

Green synthesis and characterization of metallic nanoparticles of Aconitum heterophyllum as antifungal agents

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Abstract

Nanotechnology has become one of the most auspicious technologies applied in all fields of science. Nanoparticles gain attention of researchers because they possess distinctive properties like their small size i.e. 1–100 nm large surface-to-volume ratio and high rate of reaction. Nanoparticles synthesis by physical, chemical and biological methods have their considerable applications in the biomedical and physiochemical areas. Green synthesis of metal nanoparticles are plant mediated and these are gaining more attention due to their easy, rapid and ecofriendly nature. Biomolecules present in the plant extract can be used to reduce metal ions to nanoparticles in a plant extract mediated process. The mono and Bi-Metallic nanoparticles were prepared from the plant extract of Aconitum heterophyllum. The synthesized Bi-Metallic nanoparticles were then characterized to their study. The gold, Silver and bimetallic nanoparticles showed that the effective antifungal achievement against *C. albicans*. The antifungal activity of the BMNPs was found to be improved in comparison to the AgNPs and AuNPs due to the additional properties of the silver nanoparticles present in the BMNPs.

Keywords: Aconitum extract,, silver nanoparticles, gold nanoparticles, TEM, bioactivities antifungal activity.

Introduction

Natural products and traditional medicines are of great importance. These products and their derivatives have been recognized for many years as a source of therapeutic agents and structural diversity with multidimensional chemical structures. The utility of natural products as biological function modifiers has also won considerable attention. From earlier decades medicinal plants/herbs existed on earth, having global and paramount importance. The world is decorated with medicinal herbs, which is a rich wealth of endurance. Every plant is identified by its own different therapeutic properties due to active bioactive molecule. In the modern system of medicine, natural drug substances are reported to be vital and have appreciable roles. Their therapeutic role was justified by the presence of their bioactive molecules. Due to disease- inhibiting capabilities, they are extremely useful as natural drugs, provide basic bioactive compounds that are less toxic and more effective, and bring biological and chemical means of modification and pave the way into development of potent drugs ^{1,2}

Ativisha (*Aconitum heterophyllum wall*) of family Ranunculaceae is an Ayurvedic herb which is known for its important medical properties. The root of the plant find use in one form or the other in various Ayurvedic preparations. Ativisha (*Aconitum heterophyllum*) is a popular Ayurvedic herb for treating diarrhoea, fever, and inflammation. Ativisha is a critically endangered species found exclusively in the subalpine Himalayas. The commercialization of Ativisha far exceeds the plant's natural supply, resulting in extensive substitution and adulteration. Atisine is generally regarded non-poisonous due to its significantly lower toxicity than aconitine and pseudaconitine. Despite the fact that the alkaloid atisine produces hypotension, a full aqueous extract of the root generated considerable hypertension, most likely as a result of an effect on the sympathetic nervous system. Aconitine acts on the central nervous system, cardiovascular system, and respiratory system due to the presence of benzyl ester and OH- groups in the molecular structure. Celastrus paniculatus polyesters include this system, and their esters operate similarly to aconitine. The nanoparticles of

plant extract are better than pure extract for pharmacological activity. Nanotechnology is defined as the creation of particles with a size range of 1.0 to 100 nanometers. Because of their huge surface area, certain medicinal nanoparticles are thought to have strong antifungal characteristics. Because of their antioxidant and reducing characteristics, microorganisms and plants are commonly used in the biological synthesis of silver nanoparticles. The downside of microbe-mediated synthesis is that it requires extremely strict aseptic conditions to maintain. As a result, plant extracts may be preferable to microbes for the green manufacturing of nanoparticles. Proteins, amino acids, enzymes, and phytoconstituents are involved in the reduction of silver ions as well as the stabilization of Nano-material synthesis. Green metallic nanoparticles are biocompatible, environmentally benign, and cost-effective. The Antimicrobial effect could be due to the electrostatic attraction between nanoparticles and fungal cells, or it could collect inside the cells, causing damage to the cell walls and membranes. It's also possible that silver can intercalate between the purine and pyrimidine base pairs, causing the DNA molecule to denaturize ^{3,4}.

Bimetallic nanoparticles, which are composed of two distinct metals, have garnered more attention than monometallic nanoparticles in both the scientific and technological communities. These are created by mixing metallic nanoparticles with different architectural characteristics. These properties may be different than those of pure elemental particles and may include size-dependent optical, electrical, thermal, and catalytic effects. Indeed, metallization allows for significant enhancements in the catalytic characteristics of the resultant nanoparticles that are not possible with monometallic catalysts. The electronic effect is critical in bimetallic catalysts because it describes charge transfer. By alloying the constituent elements, the structure of the bimetallic nanoparticles can be altered. An additional degree of freedom is introduced when monometallic nanoparticles are converted to bimetallic nanoparticles. Following that, the catalytic activities of various bimetallic nanoparticles were compared. With the aid of physical and spectroscopic measurements, many methodologies and correlations have been created. The circumstances of preparation dictate the structure and miscibility of the two metals within the bimetallic nanoparticle. Bimetallic nanoparticles are frequently synthesised by simultaneously reducing two metal ions in the presence of an appropriate stabilising technique, such as steric hindrance or static-electronic repulsive force. Between the core shell and the homogeneous alloy, this process produces a particle structure that is dependent on the reduction situation ⁵⁻⁷.

