nano silver particles using different phyto-extracts

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Abstract
Nanoparticles (NP) have been shown to have various useful applications. They are generally synthesized using chemical processes involving hazardous chemicals. Therefore, green synthesis of NPs using natural products can be an environmentally friendly alternative. Plants contain different important phytochemicals that can be used as a potential treatment for various ailments. The green synthesis of silver nanoparticles from the extract of different plant parts has gained a wide range of engrossment among the researchers due to its unique optical and structural property. The aim of this study is green synthesis of silver nanoparticles from the ethanolic leaf extract of Punarnava (*Boerhavia diffusa*), pomegranate (*Punica granatum*), Tulsi (*Ocimum sanctum*) and Moringa (*Moringa oleifera*) was used for the green synthesis of silver nanoparticles (AgNPs) emerges as a costeffective and eco-friendly approach. Characterization of nanoparticles was done using different methods, which include; ultraviolet–visible spectroscopy (UV-Vis), powder X-ray diffraction (XRD), scanning electron microscope (SEM), Fourier- transform infrared spectroscopy (FTIR), dynamic light scattering (DLS). UV–visible spectrum of the silver nanoparticles showed absorption peak at around 420 nm. Scanning electron microscope micrographs showed the formation of well-separated silver nanoparticles of size in the range of 90-111 nm. XRD study showed the particles to be crystalline in nature, with a face-centered cubic (fcc) structure. FT-IR spectrum of the *Boerhavia diffusa* roots extract showed Several Peaks. The peak in the region of 1456.30 to 3439.19 cm^{-1}. The size and stability were detected using DLS. Hence, this AgNPs might be used as antibiotics and antidiabetic agent in future and can be used in many medicinal and technological applications due to non-toxic, cheap and eco-friendly.

Keywords: Silver nanoparticles, ethanolic leaf extract pomegranate, methanolic extract of Punarnava, moringa and Tulsi seed, green synthesis and charcterization

Introduction
Nanotechnology is a known field of research since last century. Since ‘‘nanotechnology” was presented by Nobel laureate Richard P. Feynman during his well famous 1959 lecture ‘‘There’s Plenty of Room at the Bottom” (Feynman, 1960), there have been made various revolutionary developments in the field of nanotechnology. Nanotechnology produced materials of various types at nanoscale level. [1] The word “nano” is used to indicate one billionth a meter or 10-9. The term Nanotechnology was coined by Professor Noria Taniguchi of Tokyo Science University in the year 1974 to describe precision manufacturing of materials at the nanometer level. “Nano” is a Greek word synonymous to dwarf meaning extremely small. Nanoparticles are beginning viewed as fundamental building blocks of nanotechnology [2]. In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc. Generally the nanoparticles are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for. Nanoparticles can be broadly grouped into two, namely, organic nanoparticles which include carbon nanoparticles (fullerenes) while, some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles (like titanium oxide and zinc oxide). There is a growing interest in inorganic nanoparticles of noble metal nanoparticles (Gold and silver) as they provide superior material properties with functional versatility. Due to their size features and advantages over available chemical imaging drug agents and drugs,

inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. [

Nanoparticles

The prefix nano is derived from Greek word nanos meaning "dwarf" or extremely small. Nano- sized materials, known as NPs, possess unique and improved properties because of their larger surface area to volume ratio. NPs can be broadly grouped into two, namely, organic NPs and inorganic NPs which include noble metal NPs (like silver and gold), semi-conductor NPs (Liketitanium oxide and zinc oxide) [7]. Nanotechnology can be applied to medicine, therapeutics, drug delivery and also in treatment for many diseases and disorders

Materials and Methods

Materials

Glassware: Solvent extractor, conical flask, beaker, tube test, glass rod, test-tube stand, pipette, round bottom flask, flat bottom flask, slides, reagent sprayer, china dish, volumetric Cylinder, volumetric flask, UV - Vis spectrometric cuvettes, glass cutter, morter pestle, aluminium foils, cotton, burette holder & clips, pH paper, petridish, soxhlet extractor, condenser.

Chemicals

Silver nitrate (Merck specifications pvt. Indore Ltd. Mumbai) Tulsi (*Ocimum sanctum*) Seed,
Pomegranate (*Punica grantum*) Leaves, Punarnava (*Boerhavia diffusa*) roots, Indore *Moringa oleifera* seed, Indore.

Methods

Herbal Component of Plant

Procurement of Plant

Plant Material i.e. leaves of Pomegrante was collected from Medicinal Plant of Swami Vivekanand College of Pharmacy, Indore. The plant leaves were dried in a hot air oven at 25oC and Powdered. Fine powder was obtained and stored in container for future use. The seed of Tulsi and Moringa and Powder of Punarnava Root was Purchased from Market Indore. While Silver Nitrate Was Purchased From Merck Specifications Pvt. Ltd., Mumbai

Preparation of Plant Extract

The Punica grantum extract was prepared using Soxhlet extractor using Ethanol as a solvent. Similarly, Ocimun sanctum, Moringa oleifera and Boerhavia diffusa extract was prepared using methnol as a solvent crude plant extract was prepared by Soxhlet extraction method. About 100 gm of powder material was uniformly packed in to a thimble and run in Soxhlet extractor. It was extracted with a solvent for the period of about 48 hour around 28 cycles till the solvent in the siphon tube of an extractor become color less. The extracts were filtered with the help of filter paper and solvent was evaporated from extract in Rotary
evaporator to get the syrupy consistency. Then extracts were kept in refrigerator at 4oC for future experiments.

