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Biosynthesis of Silver Nanoparticles using *Moringa Oleifera* Leaf Extract and their Application

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Abstract

In the present study synthesis of silver nitrate nanoparticles, with the *Moringa olifera* leaf extract, green synthesis it's an interesting and expanding research due to the potential application as eco-friendly development and novel technologies. To silver nitrate nanoparticles was characterized and its size, structure of nanoparticles was confirmed with XRD technique. The synthesized silver nanoparticle its particle size, is calculated as 111nm. This Scanning Electron Microscopic (SEM) study, revealed spherical shape nanoparticle, showing the spherical between each nanoparticle. The anti-microbial activity of silver nanoparticle of *moringa olifera* revealed inhibition against pathogens, *Staphylococcus aureus*.

Keywords: Nanoparticles, XRD, SEM, *Moringa olifera* and *Staphylococcus aureus.*

Introduction

Nanotechnology is the faster growing area of manufacturing in the world today and there is an increasingly frantic search for new nanomaterials and methods to make them. It has been well known that living cells are the best examples of machines that operate at the nanolevel and perform a number of jobs ranging from generation of energy to extraction of targeted materials at very high efficiency (Goodsell, *et al*., 2004).

One of the fields in which nanotechnology finds extensive applications is nanomedicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. Nanotechnology can improve our understanding of living cells and of molecular level interactions. A number of nanoparticles based therapeutics has been approved clinically for infections, vaccines, and renal diseases (Kulkarani and Muttapur, 2014).

Nano silver one of the highly commercialized nanomaterials produced about 320 tons for year. Nanomaterials have a long list of applicability in improving human life and its environment. The first relation between human life and nano scale was developed naturally in ayurveda, which is a 5000 year old Indian system of medicine. The wide applications have attracted the attention of scientists to produce them by different methods. The chemical methods are tedious, more time consuming and expensive (Gottschalk, *et al.*, 2010).

Silver nanoparticles are toxic to bacteria and can destroy antibiotic resistant bacteria such as methicillin resistant *Staphylococcus aureus*. In fact bacteria are not able to develop resistance against silver like they do with antibiotics. Silver nanoparticles are better than silver based compounds and silver ions kill microbes effectively (Sondi, 2004).

Nano-biotechnology is the most active area of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution, and morphology (Singh, *et al.*, 2010). Nanoparticle bound drugs have an extended half –life in vivo, longer circulation times and can convey a high concentration of a potent drug to where it is needed (Sahoo *et al*., 2007). The size of the drug nanoparticles and its surface characteristics can be modified to achieve the desired delivery characteristics (Mohanraj and Chen, 2007).

Plant extract may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. The source of the plant extract is known to influence the characteristics of the nanoparticles (Kumar and Yadav, 2009). This is because different extracts contain different concentrations and combinations of organic reducing agents (Mukunthan, 2012).

Biological methods of synthesis have paved way for the “greener synthesis” of nanoparticles and these have proven to be better methods due to slower, kinetics, they offer better manipulation and control over crystal growth and their stabilization (Dubeya, *et al*., 2010).

The advantages of using plant material for synthesis of nanoparticles is that it is easily available, safe, to handle and possesses a broad variety of metabolites that may aid in reduction. Plant mediated synthesis of nanoparticles is formed due to the presence of biomolecules such as protein, amino acids, vitamins, polysaccharides, polyphenols, Terpenoids and organic acids such as citrates etc present in the plants as their phytochemicals (Hutchinson, *et al*., 2008).

The presence of antioxidants such as phenolic, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases; for example, ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Ganatra, *et al*., 2012). Medicinal plants are therefore being investigated for their antioxidant properties, and the demand for natural antioxidants and food preservatives is increasing (Pesches, *et al*., 2006).

In the present study, much focus of attention was given to elucidate the role of medicinally important leaves of *Moringa oleifera*, to be synthesized with AgNPs. The preliminary study was carried out to asses Active principles of *Moringa oleifera* by phytochemical analysis. Green synthesis of *Moringa oleifera* with nanoparticles synthesized and to study the X-ray diffraction analysis to predict crystal structures and scanning electron microscopic studies pertaining to identify size, shape and structure of nanoparticles. The study extent to evaluate the functional groups present in leaves of *Moringa oleifera* by FTIR. The further extended to assess the role of synthesized nanoparticles to be used on Antioxidants. In order to estimate presence of phenol content, total phenol estimates was performed. The study was intended to evaluate the leaf extract of *Moringa oleifera* as antimicrobial agent, which can be safety used to indicate the pathogens.

Material and Methods

Collection of Samples

The *Moringa oleifera* leaves collected from venture farm house, Madurai. The plant material was air dried for over a night. Indian medicinal plant *Moringa olifera* was selected on the basis of cost effectiveness. Ease of availability and medicinal property. Fresh and healthy leaves were collected locally and rinsed thoroughly first with distilled water, to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature . About 50 g of leaves weighed separately and transferred into beakers and 100ml distilled water dissolved in filtered by filter by filter paper and get to clear solutions was extract collected (Plate:1).