Candidiasis is a fungal infection that is spread by yeasts belonging to the species *Candida albicans*. *Candida* yeasts are normally found in abundance in the gastrointestinal tract, mucous membranes, and skin without causing infection; nevertheless, an excess of these organisms can cause symptoms. *Candida* symptoms vary according to the location of infection on the body. Thrush is the medical word for a mouth condition. Signs and symptoms include white patches on the tongue and other areas of the mouth and throat. Discomfort and trouble swallowing are additional signs and symptoms. When it affects the vaginal area, it is called a yeast infection. Biofilms have been linked in a variety of microbial diseases throughout the body, accounting for up to 80% of all infections, according to one estimate. Biofilms have been associated with infectious processes such as bacterial vaginitis, urinary tract infections, catheter-associated infections, middle-ear infections, dental plaque development, gingivitis, and contact lens coating. Current treatments are ineffective against multidrug-resistant pathogens. Resistance has developed into a significant issue, necessitating the development of new antimicrobials ⁸⁻⁹.

Additionally, the metallic nanoparticles are successfully used to diagnose and cure cancer. Nanoparticles are frequently synthesised using a series of chemical and physical methods that are both expensive and potentially hazardous to the environment, as they include the use of poisonous and dangerous substances that can result in a variety of biological risks. The development of biologically inspired experimental methodologies for nanoparticle production is rapidly growing in importance as a field of nanotechnology ¹⁰.

As a result, plant extracts may be preferred to microbes for this purpose because to their ease of development, lower toxicity, and more complicated way of cell culture upkeep. It is the optimal platform for nanoparticle synthesis since it is free of toxic substances and contains natural capping agents that enhance the stability of metal nanoparticles ¹¹.

MATERIALS AND METHODS

The reagents used were of analytical quality, and they were obtained from Merck (Mumbai, India). The chemicals that were employed in the synthesis were of the highest analytical quality. Merck (India) provided silver nitrate (AgNO_3), while Sigma Aldrich, Pvt. Ltd. in Mumbai, provided chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$). Room temperature is used to carry out the biological reduction of silver nitrate and chloroauric acid for the preparation of a nanocomposite using aconitum root extract. For phytochemical extraction, hexane, ethyl acetate and methanol were utilized as solvents. All

of the solvents were purchased from Sigma Aldrich, Pvt. Ltd. in Mumbai, India. Sabouraud's dextrose broth (SDB) and potato dextrose agar (PDA) were purchased from Hi-Media, India.

Collection of plant

In September (flowering season), the *Aconitum Heterophyllum* species was obtained from Razdhan pass (Bandipora), Jammu and Kashmir, India.

Cold Extraction of root of *A. Heterophyllum*

Considering its critically endangered status, root (5 g), stem, was cleaned and cut into tiny pieces, then shade-dried at ambient temperature before being processed into powder with a mechanical grinder. The root powder was then sequentially extracted using a series of solvents, including hexane, ethyl acetate and methanol, over a period of 48 h, with occasional shaking and using intermittent heating over a water bath at their respective boiling temperatures. Whatman filter paper No. 1 was used to filter the extracts. The filtrate was collected and concentrated in a water bath, with the leftovers being discarded. The dried extracts were labeled and kept in glass vials at 4 °C for subsequent testing¹²⁻¹⁴.

Qualitative Detection of Phytochemical Constituents:

Detection of active phytochemical constituents was carried out for all the extracts using the standard procedures. In order to determine the active phytochemical contents in all of the extracts, the usual methodologies were used. The subsequent fractions obtained with hexane, ethyl acetate and methanol were qualitatively analyzed for secondary metabolites, such as phenolics, alkaloids, glycosides, tannins, flavonoids, terpenes, saponins and steroids, using conventional procedures¹⁵.

