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DEVELOPMENT AND CHARACTERIZATION OF HERBAL NANOPARTICLES

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Abstract: The objectives of the present study are biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical applications. This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route. So, in the search of cheaper pathways for nanoparticles synthesis, scientist used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles. Green synthesis provides advancement over chemical and physical method as it is cost.

Keywords: Nanoparticles, phytochemicals,

Introduction*:* Nanotechnology is the modern research field which deals with design, synthesis and manipulation of particles structure range from 1-100nm in dimension. A nanoparticle is ultra- fine unit with dimension less than 100 nm (1 nm=10-9 metre). Because of their small size they have unique characteristics and their applications in various field such as medicines, engineering and environmental remediation. International Organization of Standards defined nanoparticle as the discrete nano object with dimensions are less than 100nm.The ISO also defined two dimensional nano objects as nano discs & nano plates, and one dimensional nano objects as nano fibres & nano tubes. The applications of nanotechnology are suitable for biological molecules due to their exclusive properties. They undergo highly controlled assembly for making them suitable for the metal nanoparticle syntheses which are found to be reliable and eco-friendly. The synthesis of metal nanoparticles is a vast area of research because of its potential applications which are implemented in various development of novel technologies. The field of nanotechnology is the upcoming areas of research in field of material science. The applications of nanoparticles and nano materials are emerging rapidly in different fields. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms due to their unique properties such as catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties [5-8,] which are gaining the interest of scientist for production of nanoparticles. The last few years, the majority of research in material science is the synthesis of nanoparticles. Nano-crystalline silver particles have been found in various applications in the fields of molecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics. However, there is still need environmentally clean route of synthesis for preparing the silver nanoparticles. Silver possesses inhibitory effects against so many strains of bacteria and microorganisms which are commonly present in medical and industrial processes. Silver and silver nanoparticles are used in skin ointments and creams contain silver to prevent infection of burns and open wounds, medical

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devices and implants prepared with silver-impregnated Polymers [11]. In textile industry, silver-embedded fabrics are used in sporting equipment [12].

Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles.

Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non- ecofriendly byproducts. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for green nanotechnology".

Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants [14, 15].

Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganism's isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms .

Sometimes the synthesis of nanoparticles using various plants and their extracts can be advantageous over other biological synthesis processes which involve the very complex procedures of maintaining microbial cultures [16, 17]. Many such experiments have already been started such as the synthesis of various metal nanoparticles using fungi like *Fusarium oxysporum*

But, synthesis of nanoparticles using plant extracts is the most adopted method of green,eco - friendly production of nanoparticles and also has a special advantage that the plants arewidely distributed, easily available, much safer to handle and act as a source of several metabolites [22]. There has also been several experiments performed on the synthesis of silver nanoparticles using medicinal plants such as *Oryza sativa, Helianthus annus, Saccharum officinarum, Sorghum bicolour, Zea mays, Basella alba, Aloe vera Capsicum annuum, Magnolia kobus, Medicago sativa* (Alfalfa), *Cinamomum camphora* and *Geranium* sp. in the field of pharmaceutical applications and biological industries. Besides, green synthesis of silver nanoparticles using a methanolic extract of Eucalyptus hybrida was also investigated [23].

Methodology

Materials: Fresh leaves of *Azadirachta indica* were collected from local area of Muzaffarnagar, and washed several times with water to remove the dust particles and then air dried at room temperature to remove the residual moisture and ground to form powder. Then plant extract was prepared by mixing extract (1%) with deionized water in a 250ml conical flask. The above solution was incubated at about for 30 minutes. Then the solution was subjected to centrifuge for 30 minutes at 5000 rpm. The obtained supernatant was separated by filtered with the help of vacuum filter. Further the solution was used for the reduction of silver ions Ag+) to silver nanoparticles (Ago).

Fig. 4: Picture of *Azadirachta indica* leaves

Dried leaves of *Momordica charantia* were ground to form powder. The extract of *Momordica charanti*ana was prepared by using 1% of plant extract mixed with deionized water in a 250ml conical flask. Then the solution was incubated for 30 minutes and then subjected to centrifuge for 30 minutes in room temperature at 5000 rpm. The supernatant was separated, filtered with filter paper with the help of vacuum filter. Then the solution was used for the reduction of silver ions (Ag+) to silver nanoparticles (Ago).