Extraction of *Moringa olifera* leaves by Soxhlet Apparatus

The chemical extraction was done by following the method of Sharma, *et al.*, 2008. 50g of *Moringa olifera* leaf was extract in soxhlet apparatus using methanol (100ml) as solvent for 8 hours at 65^0c. Soxhalation is a process of continuous extraction in which the same solvent can

be circulated through the extracted several times. The process involves extraction followed by evaporation of the solvent. The vapors of the solvent were taken in a condenser and the condensed liquid will be returned to the same for continuous extraction.

Soxhlet consists of a body of extractor attached with a side and siphon tube. The extractor from the lower side can be attached to distillation flask and the mouth of extractor is fixed to a condenser by standard joints. The aloe veraleaf extract as thimble is placed in the soxhlet apparatus.

Phytochemical Analysis

Confirm the presence of active principles (or) secondary metabolites, such ascarbohydrate, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, terpenoids, phenol, proteins are isolate from colour appearance of solution. Performed with different tests.

1.Carbohydrate test

2 ml of *moringa oleifera* leaf methanolic extract was added to the test tube. 1 ml of molich's reagent was added and treated with few drops of conc H_2SO_4.Appearance of purple colour indicated positive results.

2. Tannins test

1ml of leaf methanolic extract was added to the test tube. 2 ml of 5% ferric chloride was added. Appearance of greenish black indicated positive results.

3. Saponins test

2 ml of leaf methanolic extract was added to the test tube. Add 2 ml of distilled water, shaken in a graduated cylinder for 15 minutes. Formation of foam.

4. Flavonoids test

1 ml of leaf extract was added to the test tubes and add 5 ml of diluted ammonia solution and conc H_2SO_4. Appearance of yellow colour indicated positive results.

5. Alkaloids test

2 ml of leaf extract was added to the test tubes. Add 2 ml of conc HCL and a few drops of mayer's reagent. Appearance of green colour indicated positive results.

6. Quinones test

1 ml of leaf extract was added to the test tubes. Add 1 ml of conc H_2SO_4. Appearance of red colour indicated positive results.

7. Glycosides test

2 ml of leaf extract was added to the test tubes. Add 3 ml of chloroform and 10% ammonia solution. Appearanceof pink colour indicated positive results.

8. Terpenoids test

0.5 ml of leaf extract was added to the test tubes. Add 2 ml of chloroform and conc H_2SO_4. appearance of red brown colour indicated positive results.

9. Phenol test

1 ml of leaf methanolic extract was added to the test tubes. Add 2 ml of distilled water and few drops of 10% ferric chloride. Formation of green colour indicated positive results.

10. Proteins test

1 or 2 ml leaf methanolic extract was added to the test tube. Add 10% sodium hydroxide solution. Formation of purple colour indicated positive results.

Green Synthesis of Nanoparticles

Synthesis of silver nanoparticles was carried out in 250 ml Erlenmeyar flask containing 90 ml of 1 mM silver nitrate ($AgNO_{3)}$ and 10 ml of leaf extract. The solution was kept at dark room at 37ºC with continuous agitation at 100 rpm for 24-48 hrs for the reduction of Ag+ions. The colour change of the solution dark green to dark brown indicates the synthesis of silver nanoparticles (AgNPs).

Characterization of Silver Nanoparticles

UV Visible Absorbance Spectroscopy

Uv visible spectroscopy analysis was carried out on a systronic UV Visible absorbance spectrophotometer 117 with a resolution of ±1nm between 200 and 1000nm processing a scanning speed of 200nm/min. Equal amounts of the suspension (0.5mL) were taken and analyzed at room temperature. The progress of the reaction between metal ions and the leaf

extract was monitored by UV-visible spectra of silver nanoparticles in aqueous solution with different wavelength in nanometers from 340 to 800 nm. The reduction of silver ions and formation of silver nanoparticles occurred within an hour of reaction. Control was maintained by using $AgNO_3$.

X-Ray Diffraction (XRD) Analysis

The silver nanoparticles solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by red dispersion of the pellet of silver nanoparticles into 10 ml of deionized water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD and SEM.

Scanning Electron Microscopic (SEM) Analysis of Silver Nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grit by just dropping a very Small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Fourier Transform Infra Red Spectroscopy (FTIR)

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 30 ml after reaction was centrifuged at 10000 rpm for 10 min and the resulting suspension was redisposed in 2 ml sterile distilled water. The centrifuging and redisposing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried power. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

EDX Analysis

In order to carry out EDX analysis, the bark extracts reduced silver nanoparticles were dried and drop coated on to carbon film and performed on Hitachi S-3400 N SEM instrument equipped with a Thermo EDX attachments.

Determination of Antimicrobial Activity of Nanoparticle Compounds Treated Against Bacterial Pathogens Using Well Diffusion Method

Antimicrobial activity of the extract of compounds was determined using well diffusion method. It was performed by sterilizing Mueller Hinton agar media. After solidification, wells were cut on the Mueller Hinton agar using cork borer. The test bacterial pathogens like *E.coli, Shigella flexneri, Staphylococcus aureus,* and *Klebsiella pneumonia* were swabbed onto the surface of Mueller Hinton agar plates. The silver nanoparticles (AgNPs) synthesized from *moringaoleifera* leaf extract were transfer into wells were impregnated with 25 µl of the test samples. The plates were incubated for 30 min to allow the extract to diffuse into the medium. The plates were incubated at 30^{o}C for 24 hours, and then the diameters of the zone of inhibition were measured in millimeters. Each antibacterial assay was performed in triplicate and mean values were reported.