Synthesis of nanoparticles

Nanoparticles can be created in a variety of shapes and sizes, based on the needs that have been established. In order to synthesise nanoparticles, there are two basic approaches: top-down processes and bottom-up processes. The latter is the preferred way since it involves the accumulation of atoms into molecules, which then cluster together to produce nanoparticles^{16,17}.

a) Synthesis of silver nanoparticles

An aqueous solution (0.01mM) of silver nitrate (AgNO_3) and various concentrations of root extract of *Aconitum heterophyllum* from 1 to 5 ml were formed independently for the preparation of silver nanoparticles. Each concentration of the root extract was added to a 10 ml solution of 0.01mM AgNO_3 that had been previously prepared. It took 20 minutes for the colour of the solution to shift from light yellow to dark brown, indicating the production of AgNO_3 in the solution. The resulting colloidal silver solution was subjected to UV – Vis spectroscopy to determine its composition^{18,19}.

b) Synthesis of gold nanoparticles

Chloroauric acid [HAuCl_4], 0.5mM was mixed with the plant extract of *Aconitum heterophyllum* root (40mg/ml). The final reaction mixture was made upto 1ml and incubated in water bath at 70°C for 10mins. The formation of Au nanoparticles was observed with a color change from yellow to pink. The mixture was centrifuged at 12000 rpm for 20 mins to separate the nanoparticles which were then dried to obtain their powdered form²⁰.

c) Synthesis of gold-silver bimetallic nanoparticles

Chloroauric acid [HAuCl_4], 1.5mM and silver nitrate [AgNO_3], 1.5mM were mixed in the ratio of 1:1 with the plant extract (40mg/ml). The final reaction mixture was made upto 1ml and incubated in water bath at 80 °C for 10mins. The formation of bimetallic nanoparticles can be monitored by the change in color from yellow to a dark purple^{21,22}.

Characterization of biosynthesized nanoparticles

UV-visible spectroscopy

The UV-vis spectra of the biosynthesized Ag, Au, and Ag/Au bimetallic nanoparticles in the 200-850-nm wavelength range was investigated. A UV-Visible spectrophotometer was used to observe the reduction of Ag, Au, and Ag/Au ions in the reaction mixture. A pipette was used to pipette 2 ml of the sample into a cuvette, which was then evaluated at room temperature²³.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is used to observe the surface morphologies of Ag, Au, and Ag/Au nanoparticles in greater detail. Because the surface of the samples is not sufficiently conductive, charge-up can occur during irradiation with the electron beam when the samples are exposed to it. The surface of the samples is coated with a very thin film sample prepared using the silver, gold, and silver/gold bimetallic nanoparticles via a method of spin coating (1500 rpm)

on a 1x1-cm aluminium foil, after which the samples are allowed to dry for 30 minutes at room temperature ^{24,25}.

X-ray diffraction (XRD) technique

PAN analytical X-PERT PRO diffractometer at room temperature was used to determine the crystalline structure and particle size of nanoparticles. Cu K radiation ($\lambda = 1.54060 \text{ \AA}$) was used in conjunction with a nickel monochromator in the 2° angle, 100-800 range to acquire the results. It is possible to execute this test procedure by shining an x-ray beam onto a sample and measuring the scattered intensity as a function of the outgoing direction. Once the beam has been separated, the scattering pattern, also known as a diffraction pattern, displays the crystalline structure of the material. The Rietveld refinement approach is then applied in order to characterise the crystal structure that is most likely responsible for the pattern that was observed ^{26,27}.

Fourier transformed infrared spectroscopy (FTIR)

The chemical arrangements of AgNPs, AuNPs and bimetallic nanoparticles were carried out using the FTIR spectrometer (Perkin Elmer Spectrum 100 FTIR Spectrometer) at room temperature. FTIR experiment was carried out to identify the biomolecules present in the extract responsible for the reduction of silver and gold ions. The Infrared spectra were noted in the range of $4000\text{--}500 \text{ cm}^{-1}$ at 4 cm^{-1} resolutions and assigned peak numbers. At room temperature, the chemical compositions of NPs were determined using an FTIR spectrometer (Perkin Elmer Spectrum 100 FTIR Spectrometer) and an FTIR spectrometer. It was decided to conduct an FTIR experiment in order to determine the biomolecules contained in the extract that were responsible for the reduction of silver and gold ions ²⁸.

Microscopic (TEM) analyses

A transmission electron microscope (TEM) was used to examine the morphology of the biosynthesized silver, gold, and silver/gold bimetallic nanoparticles. For TEM investigation, we prepared the samples by drop coating the nanoparticle solutions onto carbon-coated copper grids and allowing them to cure at room temperature for several hours. The TEM study was carried out using a Tecnai F20 model that had been set up with an accelerating voltage. The transmission electron microscope (TEM) images a nanoparticle sample using an electron beam, which provides significantly higher resolution than is feasible with light-based imaging techniques. The transmission electron microscope (TEM) is the method of choice for directly measuring nanoparticle size, grain size, size distribution, and morphology ²⁹⁻³¹.