Preliminary Phytochemical Screening of Plant Extract of Active Components

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "Secondary metabolites" which includes tannins, saponins, flavonoids, alkaloids, proteins, steroids, Quinones, terpenoids, cardio glycosides and phenol. 120
Alkaloids – 1 % HCl was added to the extract in a test tube reacted for 20 minutes, cooled and filtered. About 2 drop of Mayer's reagent was added to rhe extract. A creamy precipitate was an indication of the presences of alkaloids. Tannins- Freshly prepared 10% KOH was added to the extract. A dirty white precipitate shows the presences of tannins. Phenolics- Two drops of 5% Fecl3 were added to the extract in a test tube. Absence of greenish precipitate indicates the absence of phenolics.
Glycosides- 50% H_2SO_4 was added to the extract and the mixture heated in boiling water for about 15 minutes. Fehling's solution was then added and the mixtures boiled. A brick-red precipitate was confirmatory for the presence of glycosides
Emulsion test: 5 drops of olive oil was added to the extract in the test tube and vigorously shaken. Absence of stable emulsion formed indicates the absence of saponins.

Nano-silver Component of Plant

Procurement of Chemicals

Silver Nitrate ($AgNO_3$) was used as purchased from Merck Specification Pvt. Ltd., Mumbai.

Preparation of 1x 10 -3 M Silver Nitrate Solution

1 x 10^{-3} M solution of silver Nitrate was prepared by dissolving 0.170 g of silver nitrate in sufficient water to produce 1000 ml.

Preparation of Plant Extracts Solution

A solution of plant extract was prepared by dissolving extract in minimal quantity of organic solvent, to get slurry. The prepared slurry was mixed by a homogenizer in water to get a suspension. The stock solution of 30 mg/30 ml was prepared from ethanolic extract of Punica grantum, Methanolic extract of Moringa, Occimun sanctum, and Boerhavia diffusa. From the stock solution different dilutions are prepared ranging from 40, 60, 80 and 100µg/ml, using water as diluents.

Biosyntesis of Nano- Silver Particles

100-ml aqueous solution of 1.0 x 10 -3 m silver Nitrate was mixed with plant extract solution of different concentration In Silver Nitrate Solutions. In Silver Nitrate Solution plant extract was added followed by intermittent strring at room temperature. By mixing both solutions, Ag ions were reduced and clustered together to form monodispersed nanoparticles as a transparent sol in aqueous medium. The Ag solution was yellow and gives absorption at 390 nm. The solutions was stirred repeatedly after so up to brown dark color appeared for approximately after an hour. It keeps in observation until it became stable. At this point this solution of Ag nanoparticles was so stable that it did not change color for as long a period of time without any stabilizing agent.
Similar procedure was followed for every dilution i.e. 40, 60, 80 and 100µg/ml. after every interval sampling was done and studied by UV-Vis analysis for the formation no of particles with time.
Characterization of Nano - Silver particles

UV-Vis spectra analysis of nano silver particles

The bioreduction of Ag+ in aqueous solution was monitored by periodic sampiling of aliquets (0.2 ml) of the suspension, then diluting the samples with 2 ml deionized water and subsequently measuring UV - vis spectra of the resulting diluents. Water is taken as blank solution for analysis. UV-vis spectroscopy analyses of silver nanoparticles produced were carried out as a function of bio reduction time at room temperature on Shimadzu UV - 1800 spectrophotometers at a resolution of 1 nm. Sampling was done starting from 0, 15,30, 45, 60, 90, 120 and 24 hrs. And then the spectra were taken Stability of solution was observed periodically after every 15 days by above mentioned method.

2. SEM analyses of nanosilver particles

Scanning Electron microscopic (SEM) analyses was done using Model: JEOL JSM 5600 SEM machine. The silver nanoparticle solution obtained was purified by repeated centrifugation at 5000 nm. For 20 min followed by redispersion of the pellet of silver nanoparticles into 10 ml of deionzed water. Thin films of the sample were prepared on a side by dropping a very small amount of the sample on the slide, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under lamp for 5 min and analyzed by Scanning Electron Microscopy (JEOL JSM 5600).

3. X - Ray Diffraction analysis of nanosilver particles

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of silver nanoparticles into 10 ml of deionized water. A thin film was formed in glass slides by drying the purified silver nanoparticles; the structure and composition were analysed by XRD SEM. The film of Silver nanoparticles was prepared for the determination of the formation of Ag nano particles by Thin Film X - ray Diffr actomer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu Ku radiation in a Ɵ-2Ɵ configuration

4. TEM analyses of Nano Silver particles

The size and morphology of synthesized AgNPs are investigated by HR-TEM image indicates that the particles are predominantly globular shaped. Selected area electron diffraction (SAED) image reveals that the AgNPs are polycrystalline in nature. It is evident that the average particle size distribution of the synthesized AgNPs ranges from 11–15 nm 119

5. Fourier Transform - Infra red spectroscopy of nano silver particles

By centrifugation, free biomass residue or compound that is not the capping ligand of the nanoparticles were removed. 30 ml of solution was after reaction was centrifuged at 10000 rpm for 10 min and the resulting suspension was redispersed in 2 ml sterile distilled water. Finally, the purified suspension of nanoparticles was analyzed by FTIR Bruker Model Vertex 70 in ATR mode.

6. Particle size determinations of nano silver particles

Physicochemical characterization of prepared nanomaterial is an important factor for the analysis of biological activities using radiation scattering techniques. DLS can probe the size distribution of small particles a scale ranging from submicron down to one nanometer in solution or suspension. of narrow particle size distributions, especially in the range of 2–500 nm

Results and Discussion

The Preparation of Plant Extracts

The plant extract was prepared by drying the samples in a hot air oven at 25^{O}c and blended into powder. The powder was extracted with methanol/ethanol solvent by soxhlet extractor for 48 hours and filtered. Extraction was repated until obtaining colorless extract, all extracts were combined and the solvent was evaporated under vaccum. Finaly, the percentage yield of the crude extract was calculated. The obtained crude extract was sticky and viscous dark greenish brown semisolid.