Determination of Free Radical Scavenging Activity by Plant Leaf Extract *Moringa oleifera*

The ability of the AgNPs solution to scavenge hydrogen peroxide was determined by the method.A solution of hydrogen peroxide (H_2O_2) (40µm) was prepared in phosphate buffer (P^H 7.4). 4 ml of the AgNP solution at different concentrations (250µg/ml, 500µg/ml, 750µg/ml, and 1000µg/ml) was added to 0.6 ml of previously prepared H_2O_2solution. The absorbance of the solution was measured at 450nm after 10 mins against a blank containing phosphate buffer without H_2O_2 using Uv-vis spectrophotometer.

Determination of Total Phenol Content from Leaves of *Moringa oleifera*

The methanolic leaves extract was subjected to preliminary phytochemical screening as the reported methods. Folin-ciocalteu reagent was used to estimate total phenolic content.

Result

Green Synthesis of Silver Nanoparticles

In the present investigation synthesis of $AgNO_3$ using plant extract. Green synthesis of nanoparticles was carried out. The results were showed in Plate:2. The biosynthesis of nanoparticles initially detected by colour as green and challenged to honey brown. Nanotechnology is a most promising field to generate new applications in several fields including biotechnology and nanomedicine and the nanomaterials could be used widely due to

their. Nanoparticles enhanced reactivity, strength and electrical characteristics. Recently, biosynthesis of nanoparticles received considerable attention due to its unique physicochemical characteristics, electronic, magnetic, optical, catalytic properties as well as antibacterial, antioxidant and antiproliferative properties. Plant mediated synthesis of metal nanoparticles are cost-effective, environmental amicable, safe to handle and offers broad range of biomolecules which was kenned to mediate synthesis of nanoparticles.

Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals and provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganism isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms. The results were depicted in Plate: 3 and 4.

Nanobiotechnology is the most active area of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology.

Phytochemical Analysis of Nanoparticles from *Moringa olifera* Leaf Extract

The phytochemical analysis of Moringa leaf extract and nanoparticles synthesized compound extract was studied. The results revealed presence of carbohydrate, tannis, flavanoids, sapponins, alkaloids and phenols. The green synthesis nanoparticles absence of Quinones, Glycosides, terpenoids and Proteins. It is justified that phenolic compounds present in *Moringa* leaf extract inhibit glucose transports there by inhibit sodium glucose co-transporter. Our finding was supported by Heim *et al.*, 2002. (Table:-1, Plate:-5).

Table-1 Phytochemical Constituents Obtained From Methanolic Extract of *Moringa Oleifera* Leaves

S. No	Test	Result
1	Carbohydrate	Positive
2	Tannins	Positive
3	Saponins	Positive
4	Flavonoids	Positive
5	Alkaloids	Positive
6	Quinones	Negative
7	Glycosids	Negative
8	Terpenoids	Negative
9	Phenol	Positive
10	Proteins	Negative

Uv-vis spectral analysis

The highest peak that is observed at 412 nm (Figure:1) corresponds to surface Plasmon re29sonance (SPR) and indicates formation of AgNPs. AgNPs have free electrons, which give SPR absorption band, due to the combined vibration of electrons of AgNPs in resonance with light wave (Bankar, *et al*., 2010). In the present study, a single SPR band is exhibited, which shows no agglomeration of AgNPs.

From Figure:1, it is noted that absorbance of AgNPs increases with time as increasing the incubation time up to 24h. Above 24 h of reaction time; there is no significant change in the absorbance, which indicates the maximum attainability in the stability of the AgNPs. There is no obvious change in colour intensity, spectral peak position and absorbance of AgNPs. The results were noted in Table:2.

Figure:1. Uv Treated with *Moringa olifera* Leaves Treated With Different Aspect of Studies

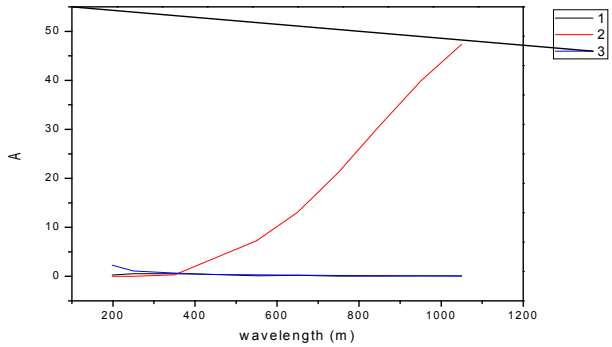


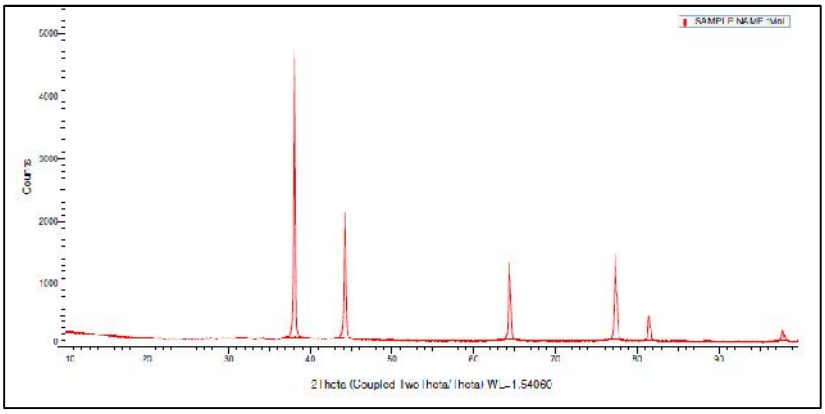
Table:2. 1- leaves extract 2-3ml of leaves extract & 4ml of silver nitrate solution 3-5ml of leaves extract & 4ml of silver nitrate solution