Antifungal activity determination of prepared samples

Preparation Potato dextrose Agar media (PDA)

For the fungal growth the easiest and most common culture media is potato dextrose agar media which can be easily prepared in laboratory. Potato infusion and dextrose promote luxuriant fungal growth. ³²

Determination of antifungal susceptibility:

Fungal Culture: Fungus in freeze dried condition and revived the culture on agar plate in laboratory.

In vitro antifungal test:

The antifungal activity was performed on petridishes containing potato dextrose agar when temperature of the medium reached at 40°C i.e. slightly above the room temperature. The specific concentration of 100% stock solution nanoparticles (diluted in double distilled water) were added to petriplates of 9 cm diameter containing PDA. The rate of mycelial growth was measured after placing an active mycelial plug of fungi on petridish. Fluconazole was used as positive control. Finally the petriplates were incubated at 28°C . The observation was recorded after 48 hours. The rate of mycelial growth inhibition was calculated by formula ³⁴:

$$\text{GI\%} = \frac{\text{DC}-\text{DT}}{\text{DC}} \times 100$$

Here DC = diameter of zone of inhibition of control, DT is the diameter of zone of inhibition of antimicrobial agent

RESULTS

Phytochemical Characterization of Plant Extract:

Table1: Qualitative phytochemical screening of aconitum heterophyllum

S.no.	Plant constituents	Hexane extract	Ethyl acetate extract	Methanolic extract
1.	Alkaloids	—	+	++

2.	Steroids	+	+	+
3.	Terpenoids	—	+	+
4.	Terpenes	—	—	—
5.	Phenols	+	+	++
6.	Tannins	—	+	+
7.	Saponins	+	+	++
8.	Flavonoids	+	+	++
9.	Glycoside	+	+	+

Note: ‘++’ = Strong presence, ‘+’ moderate presence, and ‘—’ = absent;

Synthesis of gold nanoparticles:

The synthesis of gold nanoparticles can be observed with change in color from yellow to pink. The formation of bimetallic nanoparticles can be monitored by the change in color from yellow to a dark purple. The Preparation or development of bimetallic nanoparticles was observed by the change in color from yellow to dark purple.

Characterization of gold and gold-silver bimetallic nanoparticles:

UV-vis spectroscopy

The formation of monometallic and bimetallic nanoparticles was monitored using a Microplate reader Epoch 2 (Biotech) to obtain the UV-vis spectra. The nanoparticle solution was scanned between the wavelengths of 300 to 850nm. The peak for silver, gold and bimetallic nanoparticles was found to be at 437nm, 537nm and 468nm respectively. The resemblance in the peak of the BMNPs may be due to the high concentration of the gold nanoparticles due to their shorter synthesis time in comparison to Ag-nanoparticles.

Phyto-fabricated mono and bimetallic nanoparticles:

The phyto-fabrication of mono (Au, Ag) and bimetallic (Au-Ag) nanoparticles loaded with flavonoids has been validated using UV spectroscopy. Synthesis of AuNps was carried out as functions of pH 3.5, 5.5 and 7.5 . No desirable SPR peak was obtained at high acidic and basic conditions. Therefore, pH 5.5 has been considered optimum pH, due to its high absorbance λ max: 537 nm.

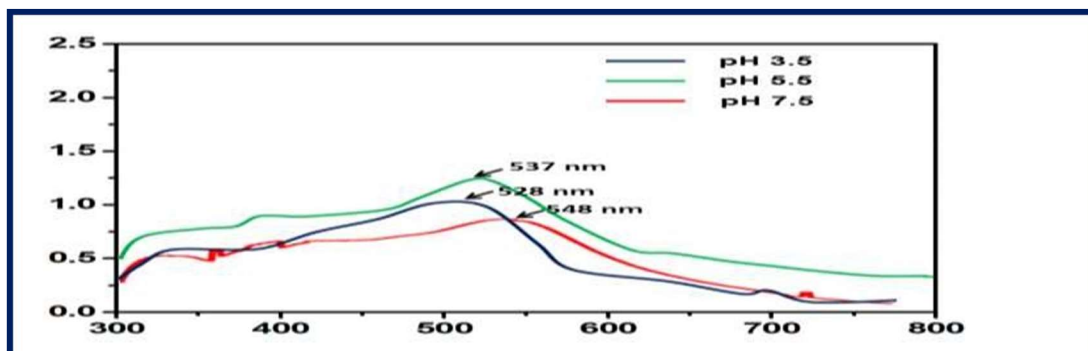


Fig.1 : UV-Vis spectra of AuNps at different pH

The phyto-fabrication of AgNps was carried out as functions of pH 7.5, 9.5 and 11.5. No perceptible peak was obtained at acidic conditions. As the pH increases (7.5-11.5), the wavelength shifted (447 - 437 nm) towards a lower wavelength. The hypsochromic shift from 447 to 437 nm attributed to the formation of small-sized nanoparticles and having high absorbance intensity. Therefore, pH 11.5 has been considered as optimum pH, having a characteristic peak at λ max: 437 nm. The pH has a significant role in the phyto-fabrication of metal nanoparticles by altering the charge

on the molecule, affecting their properties and behavior. The change in the pH of the solution can alter peak absorption, wavelength and intensity.