The percentage yield of crude extract was calculated by following equation:

$$\% \text{ Yield} = \frac{\text{weight of crude extract}}{\text{Weight of dry Powder}} \text{ x100}$$

The percentage yield of batches of Ethanolic extract of *Punica grantum*, methanolic extract of *Boerhavia diffusa*, *Ocimum sanctum* and *Moringa oleifera* were 18.12%, 9.03%, 1.23% and 3.95% respectively.

Table 1: Percentage Yeild of Plant Extract

Plant extract	Weighed of powder (gm)	Weight of crude Extract(gm)	%Yield
Ethanolic extract of *Punica grantum*	100	18.12	18.12
Methanolic extract of *Boerhavia diffusa*	100	9.03	9.03
Methanolic extract of *Ocimum sanctum*	100	1.23	1.23
Methanolic ext*ract of Moringa oleifera*	100	3.95	3.95

Fig 7: Photograph of Procurement of Plants, A) Plants Leaves B) Plant Powders, C) PlantsExtracts

Fig 8: Photograph of biosyenthesis of Silver Nanoparticles from *Punica grantum* extract A) Before synthesis, B) After 15 min & C) After 24 hrs.

Fig 9: Photograph of biosyenthesis of Silver Nanoparticles from *Boerhavia diffusa* extract A) Before synthesis, B) After 15 min & C) After 24 hrs

Phyto - Chemical Studies

Methanolic extract of *Moringa Oleifera*, methanolic extract of *Boerhavia diffusa* and *Ocimum sanctum*, Ethanolic extract of *Punica grantum* were subjected to various chemical tests for the preliminary determination of Phytoconstituents. Methanolic extract of *Moringa oleifera*, methanolic extract of *Boerhavia diffusa* and *Ocimum sanctum*, Ethanolic extract of *Punica grantum* were mixed with equal proportion of alcohol and water (to get hydro alcoholic sample) before subjected to various chemical test. Results of phytochemical studies were shown in table 2.

Above studies confirms the presence of Tannin in methanolic extract of *Boerhavia diffusa* and *Moringa*. Glycosides are present in methanolic extract of *Boerhavia diffusa*, *Moringa oleifera Ocimum sanctum* and ethanolic extract of *Punica grantum*. Methanolic extract of *Boerhavia diffusa* and *Moringa oleifera* and ethnaolic extract of *Punica grantum* are showed the presence of flavonoids and steroids. Tannin is presence in *Punica grantum* and *Moringa oleifera* except *Ocimum sanctum and Boerhavia diffusa*.

Table 2: *Chemical Tests*

Test Performed	Punica Grantum	*Boerhavia diffusa*	Ocmium Sanctum	Moringa
Tannins	+	-	-	+
Phenolic compound	+	+	-	-
Glycosides	+	+	+	+
Saponin	+	+	-	-
Flavonoids	+	+	+	+
Phlobatannins	-	-	-	-
Terpenoids	+	-	-	-
Steriods	+	+	+	+

(+) Presence, (-) Absence

Thin Layer Chromatographic Analysis of Plant Extract

For qualitative analysis and to identify the phytoconstituent thin layer chromatography was performed as given in individual monograph. Table 1, 2, 3 and 4 shows R_F value for identified spot in the plant extract.

For *Boerhavia diffusa* Extract

TLC of methanolic extract of *Boerhavia diffusa* was carried out using solvent system containing volumes of 21 volumes of toluene, 3 volumes of ethyl acetate and 6 volumes of methanol and detected by under UV light. The spot were found to be R_F value 0.79.

Table 3: TLC profile of methanolic extract of *Boerhavia diffusa*

S. NO	Distance travelled by solvent front (S) cm	Distance traveled by sample (X)cm	R_f value= X/S
1	11	8.5	0.77
2	11	8.7	0.79
3	11	8.7	0.79

For *Punica grantum* Extract

TLC of ethanolic extract of *Punica grantum* was carried out using solvent system containing volumes of 9 volumes of chloroform, 1 volumes of methanol and 1 volumes of acetic acid and detected by under UV light. The spot were found to be R_f value 0.47.

Table 4: TLC Profile of Methanolic Extract of *Punica grantum*

S.NO	Distance travelled by solvent front (S) cm	Distance traveled by sample (X)cm	R_f value= X/S
1	11	5.5	0.5
2	11	5.2	0.47
3	11	5.2	0.47

For Ocimum sanctum extract

TLC of methanolic extract of *Ocimum sanctum* was carried out using solvent system containing volumes of 9 volumes of toluene, 9 volumes of ethyl acetate and 3 volumes of acetic acid and detected by anisaldehyde under UV light. The spot were found to be R_F value 0.37.

Table 5: TLC Profile of Methanolic Extract of *Ocimum sanctum*

S.NO	Distance travelled by solvent front (S) cm	Distance traveled by sample (X)cm	R_f value= X/S
1	9	3.8	0.42
2	9	3.4	0.37
3	9	3.4	0.37

For Moringa oleifera extract

TLC of methanolic extract of *Moringa oleifera* was carried out using solvent system containing volumes of 14 volumes of toluene, 4 volumes of ethyl acetate and 2 volumes of methanol. And detected by anisaldehyde under UV light. The spot were found to be R_F value 0.47.