Λ	**Plant extract (without silver nitrate)(MOL)**	**Silver nitrate ($AgNO_3$)**	**Green synthesis (AgNPs)**
198	0.2704	0.0117	2.262
250	0.5273	0.0087	1.0863
350	0.5467	0.3184	0.6727
450	0.3841	3.7966	0.3792
550	0.0964	7.2571	0.3033
650	0.2075	13.080	0.2351
750	0.0604	21.247	0.1721
850	0.0625	30.752	0.1291
950	0.0692	39.833	0.1011
1050	0.0723	47.379	0.0809

XRD Analysis

Miller indices (hkl) are necessary to be assigned for each peak to index. The distict diffraction peaks at 37.78, 44.04, 64.4 and 77.46˚ and 82.11 corresponds to 111, 200, 220, 311 and 477 facets of the face centered cubic (FCC) crystal structure, respectively (Fig-2). A typical XRD pattern of the synthesized AgNPs using aqueous extract of *moringa oleifera* leaves is found to possess a FCC structure, which go very well with the values manipulated for FCC structure of silver nano-crystals (Joint committee on powder diffraction Standards: Brankar-ECODB Advanced. The XRD-V alue exhibit 2 theta (couple two theta/Theta) WL=1.54˚060.D=0.9λ/β cosϴ. Where, "D" is particle diameter size, λ is wave length of X-ray (0.1541 nm), β is FWHM, and Ɵ is the diffraction angle.The value of d (the interplanar spacing between the atoms) is calculated using Bragg's Law.d=λ/2xsinϴ; λ=0.1541 nm for CuKα. The results were shoed in Figure:2.

Figure: 2.XRD analysis for Green synthesis of silver nanoparticles



SEM Analysis of Silver Nanoparticles

The silver nanoparticles synthesized by the help of moringa oleifera leaves extract were scanned by SEM asshown in figure (3). It reveals that silver nanoparticles seen to be spherical morphology and particles form cluster. It is easy to notice that the examined particles consist of a number of smaller objects of a few micrometers in size. However, we did not manage to examine the structure of the observed nanoparticles because of difficulties connected with getting higher magnification with X5000, X 10000, X20000 and X30000 of mm at 20 kr showed SEM image of Green synthesis with silver nanoparticles. Figures (a) and (b) and (c) and (d) show the images of the silver nanoparticles in *moringa oleifera* leaves with silver nitrate. Uniformly distributed silver nanoparticles on the surface of the cells are observed.

Figure.3 SEM analysis of silver nanoparticles

Fig-a

SEM Analysis images observed at 1,000 magnification

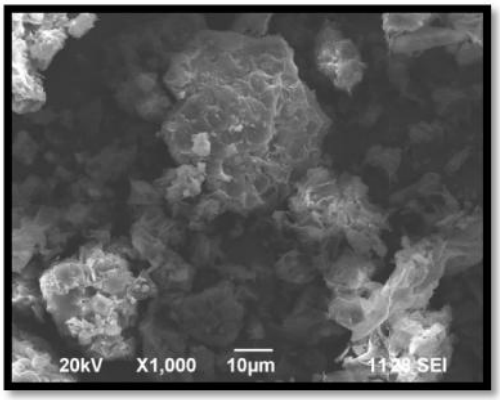


Fig-b

SEM Analysis image observed at 2000 magnification

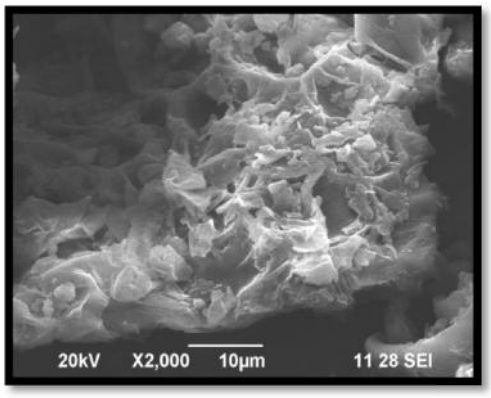


Fig-c

SEM Analysis images observed at 5,000 magnification

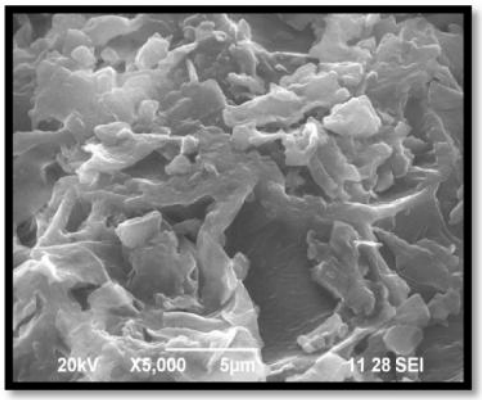
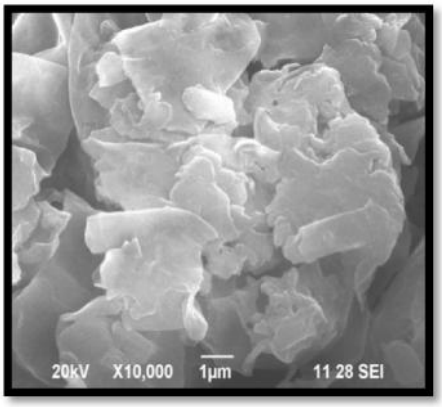