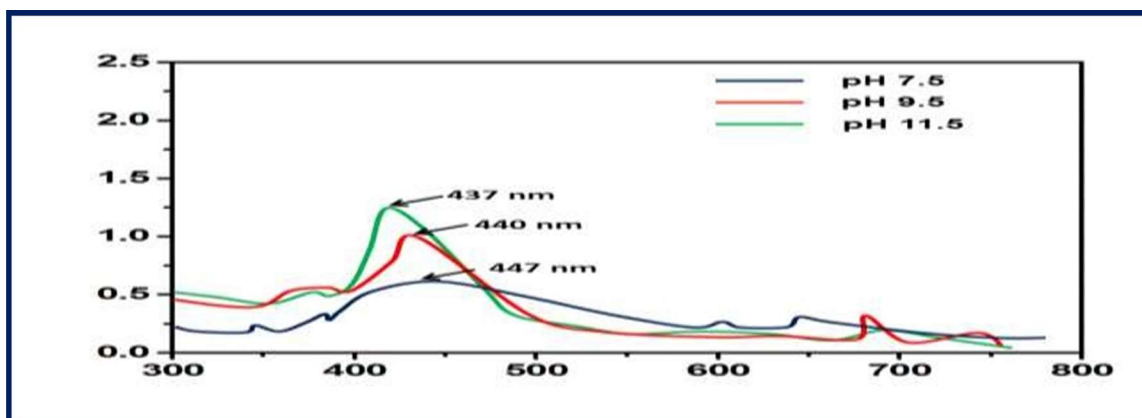


Fig.2 : UV-Vis spectra of AgNps at different pH

Phyto-fabrication of bimetallic nanoparticles has been carried out in two possible methods: co-reduction (simultaneous addition) and sequential reduction (stepwise addition). The simultaneous addition (co-reduction) is used for the fabrication of Au-Ag nanoparticles in which both metals are reduced simultaneously. The second option is the stepwise addition of sequential reduction and used to form core-shell structures of nanoparticles attached to another metal. The simultaneous co-reduction method has been widely employed for the phyto-fabrication of bimetallic nanoparticles. Synthesis of Au-AgNps was carried out as functions of pH 4, 6, 8 and 10. The confirmation of the fabrication of Au-Ag bimetallic nanoparticles (pH 10) is evident from the appearance of a single band (λ_{max} 468 nm) at a characteristic wavelength (420-495 nm) of bimetallic nanoparticles.

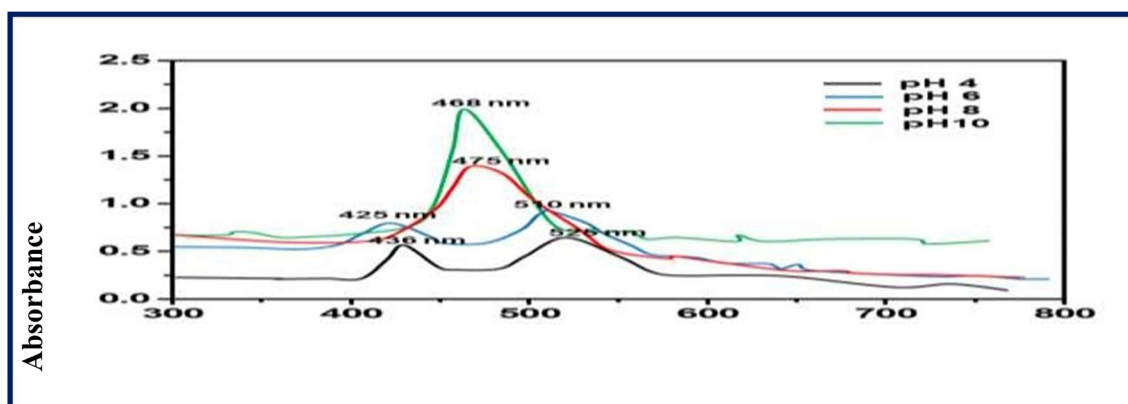


Fig.3 : UV-Vis spectra of Au-AgNps at different pH

Fourier Transform Infrared Spectra

The FTIR spectra can help in study the functional groups present in the synthesized nanoparticles. Since the nanoparticles were synthesized from the aconitum root extract, its functional group get attached to the nanoparticles and impart additional characteristics. The region below 1500 cm^{-1} is known as the fingerprint region which is due to the bending vibrations of the molecule. The band noticed between 3371 to 3290 cm^{-1} may be assigned to O-H stretching. The high peak between 1710 and 16 cm^{-1} may indicate a stretching of C=O bonds.