Table 6: TLC Profile of Methanolic Extract of *Moringa oleifera*

S.NO	Distance travelled by solvent front (S) cm	Distance traveled by sample (X)cm	R_f value= X/S
1	9	4.3	0.47
2	9	4.2	0.46
3	9	4.3	0.47

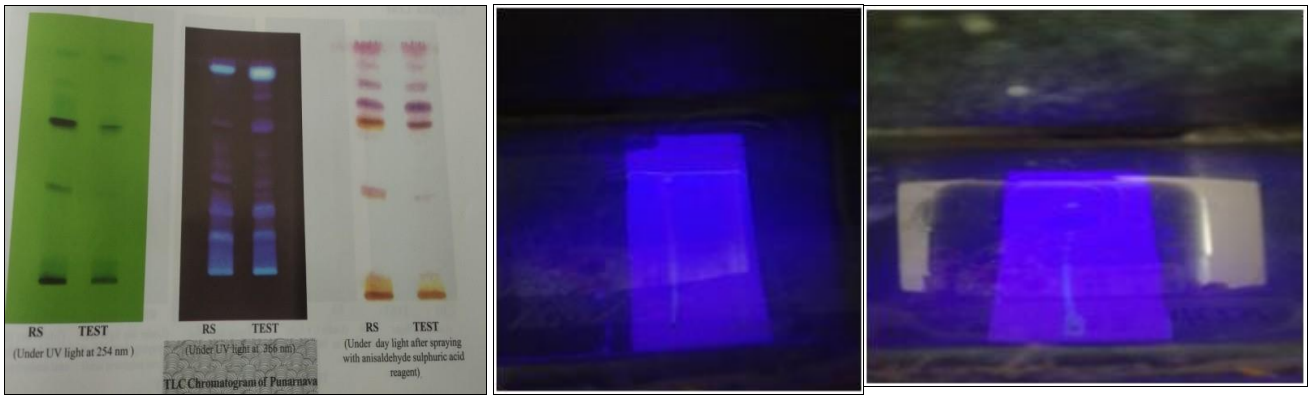


Fig 10: TLC of *B. diffusia*

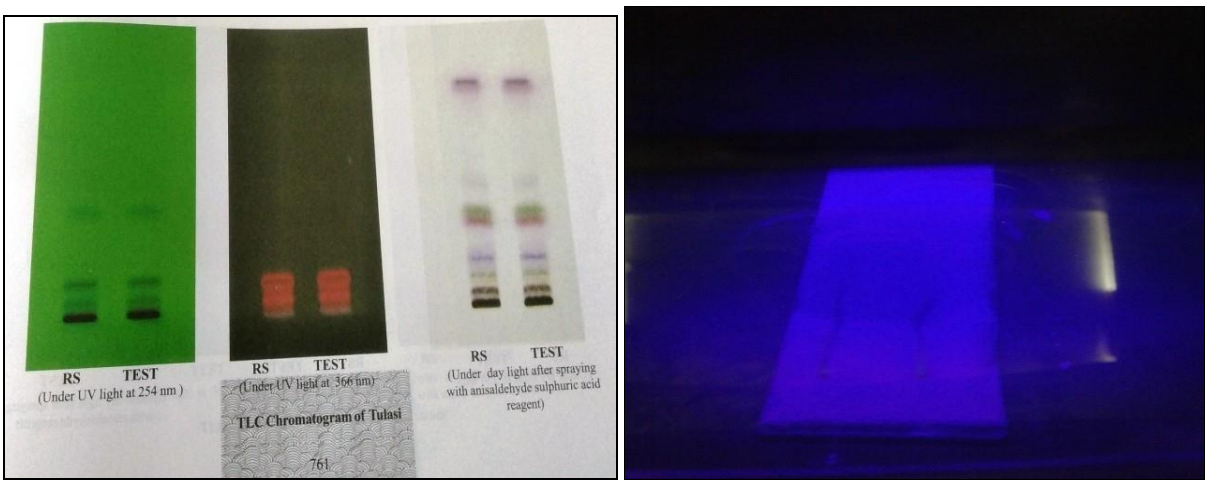


Fig 11: TLC of *Ocimum sanctum*

Table 1: Observation of Colour Development in Sample of *Boerhavia diffusa* with Time

S.no.	Sample code	Colour intensity		
		15min.	30min.	1hrs
1	BD1	+	+	++
2	BD2	+	++	++
3	BD3	+	++	++
4	BD4	+	+	+

(-)= No colour change or undesirable colour change.
(+)= Yellowish brown colour observed.
(++)= Reddish brown colour observed.

Table 8: UV-Spectrophotometric Data of Nanosilver from *Boerhavia diffusa* extract:

S.no.	Concentration (µg/ml)	0 hrs	0.15 hrs	0.30 hrs	1hrs	24hrs	48 hrs
BD1	40	236	298	298	312	395	395
BD2	60	298	298	395	410	395	420
BD3	80	248	248	248	250	298	390
BD4	100	365	365	312	336	260	260

For *Punica grantum* extract

Different concentration PG1, PG2, PG3 & PG4 i.e. 40, 60, 80 and 100 µg/ml respectively) of ethanolic extract of *Punica grantum*, was added to 1x 10 $^{-3}$ M silver nitrate solution. There was a gradual increase in color development in the partical size, dielectic medium and chemical surroundings.

Sample PG1 i.e 40 µg/ml shows the colour change from yellow to reddish brown. There was a shift in λ max with increase in reaction time. There was increase in intensity of the peak showing increase in concentration of silver nanooaticles. The colour change was from light greenish to brown. Intense peak was observed at 390nm. Sample was further used for analysis.

In samle PG2 i.e. 60 µg/ml, intense peak was observed at 292nm. But after 24 hrs, characteristic peak are Observed

In sample PG3 i.e. 80 µg/ml. intense spectrum was observed at 300nm. A stable colour change was seen from greenish yellow to reddish brown the maximum absorbance at 300 nm. Sample was rejected for further analysis.