Fig-d

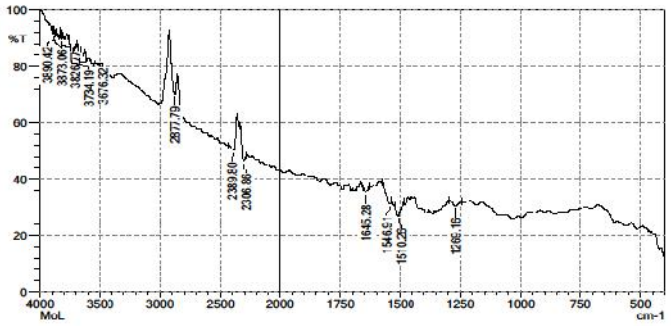
SEM Analysis images observed at at 10,000 magnification



FTIR Spectral Analysis

In the present study FTIR spectrum of biosynthesized AgNPs (Figure-4) shows the presence of different peaks. The peak at 3890.42 cm^{-1} in the FTIR spectrum indicates O-H groups in alcohols, phenols and N-H stretching vibrations of amides of protein. The peak at 2389.80 and 2877.79 cm^{-1} corresponds to aliphatic CH, CH_2, and CH_3 groups. The peak at 1645.28 cm^{-1} corresponds to C=O stretching and N-H bending in amides. The peak at 1510.26 cm^{-1} corresponds to C=C in ring system or double bond stretching in C=O and C=N. The sharp

peak at 1546.91 cm^{-1} corresponds to C-O stretching of aromatic amine groups or secondary amines. The peak at 1269.16 cm^{-1} corresponds to C-O stretching of alcohols, carboxylic acids, or C-N stretching of aliphatic amines. FTIR studies confirm that the carbonyl groups from the amino acid residues and proteins have the stronger ability to bind AgNPs to prevent agglomeration and there by stabilize the AgNPs through free amine groups in proteins.



	Peak	Inensity	Corr. Inensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	1269.16	20.59	1.57	1294.24	1242.16	3572.212	28.849	
2	1510.25	27.06	4.31	1525.69	1489.05	2509.786	88.458	
3	1546.91	21.15	2.73	1575.84	1529.20	2418.265	65.859	
4	1645.28	95.42	1.74	1654.92	1601.78	1476.231	53.757	
5	2106.86	76.40	5.97	2295.80	2081.79	2711.773	212.304	
6	2389.50	50.35	7.84	2400.31	2362.80	3176.535	284.640	
7	2877.79	70.20	11.40	2924.00	2854.65	1686.464	486.841	
8	3676.32	84.00	2.80	3691.76	3666.68	960.046	95.777	
9	3734.19	80.27	3.68	3761.10	3724.54	510.607	74.080	
10	3824.92	99.00	2.41	3832.56	3817.13	140.423	19.912	
11	3873.06	90.61	1.02	3882.71	3860.20	100.846	10.050	
12	3890.42	91.50	1.50	3898.14	3882.71	120.793	12.641	

EDX Analysis

The standard EDX spectrum recorded on the examined sample is shown. In the middle part of the presented spectrum a strong peak located at 1 to 3 kv. This maximum is directly related to the silver characteristic line. The EDA spectrum of sample silver nanoparticles is presented. The main peak is due to silver; the peaks of copper are result of samples preparation on a copper grid similarly, calcium, potassium, magnesium, chloride, silican peaks were observed in the range of 1 to 4.5 kv. The results were noted in Figure:5.

Fig-5 EDX (Electron Dispersive X-RAY)

Fig:5.a:Electron Dispersive X-Ray (EDX)

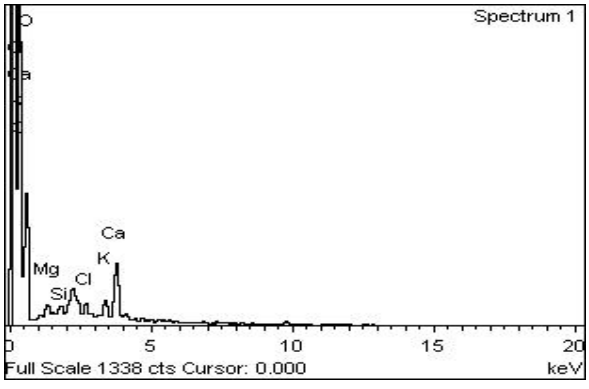
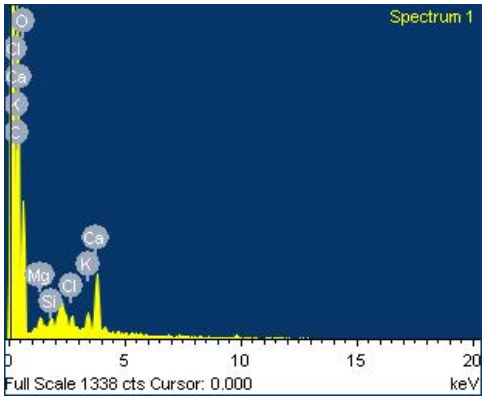