X-ray Diffraction (XRD):

The diffraction peaks of phyto-fabricated mono and bimetallic nanoparticles were depicted in Fig. The X-ray diffraction pattern of AuNps and AgNps nanoparticles were observed at 38.41°, 44.45° and 64.72°; 38.21°, 44.32° and 64.62° diffraction angles which were indexed to the (111), (200) and (220) lattice planes of face centered cubic

structure of gold and silver nanoparticles.

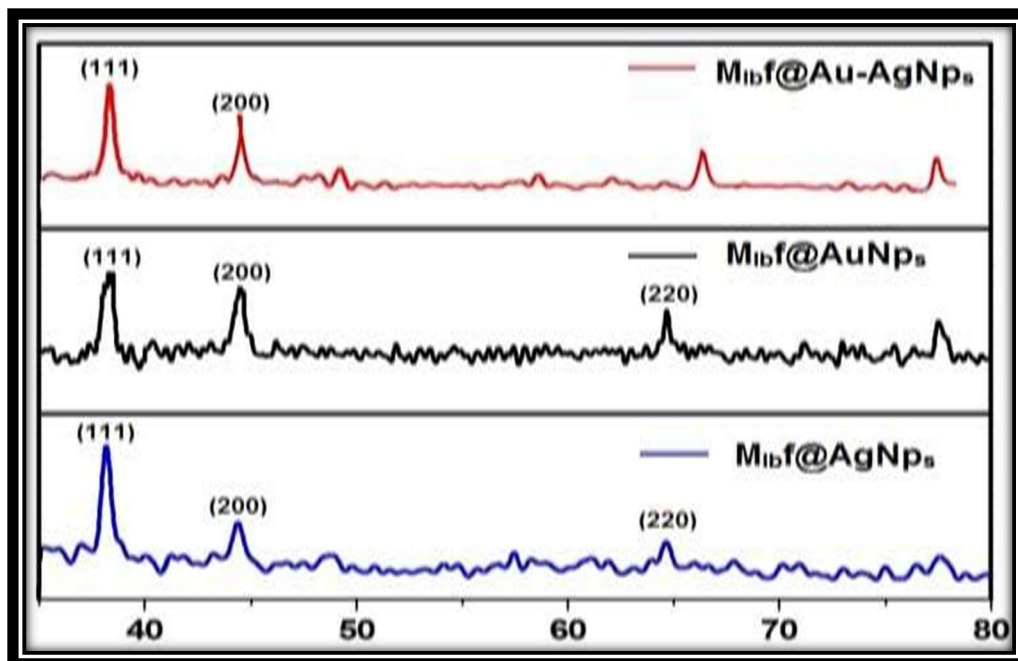


Fig.4 : XRD of mono and bimetallic nanoparticles

The two diffraction peaks of bimetallic nanoparticles appeared at 38.31° and 44.44° diffraction angles which correspond to (111) and (200) lattice planes of face centered cubic structure of bimetallic Au-AgNps. Among all diffraction peaks, (111) plane has the highest intensity comparative to other ones. The high reactivity of (111) plane is attributed to the elevated atom density. The unassigned peaks in the XRD pattern might be due to the crystallization of the bio-organic phase.

Scanning Electron Microscopy (SEM)

SEM images of all the phyto-fabricated nanoparticles were found in polydispersed spherical shaped surface morphology. The capability of cellular-uptake is influenced by the nanoparticle's shape. The spherical nanoparticles demonstrate the highest uptake among differently shaped nanoparticles. The spherical shaped nanoparticles require a minimum energy barrier with respect to the non-spherical counterparts.