In sample PG4 i.e .100 µg/ml, it was observed that with time there is 420 nm due to the surface Plasmon resonance (SPR) phenomenon of silver nanoparticles. The colour was also intense. The peak intensity was stable with time and hence sample was chosen for further studies.

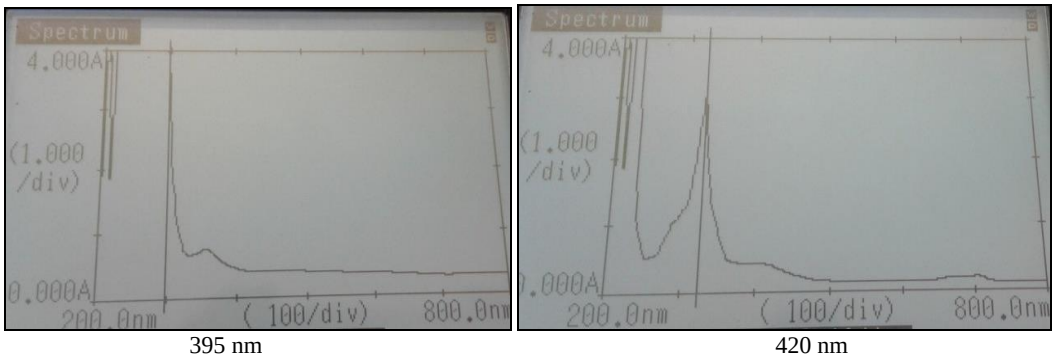
Table 9: Observation of Colour Development in Sample of Punica grantum With Time

S.no.	Sample code	Colour intensity		
		15min.	30min.	1hrs
1	PG1	+	+	++
2	PG2	+	+	+
3	PG3	+	+	+
4	PG4	+	++	++

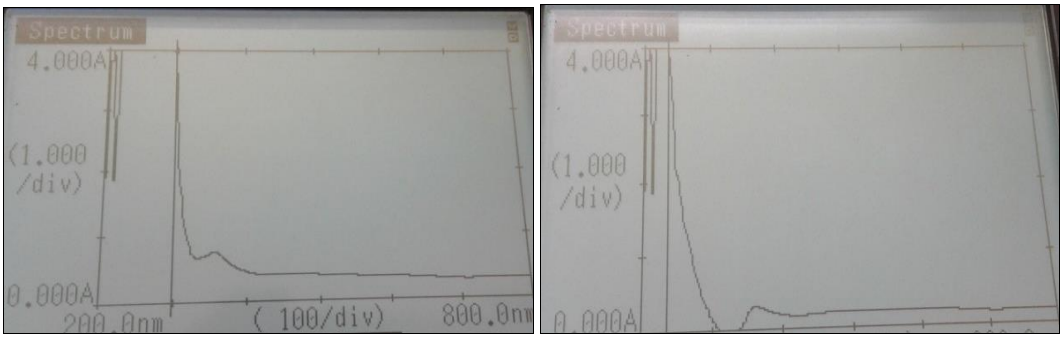
(-)= No colour change or undesirable colour change.
(+)= Yellowish brown colour observed.
(++)= Reddish brown colour observed.

Table 10: *Uvspectrophotometric Data of Nanosilver from Punica grantum Extract:*

S.no.	Concentration (µg/ml)	0 hrs	0.15 hrs	0.30hrs	1hrs	24hrs	48 hrs
PG1	40	284	284	286	286	390	390
PG2	60	286	292	292	292	220	218
PG3	80	300	300	300	296	292	292
PG4	100	324	324	324	322	324	420

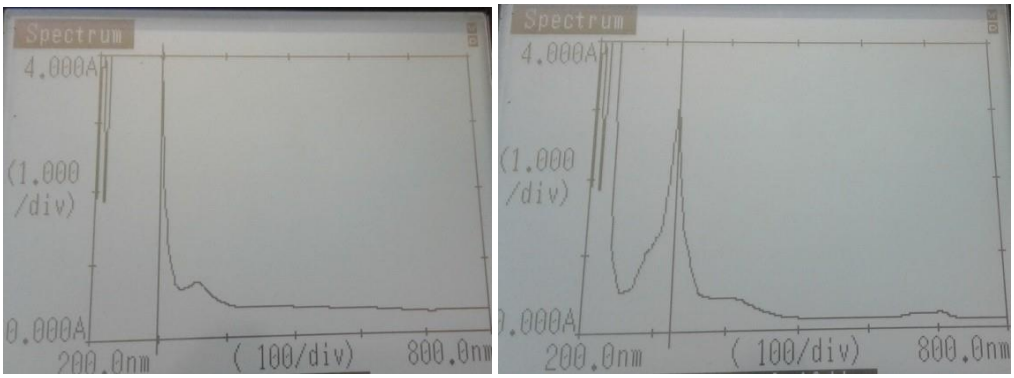


395 nm 420 nm



390 nm 260 nm

Fig 12: UV-Vis Absorption Spectra of Sample after 48hrs. Of Reaction, A) Sample BD1, B) Sample BD2, C) Sample BD3, D) Sample BD4



390 nm 420 nm

Fig 13: UV-Vis Absorption Spectra of Sample after 48 hrs. Of Reaction, A) Sample PG1, B) Sample PG

For *Ocimum sanctum* extract

Different concentration (OS1, OS2, OS3 & OS4 i.e.40, 60, 80 and 100µg/ml respectively) of methanolic extract of *Ocimum sanctum* was added to 1x 10^{-3} M silver nitrate solution. There was a gradual increase in color development in the partical size, dielectic medium and chemical surroundings.