Fig:5.b:-Electron Dispersive X-Ray (EDX)



Antimicrobial Activity and Synergistic Effect of Nanoparticle Synthesized Compound

In order to check the efficacy of synthesized nanoparticles against selected pathogens such as *E.coli, Staphylococcus aureus, Klebsiella pneumonia* and *Shigella flexneri* was studied. Evaluation of antimicrobial activity revealed AgNps exhibit against standard(Amickacin-30µg) showed a zone of inhibition at 27mm whereas, nanoparticle synthesized extracts with antibiotic exhibited a synergistic effect with zone of inhibition mounted to 28 mm in diameter against *Staphylococcus aureus*, which is followed by *Shigella flexneri* exhibited a synergistic effect of 25mm in zone of inhibition, similarly *Klebsiella pneumonia* exhibited 22mm mode rent zone of inhibition and *E.coli* revealed27mm zone of inhibition of AgNPS.

Similarly antimicrobial activity was studied by Parashar, *et al*., 2009 in magnesium oxide nanoparticles against *E.coli* and *Stapahylococcus aureus* indicated a similar result. This study of green synthesis of nanoparticles showed significant activity compared to standard Amickacin. Study further showed a synergistic activity was tremendous by analyzing the zone of inhibition. The results were noted in Plate: 6 and Table:3.

Table:3 Determination of Antimicrobial Activity of Leaf Extract of *Moringa oleifera*

S. No	Pathogenic Micro organisms	Standard antibiotics(mm)	Zone of inhibition in diameter (*Moringaoleifera* leaf extract)mm
1	*E.coli*	**27**	17
2	*Shigella flexneri*	25	22
3	*Staphylococcus aureus*	28	20
4	*Klebsiella pneumonia*	22	20

Antioxidant Activity of Silver Nanoparticles

The antioxidant activity with green synthesis of silver nanoparticles. At 230nm, absorbance of different concentration such as 1 and 3µg/ml was studied. The reduction in absorbance of hydrogen peroxide at 230nm caused by the samples were measured after 10 min. experimental results are expressed as absorbance of % inhibition.

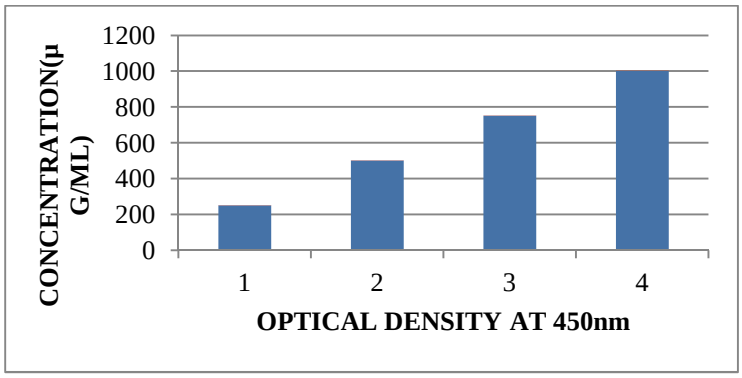
$$\% \text{ scavenged} = \text{absorbance of the control} \times \frac{100}{\text{Absorbance of sample}}$$

Hydrogen peroxide scavenging activity of AgNPs has showed maximum antioxidant activity observed at concentration at 1µg/ml (0.95%) in 3 µg/ml and maximum antioxidant activity observed 1.13%. The results were depicted in Table:4 and Figure:6.

Table:4 Determination of Free Radical Scavenging Activity by Plant Leaf Extract *Moringa oleifera*

S. No	Concentration (µg/ml)	Optical Density at 450 nm
1	250	1.61
2	500	1.59
3	750	1.57
4	1000	1.20

Fig:6. Determination of Free Radical Scavenging Activity by Plant Leaf Extract *Moringa oleifera*