Fig. 5: Picture of *Momordica charantia* leaves

Synthesis of Silver Nanoparticles

UV-Vis Analysis: The optical property of Silver nanoparticles was determined by UV-Visible spectrophotometer model Perkin- Elmer, Lambda 35, Germany. After the addition of $AgNO_3$ to the plant extract, the spectra were taken in different time intervals up to 24 hours between 350 nm to 500 nm. Then the spectra were taken after 24 hours by addition of $AgNO_3$.

FTIR analysis: The chemical composition and functional groups of the synthesized silver nanoparticles were studied by using FTIR spectrometer (Perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75o C and the dried powders were characterized in the absorbance range 4000– 400 cm-1.The sample was analyzed by using KBr pellet.

XRD Analysis: The phase variety and grain size of synthesized silver nanoparticles were determined by X-ray diffraction spectroscopy (Philips PAN analytical). The synthesized silver nanoparticles were studies with CUKα radiation at voltage of 30 kV and current of 20 MA with scan rate of 0.030/s. Different phases present in the synthesized samples were determined by X′ pert high score software with search and match facility. The particle sizes of the prepared samples were determined by using Scherer's equation as $0.9\boldsymbol{\lambda} \approx \beta \cos\theta$

Where D is the crystal size, λ is the wavelength of X-ray, ϴ is the Braggs angle in radians and B is the full width at half maximum of the peak in radians.

SEM Analysis: The morphology of synthesized silver nanoparticles from neem plant extract were studied by Scanning Electron Microscope (SEM) (JSM-6480 LV). On addition of AgNO3 after 24 hours the SEM slides were prepared by using a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an acceleration voltage of 20 KV.

DLS & Zeta-Potential Analysis: Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques were employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in deionized water followed by ultra- sonication. Then solution was filtered and centrifuged for 15 minutes at 25^oC with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer (ZETA Sizer Nanoseries, Malvern instrument Nano Zs).

Assessment of Antimicrobial Activity: Disc Diffusion method is used to assess antibacterial activity. In this method antibacterial assays were studied by using human pathogenic bacteria such as Escherichia coli and Staphyloccocus aureous, Mackonkey broth (HiMedia) medium was used to sub culture of bacteria and were incubated at temperature 370C for 24 hours. Fresh overnight cultures were taken and spread on the Mackonkey agar plates for cultivating bacteria. Previously sterilized paper discs of 5 mm diameter saturated with plant extract, silver nanoparticle and double distilled water as control were placed on each plate and were incubated again at temperature 37^oC for 24 hours and the antibacterial activity was measured based on the zone of inhibition around the disc impregnated with silver nanoparticle and plant extracts with time.

Results and Discussion: On the behalf of UV-vis data it was cleared that Azadiratcha indica reduced metal ions better than *Momordica charantia*, so further characterizations were carried out with Azadiratcha indica. (Fig 1-9).

Sem Analysis: SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results shown that the diameters of prepared nanoparticles in the solution have sizes of several µm in case of 30:1, 120:1 & 240:1 ratios where as in 60:1 ratio the size is of several nm. The size of the prepared nanoparticles was more than the size of nanoparticle which should be between 1-100 nm. The size was more than the desired size as a result of the proteins which bound in the surface of the nanoparticles. The result showed that the particles were of spherical shape in case of 30:1, 60:1, and 120:1 ratios but sheet shape in case of 240:1 ratio. The shape varies due to the concentration increased in ratio of 240:1.

DLS Analysis: The particle size distribution (PSD) of synthesized silver nanoparticles of different ratios like 30:1, 60:1, 120:1, and 240:1 are shown in the figures. According to the figure: 15 the colloidal solution of silver nanoparticles of ratio 30:1 contains particles of different sizes some were with average sizes ranging from 5 nm to 180 nm. But in case of 60:1, the solution contains particles of uniform sizes ranging from 68 nm to 396 nm. The average size of nanoparticles is 160 nm. The particle size in case of 120:1 ratio ranges from 78 nm to 255 nm with mean particle size of 169 nm. Similarly the sizes of nanoparticles in case of 240:1 ratio range from 91 nm to 220 nm with average size of 164 nm. If we compare the above four results we can conclude that the ratios like 60:1, 120:1, 240:1 give uniform distribution of particles but 30:1 ratio does not obey this principle. Among them 60:1 ratio is very appropriate since it gives lowest average size of nanoparticles.