Table 11: Observation of Colour Development in Sample of O. sanctum with Time

S.no.	Sample code	Colour intensity		
		15min.	30min.	1hrs
1	OS1	-	-	-
2	OS2	-	-	+
3	OS3	-	+	++
4	OS4	-	+	+

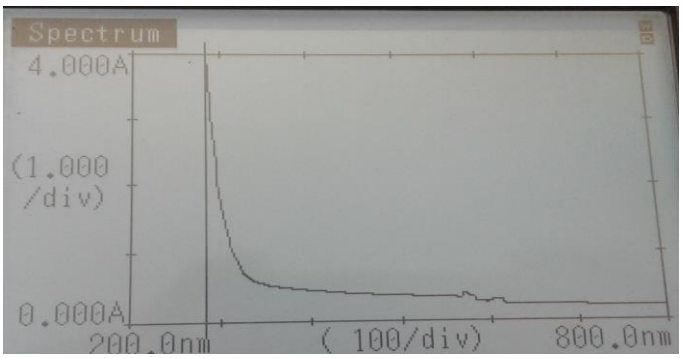
(-)= No colour change or undesirable colour change.
(+)= Yellowish brown colour observed.
(++)= Reddish brown colour observed.

Table 12: UV- Spectrophotometric Data of Nanosilver from Ocimum sanctum extract

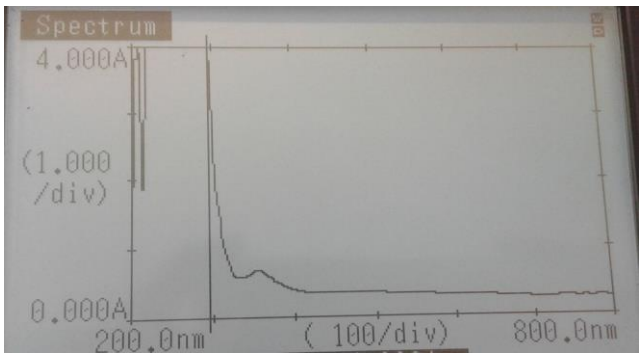
S.no.	Concentration (µg/ml)	0 hrs	0.15hrs	0.30 hrs	1hrs	24hrs	48 hrs
OS1	40	282	284	284	284	284	288
OS2	60	292	280	278	294	234	232
OS3	80	298	296	294	392	390	400
OS4	100	320	320	334	338	294	284

Table 13: Observation of Colour Development in Sample of *Moringa oleifera* with Time

S.no.	Sample code	Colour intensity		
		15min.	30min.	1hrs
1	MO1	-	-	-
2	MO2	-	-	-
3	MO3	-	+	+
4	MO4	-	+	+



UV-Vis Absorption Spectra of Sample OS3 after 48 hrs. Of Reaction



UV-Vis Absorption Spectra of Sample MO1 after 24 hrs. Of Reaction

Fig 14: Scanning Electron Microscopy

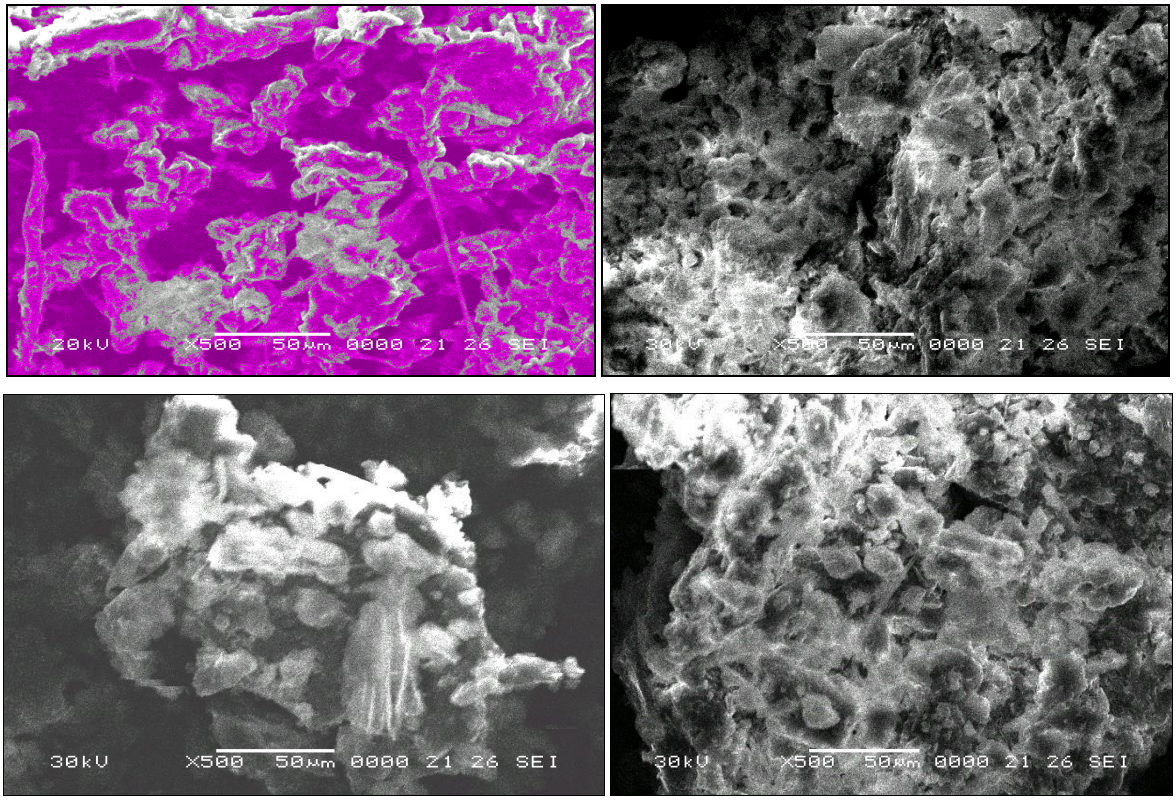


Fig 16: Scanning Electron Micrograph of Silver Nanoparticle Prepared from Methanolic Extract of Boerhavia diffusa A) Sample BD1, B) Sample BD2, C) Sample BD3, D) Sample BD4