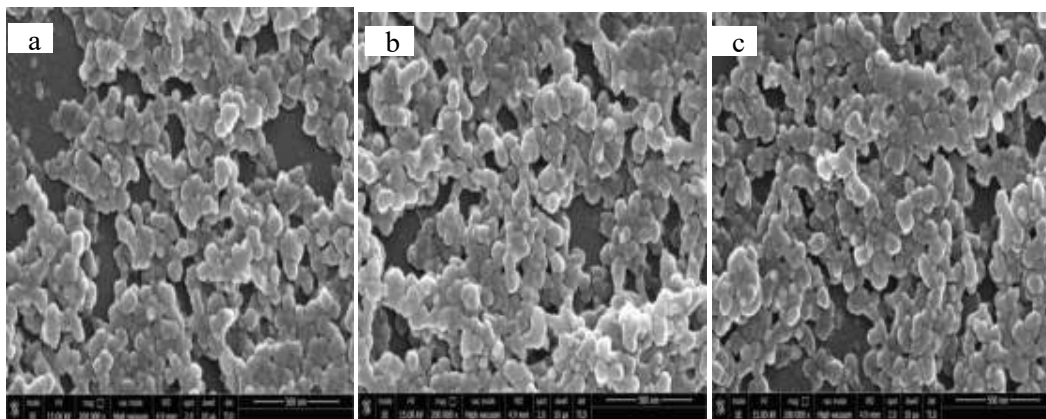
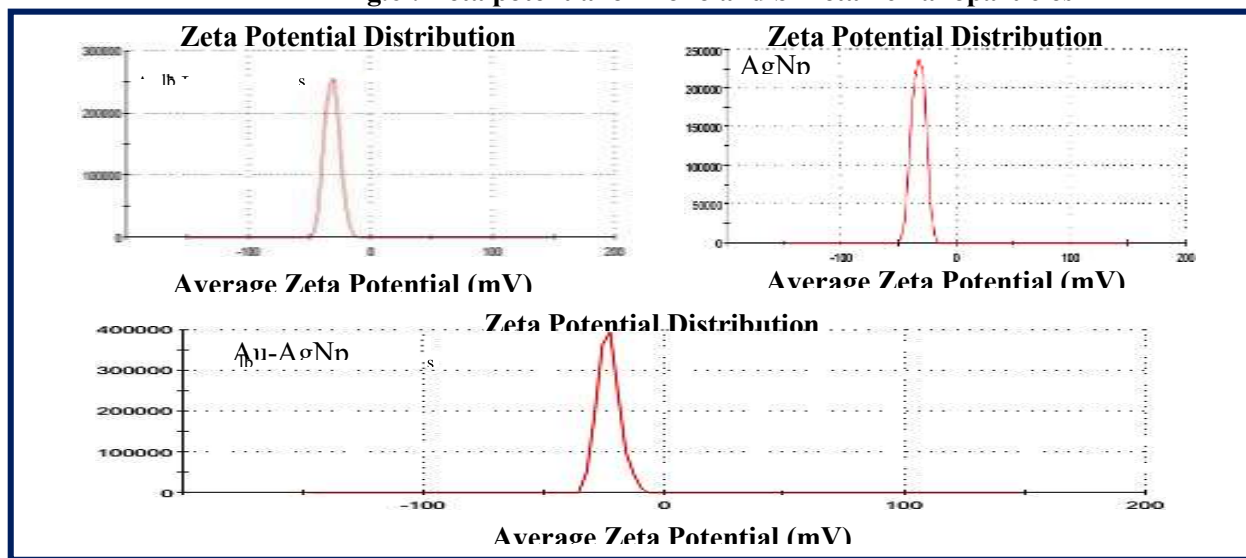


Fig.5 :SEM of (a) AuNps (b)AgNps and (c) Au-AgNps

Transmission Electron Microscopy (TEM) and Zeta sizer studies

TEM analysis confirmed the spherical shape of all the three phyto-fabricated nanoparticles having the size in the range 15-45 nm, 16-30 nm and 31-80 nm at the magnification of 300,000X. The average hydrodynamic size (Z average) of phyto-fabricated nanoparticles: AuNps, AgNps and Au-AgNps was 54.26 nm, 42.32 nm and 88.10 nm respectively. The phyto-fabricated nanoparticles AuNps, AgNps, and Au-AgNps exhibited zeta potentials as -31.0 mV, -23.2 mV and -33.6 mV respectively.

Fig.6 : Zeta potential of mono and bimetallic nanoparticles



Zeta potential values are often used as an indication of the stability of colloidal particles. The absolute values replicate the net electrical charge of the particles of functional groups present on the external surface. The high negative value indicated the stability (repulsive barrier) of the nanoparticles by preventing the agglomeration of nanoparticles. The negative potential, in the present case, might be arising from the loading of negatively charged functional groups (-OH groups of the flavonoids). The plant secondary metabolites form a coating on metal nanoparticles. This capping effect is considered valuable for shielding nanoparticles from their aggregations and keeping them in a nano state. They also contribute in the enhancement of bio-efficacy owing to their medicinal properties. There was lack of authentic evidence except for the appearance of a faded layer around nanoparticles, visible in Transmission Electron Microscopy.