Zeta Potential Analysis: The Zeta potential measurements of silver nanoparticles synthesized with different ratios like 30:1, 60:1, 120:1, and 240:1 are 15.5 mV, 1.92 mV, 6. 12 mV and 2.45 mV respectively. From the zeta potential analysis the order of stability of nanoparticles synthesized from different ratios is 30:1> 120:1 > 240:1 > 60:1. Nanoparticles are very small in size for which they are energetically very unstable. Therefore the particles undergo agglomeration/aggregation to stabilize themselves. So there were some potential charges on the surface of the nanoparticles which makes them stable. We got the potentials charges from this analysis.

Zeta potential (Surface potential) has direct relation with the stability of a form/structure as mentioned below (Table: 1)

Table: 1: A table showing the stability of the NPs according to the potential charge

Zeta potential[mV]	Stability behavior of the colloid
From 0to±5	Rapid coagulation or flocculation
From ±10 to ±30	Incipient in stability
From ±30 to ±40	Moderatestability
From ±40 to ±60	Good stability
Morethan ±61	Excellentstability

FTIR Analysis:FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles (Figure: 23 & Figure: 24) in case both of 60:1 and 120:1 ratios showed the band between 3490-3500 cm-1 corresponds to O-H stretching H- bonded alcohols and phenols. stretch for C-H bond, peak around 1450-1500 cm-1 showed the bond stretch for N- H. Whereas the stretch for Ag- NPs were found around 500-550 cm-1. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e.; capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium.

XRD ANALYSIS: XRD spectrum showed distinct diffraction peaks around 38O, which are indexed by the (100) of the cubic face-centered silver. These sharp Bragg peaks might have resulted due to capping agent stabilizing the nanoparticle. Intense Bragg reflections suggest that strong X-ray scattering centres in the crystalline phase and could be due to capping agents. Independent crystallization of the capping agents was ruled out due to the process of centrifugation and redispersion of the pellet in millipore water after nanoparticles formation as a part of purification process. Therefore, XRD results also suggested that the crystallization of the bio - organic phase occurs on the surface of the silver nanoparticles or vice versa. Generally, the broadening of peaks in the XRD patterns of solids is attributed to particle size effects. Broader peaks signify smaller particle size and reflect the effects due to experimental conditions on the nucleation and growth of the crystal nuclei.

Antimicrobial Activity: Silver nanoparticles, due to their antimicrobial properties have been used most widely in the health industry, medicine, textile coatings, food storage, dye reduction, wound dressing, antiseptic creams and a number of environmental application 130. Since ancient times, elemental silver and its compounds have been used as antimicrobial agents; and was used to preserve water in form of silver coins/silver vessels 131,132. We have examined A.indica extract mediated silver nanoparticles as possible antibacterial agents. The plant extract and those mediated silver nanoparticles were immediately tested for respective antimicrobial activities towards both gram positive (S. aureus) and gram negative (E. coli) bacterial strains showing the zones of inhibition. Based on the zone of inhibition produced, synthesized silver nanoparticles prove to exhibit good antibacterial activity against E. coli and S. aureus. On the other hand, control and plant extract alone did not exhibit any antibacterial activity. Although, it is to be presumed that the leaves extract of the plant used possess the antibacterial activities and must be reflected through greater inhibition zone but it alone shows very low activity due to its medium of extraction as well as lower concentration during experimentation. The results of antibacterial activities of prepared silver nanoparticles evaluated from the disc diffusion method are given in Table.

Table:2 Zone of inhibition (mm) obtained by disc diffusion method

Components		Zone of Inhibition
E.coli S.aureus		
Control	NZ	NZ
PlantExtract	NZ	NZ
Silvernanoparticle	9	9

Summary & Coclusion: The rapid biological synthesis of silver nanoparticles using *Azadirachta indica* leaves extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The synthesized nanoparticles were of spherical and sheet shaped and the estimated sizes were 160-180 nm. The size were bigger as the nanoparticles were surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-vis spectrophotometer, SEM, DLS, Zeta Analyzer, XRD, and FTIR techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The higher concentrated nanoparticles had sheet shaped appearance whereas the lower concentrations showed spherical shaped. The sizes of the nanoparticles in different concentration were also different which depend on the reduction of metal ions. From the data of DLS it was found that the 30:1 ratio solution had sharp nanoparticles of around 5 nm and some has around 180 nm and the had the potential of around 15.5 mV. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