Table 14: UV Spectrophotometric Data of Nanosilver from *Moringa oleifera* extract

S.no.	Concentration (µg/ml)	0 hrs	0.15 hrs	0.30 hrs	1hrs	24hrs	48 hrs
MO1	40	282	282	284	284	380	380
MO2	60	226	226	226	250	262	260
MO3	80	284	284	284	210	212	214
MO4	100	232	232	232	258	260	262

5.2 SEM (Scanning Electron Microscopy) Analysis

SEM is a technique that uses electrons instead of light to form an output image. The SEM analysis is employed to characterize the size, shape, morphology and distribution of synthesized AgNPs. The SEM micrographs also indicate the purity and polydispersity of resulting AgNPs. SEM Preparing a dry film of the prepared nanaoparticles on the glass slide. SEM analysis was performed at different resolutions and results are taken. [121]

5.3 XRD (X-Ray Diffraction Studies) Analysis

The X-ray diffraction (XRD) pattern of the prepared sample of silver nanoparticles was recorded at UGC-DAE Consortium for Scientific Research, Indore, by employing Bruker d8 Advance X- ray diffractometer, using CuKα radiation (λ = 1.5406 Å), 40 kV- 40mA, 2θ/θ scanning mode. Data was taken for the 2θ range of 30 to 80 degrees with a step of 0.0202 degree. The XRD data diffractogram (Fig. 17) has been compared with the standard powder diffraction card of JCPDS, silver file No. 04-0783.

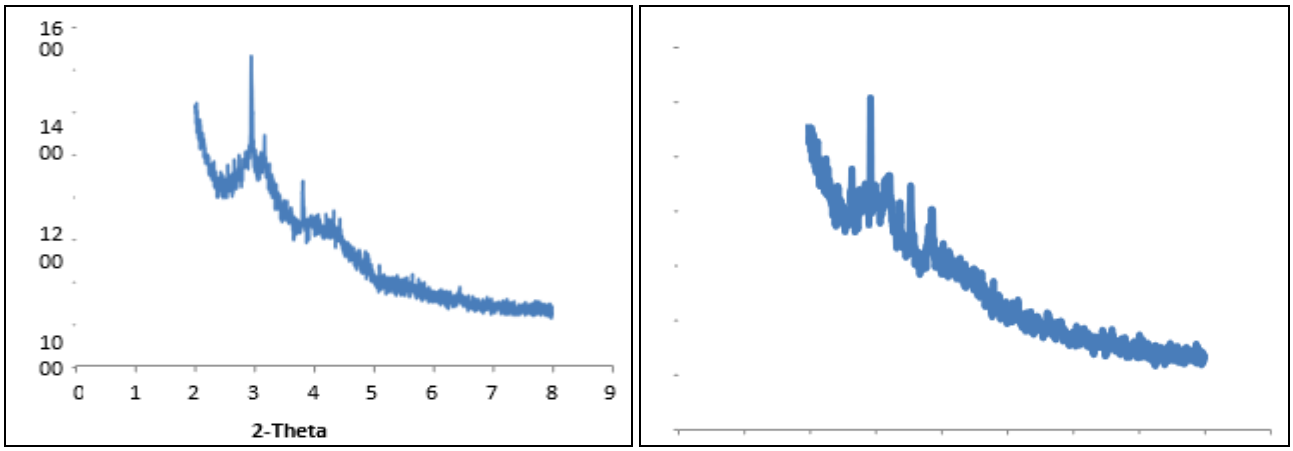


Fig 17: X-Ray Diffraction Analysis of Silver Nanoparticle Prepared from Methanolic Extract of Boerhavia diffusa, A) Sample BD1, B) Sample BD

FT-IR measurements were done to identify the major functional groups on the Boerhavia diffusa extract and their possible involvement in the synthesis and stabilization of silver nanoparticles. The spectra of Boerhavia diffusa roots after reaction with silver nitrate are shown in Fig. 18. The FT-IR spectrum of the Boerhavia diffusa roots extract shows several characteristic peaks; The peaks in the region of 1456.30 to 1458.23 cm-1, 1608.69 to 1618.33.