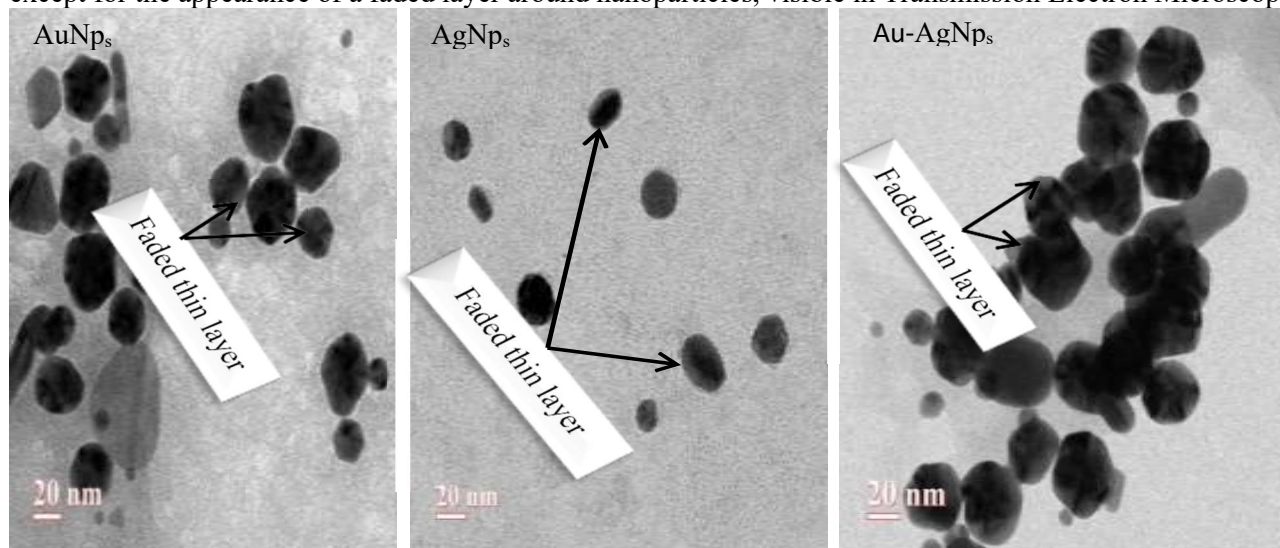


Fig. 7: TEM images of mono and bimetallic nanoparticles exhibiting faded layer around them

Antifungal activity

Antifungal tested by well diffusion method .Potato dextrose agar plates were inoculated with each fungal culture 10 days old by point inoculation. The filter paper wells 5 mm in diameter impregnated with 20 40 and 60 µL concentrations of the synthesized AgNPS, AuNPs and AuAgNPs were placed on test organism-seeded plates. Fluconazole (10 µg well) used as positive control After 72 h of incubation at 28°C and inhibition zones were measured in mm.

Table2: Antifungal activity of extract in AgNPs, AuNPs and AuAgNPs

Table 24. Antifungal activity of extract in Fig. 13, 14, 15 and 16					
A.	Nanoparticles (AgNPs)	Zone of inhibition (mm) Concentrations (µg/mL)			(Fluconazole) (mm)
	Pathogenic fungus	20	40	60	
1.	<i>Candida albicans</i>	0.60	02	04	06
2.	<i>Aspergillusfumicatus</i>	0.40	03	04	06
B.	Nanoparticles (AuNPs)	Zone of inhibition (mm) Concentrations (µg/mL)			Standard (Fluconazole) (mm)
	Pathogenic fungus	20	40	60	
1.	<i>Candida albicans</i>	0.50	03	04	06
2.	<i>Aspergillusfumicatus</i>	0.60	03	04	06
c.	Nanoparticles (AuAgNPs)	Zone of inhibition (mm) Concentrations (µg/mL)			Standard (Fluconazole)
	Pathogenic fungus	20	40	60	
1.	<i>Candida albicans</i>	02	04	05	06
2.	<i>Aspergillusfumicatus</i>	02	03	04	06

Antifungal activity of the synthesized nanoparticles:



Fig8: (a) *Aspergillus fumigatus*

(b) *Candida albicans*

CONCLUSION

On the basis of complete review of monometallic and Bi-Metallic Nanoparticles of *Aconitum Heterophyllum* it was found that nanoparticles are effective for antifungal activity. Antifungal drugs like *Aconitum heterophyllum* are highly specific against fungus and are widely used in the treatment of various fungal diseases. Now day's eco-friendly methods are gaining importance for synthesis of nanomaterial's. The synthesis process developed in this study could be forwarded for study of various metal combinations to develop most powerful novel antifungal materials.

The green synthesis of MNPs and BNPs with *Aconitum Heterophyllum* extract which acts as a reducing and capping agent. The NPs were found to be spherical in shape and polydisperse. The nanoparticles were characterized using various microscopic and spectroscopic techniques. The nanoparticles were surrounded by a thin layer of phytochemicals (Phenolic compounds, proteins and metabolites), that has different functional groups of phenols, amines, alcohols, ketones, aldehydes, etc. Morphology of NPs and their compounds were identified from the characterization using UV-Vis spectrophotometer, SEM, Zeta Analyzer, XRD and FTIR techniques. BNPs are considered to be more active antifungal as compared to their counterparts of MNPs (Monometallic Nanoparticles) because of lattice strain effects, geometric effects and electronic charge transfer effects.

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