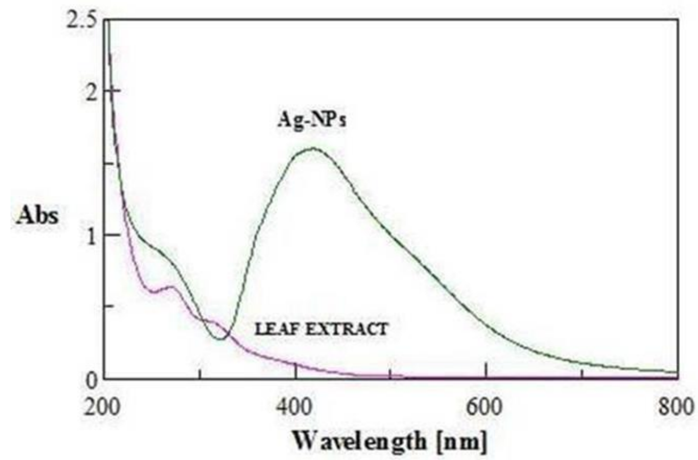

Figure 1. : UV-vis spectra of silvernanoparticle and Momodica charantia leaf extract

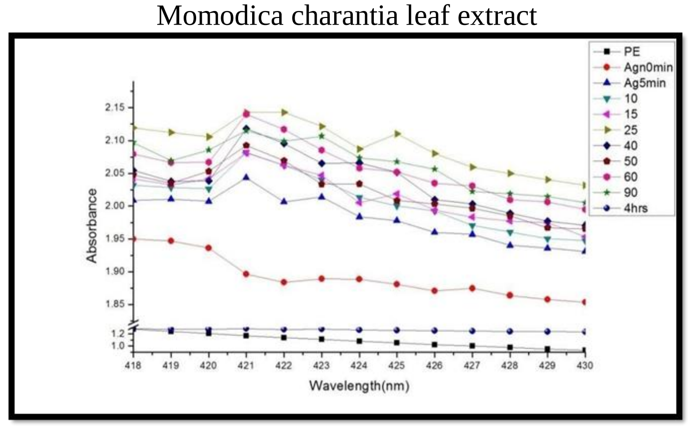

Fig.2: UV-vis spectra of *Azadirachta indica* 30:1 ratio at different time interval

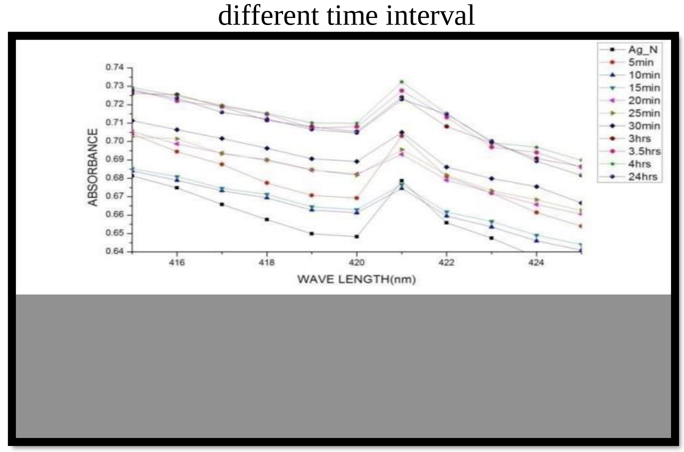

Fig.3: UV-vis spectra of *Azadirachta indica* 60:1 ratio at different time interval

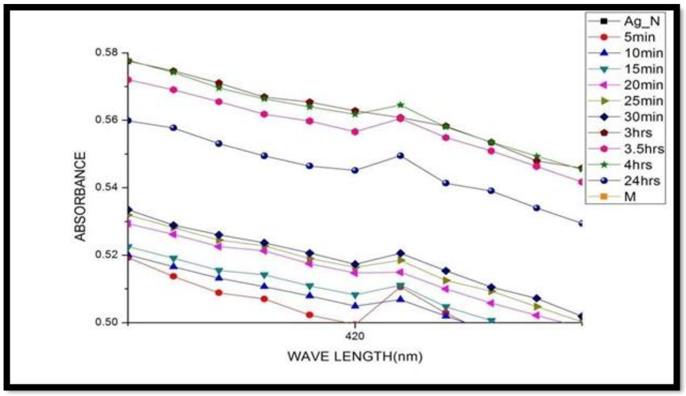

Fig.4: UV-vis spectra of *Azadirachta indica* 120:1 ratio at different time interval

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