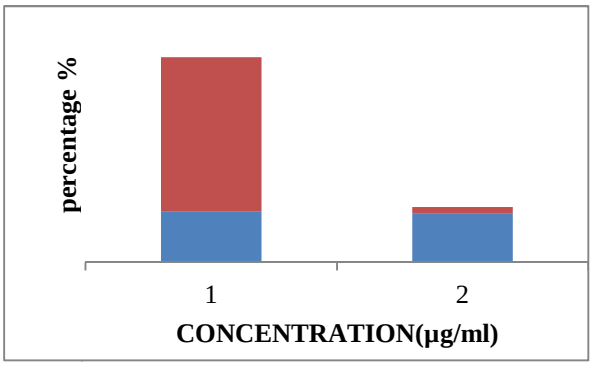
Estimation of Total Phenol Content

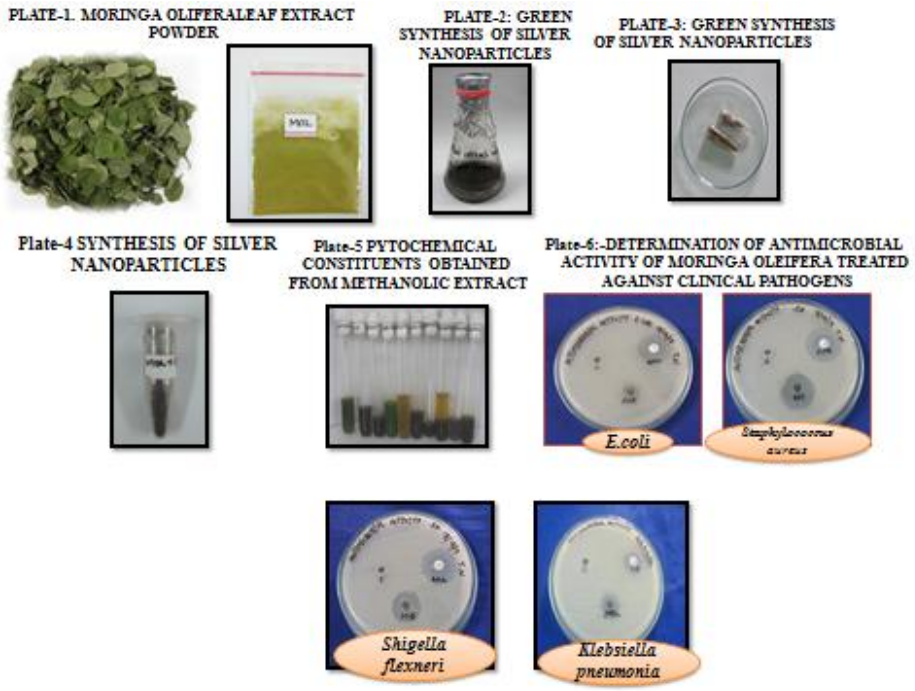
The total phenolic content was estimated by folin-ciocalteau reagent expressed in percentage at different concentration of 1 µg/ml and 3 µg/ml reveled 0.95% and 1.13 % present in the moringa oleifera leaves extract. The results were exhibited in Table:5 and Figure:7.

Table:5 Determination of Total Phenol Content from Leaves of *Moringa Oleifera*

S. No	Concentration (µg/ml)	Optical Density at 450nm
		Percentage
1	1 ml	0.95%
2	3 ml	1.13%

Fig- 4.





Discussion

The absorption spectra of metal nanoparticles were dominated by surface Plasmon resonance (SPR) particle size, dielectric medium and surface adsorbed species (Leone *et al*., 2015). According to Mie's theory, only a single SPR band is expected in the absorption spectra of spherical nanoparticles whereas, anisotropic particles could elevate to two or more SPR bands (Braca *et al*., 2002). The number of SPR peaks increases as the symmetry of nanoparticles decreases. Significant colour change of the solution before two hours indicated first reduction of $AgNO_3$ by the aqueous extract (Shankar *et al*., 2010).

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles (Shankar *etal*., 2004).

As the one leaf extract was mixed in the aqueous solution of the silver ions complex, it started to change the color from watery to yellowish brown due to reduction of silver ion; which indicated formation of silver nanoparticles. It is generally recognized that UV-Vis spectroscopy could be used to examine size-and shape- controlled nanoparticlees in aqueous suspensions (Garima, *et al.*, 2011).

The highest peak that is observed at 412 nm corresponds to surface Plasmon resonance (SPR) and indicates formation of AgNPs. AgNPs have free electrons, which give SPR absorption band, due to the combined vibration of electrons of AgNPs in resonance with light wave (Ghazwani, 2015). In the present study, a single SPR band is exhibited, which shows no agglomeration of AgNPs.

The absorbance of AgNPs increases with time as increasing the incubation time up to 24h. Above 24 h of reaction time; there is no significant change in the absorbance, which indicates the maximum attainability in the stability of the AgNPs. There is no obvious change in colour intensity, spectral peak position and absorbance of AgNPs. (Basangowda and Ashok, 2013).

XRD spectrum confirmed that, synthesized silver particles were in the form of nanocrystals as evidenced by peaks at 2Ɵ values of 34.69º, 38.40º, 44.39º and 64.62º corresponding to facets of the face centered cubic crystalline structure. The sharpening peaks clearly indicated obtained particles were in nano-regions (Dubeya, *et al*., 2010). (Montoro *et al*., 2005) described that, the more diminutive size particles perforate into the cell facilely. It is eminent to mention that the geometrically triangular nanoparticles are having very sharp vertexes and edges that would be more puissant to damage the target cells.

Scanning electron microscopy (SEM) of silver solutions, confirmed the existence of very small and uniform spherical nanoparticles. From the SEM images it can be observed that larger particles are formed due to aggregation of nanoparticles which might be induced by the evaporation of solvent during sample preparation. This could have contributed for the variation in particle size. Whereas, AgNPs from leaf extract of *Acalyphaindica* found to be more than 1000nm with spherical and cubic shapes. Most of the AgNPs are spherical in shape with moderate variation in particle sizes ranged from 8-90nm. (Sondi *etal*., 2010) stated that, silver nanoparticles obtained from C.tropicum were 20-50nm in size and the particles were poly crystalline and single orientation which formed a cluster of silver particles which was confirmed by TEM analysis (Vongsak, *et al.*, 2013).