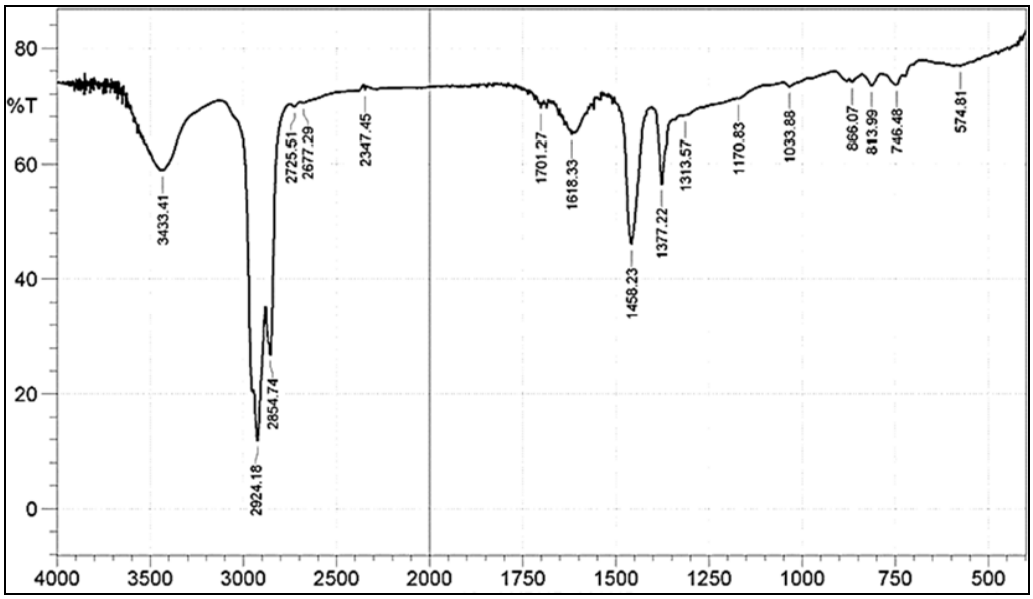


Fig 18: FT-IR spectrophotometric Analysis

Particle Size Distribution

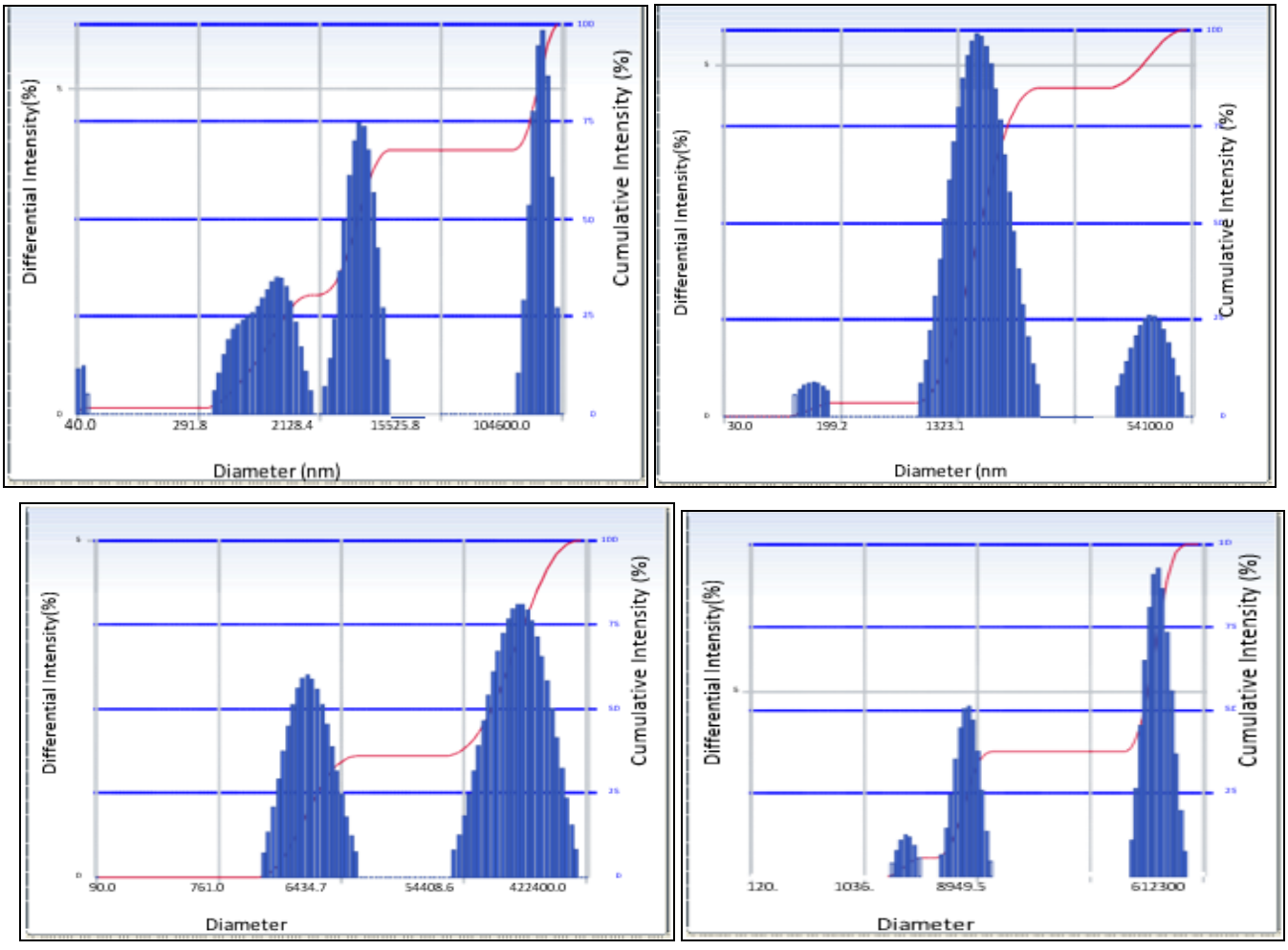


Fig 19: Particle Size Distribution Analysis of Silver Nanoparticle Prepared from Methanolic Extract of *Boerhavia diffusa*, A) Sample BD1, B) Sample BD2, C) BD3 and D) BD4 respectively.

Conclusion

The plant extract preparation involved drying samples, powdering them, and extracting with methanol/ethanol using a Soxhlet extractor for 48 hours. After filtration and solvent evaporation, the crude extract yields were calculated. The yields were 18.12% for *Punica grantum*, 9.03% for *Boerhavia diffusa*, 1.23% for *Ocimum sanctum*, and 3.95% for *Moringa oleifera*. Phytochemical analysis revealed the presence of tannins, glycosides, flavonoids, and steroids in various extracts. Thin-layer chromatography identified specific phytoconstituents, and UV-spectrophotometry and SEM characterized the synthesized silver nanoparticles. This comprehensive extraction and analysis confirm the phytochemical richness and potential applications of these plant extracts.

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