The FTIR spectrum of aqueous extract of *moringa oleifera* leavesshows the presence of different peaks. The strong peak at 3434 cm^{-1} in the FTIR spectrum indicates N-H stretching vibration of amino groups and –OH stretching of hydroxyl group in phenols (Nakkala, *et al*., 2014). A peak observed at 2,922 cm^{-1} is due to C-H stretching of alkaline amide 1 band of proteins. The peak at 1639.5 cm^{-1} corresponds to amine groups of –N-H bending vibrations of proteins and characteristic of –C=O carbonyl groups belongs to the C-H stretching vibration of $–CH_3$ And $–CH_2$ groups. The peak at 1739 cm^{-1} corresponds to –C=O stretching of carbonyl group in ketones, aldehydes and carboxylic acid. The peak at 1384.5 cm^{-1} corresponds to bending vibrations of –OH or C-N stretching of aromatic amine (Okwari, *et al*., 2015).

FTIR studies confirm that the carbonyl groups from the amino acid residues and proteins have the stronger ability to bind AgNPs to prevent agglomeration and there by stabilize the AgNPs through free amine groups in proteins (Plante, *et al*., 2010).

In our, results coincides with Aromal and Philip, 2012 and silver nanoparticles from prosesglandules and their potential application as biocontrol of Acetobactercalcoacetius and *Bacillus cereus*.

The high surface area to volume ratio of AgNPs increases their contact with micro-organisms, promoting the dissolution of Ag^+ ions and hence improving biocide effectiveness. Formation of free radicals by the AgNPs when in contact with the bacteria, and free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death (Singh and Singhet, 2009). Silver is a soft acid, and there is a natural tendency of an acid to react with a base; in this case, a soft acid to react with a soft base. Another fact is that the deoxyribonucleic acid (DNA) has sulfur and phosphorus as its major components; AgNPs can act on these soft bases and destroy the DNA which would definitely lead to cell death (Mukherjee, *et al*., 2001).

Hydrogen peroxide radical is not very reactive and it is a weak oxidizing agent; biologically, it act as a toxicant to the cell by converting it self into hydroxyl radical in the presence of metal ions in living systems which results in initiation and propagation of lipid peroxidation (Song and Kim, 2009).

The phenolic compounds may contribute directly to anti-oxidative action (Anwar, *et al*., 2007). However, antioxidant activities are attributed to the phenolic contents in plants

probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Chen and Schluesener, 2008).

Silver Nano Particle Application

Silver nanoparticles are being used in numerous technologies and incorporated into a wide array of consumer products that take advantage of their desirable optical, conductive, and antibacterial properties.

- **Diagnostic Applications:** Silver nanoparticles are used in biosensors and numerous assays where the silver nanoparticle materials can be used as biological tags for quantitative detection.
- **Antibacterial Applications:** Silver nanoparticles are incorporated in apparel, footwear, paints, wound dressings, appliances, cosmetics, and plastics for their antibacterial properties.
- **Conductive Applications:** Silver nanoparticles are used in conductive inks and integrated into composites to enhance thermal and electrical conductivity.
- **Optical Applications:** Silver nanoparticles are used to efficiently harvest light and for enhanced optical spectroscopies including metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS).

Further Development

Silver Nanoparticles for Nanotoxicology Research

There is growing interest in understanding the relationship between the physical and chemical properties of nano-materials and their potential risk to the environment and human health. The availability of panels of nanoparticles where the size, shape, and surface of the nanoparticles are precisely controlled allows for the better correlation of nanoparticle properties to their toxicological effects. Sets of monodisperse, unaggregated, nanoparticles with precisely defined physical and chemical characteristics provide researchers with materials that can be used to understand how nanoparticles interact with biological systems and the environment.

Due to the increasing prevalence of silver nanoparticles in consumer products, there is a large international effort underway to verify silver nanoparticle safety and to understand the

mechanism of action for antimicrobial effects. Colloidal silver has been consumed for decades for its perceived health benefits but detailed studies on its effect on the environment have just begun. Initial studies have demonstrated that effects on cells and microbes are primarily due to a low level of silver ion release from the nanoparticle surface. The ion release rate is a function of the nanoparticle size (smaller particles have a faster release rate), the temperature (higher temperatures accelerate dissolution), and exposure to oxygen, sulfur, and light. In all studies to date, silver nanoparticle toxicity is much less than the equivalent mass loading of silver salts.

Silver Nanowires

Silver nanowires are an exciting class of silver nanoparticles which have been studied as possible components in many advanced technology applications. Applications include:

- **Conductive Coatings**: Silver nanowires can be used to provide conductive coatings for transparent conductors and flexible electronics.
- **Plasmonic Antennas**: Metallic nanoparticles attached to silver nanowires function as antennas enhancing plasmonic activity for sensing and imaging applications
- **Molecular Sensing**: Single layers of silver nanowires have been used to construct arrays for molecule specific sensing in conjunction with Raman Spectroscopy.
- **Nanocomposites**: Silver nanowires have been studied as components of nanocomposites and can show high dielectric constants in such systems.

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