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GREEN SYNTHESIS, EVALUATION AND INVESTIGATION OF IN-VITRO ANTIFUNGAL EFFECTIVENESS OF SILVER NANOPARTICLES PREPARED WITH FRESH EXTRACT OF *ACONITUM HETEROPHYLLUM* LEAVES

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ARTICLE INFO **ABSTRACT** **ORIGINAL RESEARCH ARTICLE**

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A Silver nanoparticles of the green approach for the synthesis of well – stabilized silver nanoparticles is described the nanoparticles will be biosynthesized by adding Silver nitrate with extract in deionized water, silver nitrate and extract of Aconitum heterophyllum with different concentrations. The phytochemical extract from this plant is more suitable for large-scale biosynthesis of silver nanoparticles. Nanoparticles (NPs) prepared with plant extract are more stable, suitable and faster therapeutic efficacy against fungal infection. Moreover, the preparations of silver nanoparticles with plant extract are more uniform in shape and size. The advantages of using plant and plant-derived materials for biosynthesis of silver nanoparticles have attract the interest of researchers due to its better mechanisms in successful formation of silver nanoparticles. The metallic structurer was characterized using various methods. The AgNPs exhibit a maximum absorption at 430 nm in Ultraviolet Spectroscopy, while the X-Ray diffraction indicates that they were crystalline in nature, including high resolution transmission electron microscopy, energy dispersive X-Ray Diffraction. It was confirmed by the electronic microscopic analysis that the spheres prepared with silver metal and plant extract are uniform nanoparticle shape and size.

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INTRODUCTION

Nanotechnology is defined as the creation of particles with a size range of 1.0 to 100 nm. Because of their huge surface area, certain medicinal nanoparticles are thought to have strong antifungal 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 AgNPs are biocompatible, environmentally benign, and cost-effective. Antimicrobial properties of silver nanoparticles made from plant extracts have been described. The antibacterial effect could be due to the electrostatic attraction between nanoparticles and bacterial 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. Monometallic nanoparticles have been long used in multiple areas of research due to their antimicrobial, antioxidant, photocatalytic, anti-biofilm etc. properties. Silver nanoparticles of fresh leaves extract of *Aconitum heterophyllum* have shown antibacterial activity against microorganisms such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis*. It is also known for its applications in disease diagnosis, drug delivery. Silver nanoparticles have shown to have antifungal activity against *Candida albicans*, reduce inflammation, antiobioflim activity.

Methods for synthesis of Nanoparticles

There are two main approaches for synthesis of silver nanoparticles of fresh leaves extract of *Aconitum heterophyllum* namely Top-down and Bottom-up processes, the latter being more preferable where atoms build up into molecules which cluster together to form nanoparticles. There are three main methods by which nanoparticles are synthesized.

Physical Methods

It may involve the breakdown of bulk material into nanoparticles as is the case in the top-down approach of nanoparticle synthesis. Physical methods have certain advantages over chemical methods in that they do not make use of toxic chemicals but they are complicated, expensive and the size and shape of nanoparticles cannot be easily controlled.

Chemical methods

Chemical methods take up the bottom-up approach by which particles are synthesized by aggregation of reduced metals. They do not involve the use of costly and complicated machinery like that in physical methods but make use of toxic chemicals which may have dangerous outcomes in medical applications.

Biological methods

Biological methods for nanoparticle fabrication, is safe and hold various applications that are not possible with physical or chemical synthesis methodologies. Biological methods of synthesis may make use of microorganisms or plants in the production of nanoparticles, plants being more preferable due to the unpredictable behavior of the microorganisms. Plant based synthesis usually involves a reaction taking place in the presence of a reducing agent to produce nanoparticles.

MATERIALS AND METHODS

Chemicals and Plant Sample

The reagents used were of analytical grade obtained from Merck (Mumbai, India). The chemicals used for the synthesis were of analytical grade. We purchased silver nitrate (AgNO3) from Merck (India), Ativisha leaf extract was prepared in distilled water. All glass wares are properly rinsed with chromic acid followed by distilled water and dried. Biological reduction of silver nitrate for its nanocomposite by Ativisha leaf extract is carried out at room temperature.

Preparation of plant extract

The collected leaves of Silver nanoparticles of fresh leaves extract of *Aconitum heterophyllum* plant were washed with deionized water and dried. 4g of fresh leaves were crushed using a mortar and pestle and mixed with 200ml of deionized water. The extract was filtered using Whatman filter paper. After filtration, equal amount of ethanol was added to precipitate the mucilage present in the extract. The extract was centrifuged at 7000 rpm for 10 mins to make it mucilage free. The supernatant was collected and stored at 4^{o}C.

Synthesis of silver nanoparticles by Aconitum heterophyllum.

Synthesis of silver nanoparticles an aqueous solution (0.01mM) of silver nitrate (AgNo3) and various concentration of leaf extract of *Aconitum heterophyllum* from 1 to 5 ml were prepared separately. Each concentration of the leaf extract was added to 10 ml of 0.01mM AgNo3 prepared. After 20 minute, the color of the solution changed from light yellow to dark brown, indicating the formation of AgNO3. The resulting colloidal solution of silver was analyzed using UV-Visible spectroscopy.

Characterization of biosynthesized silver nanoparticles

UV–visible spectroscopy

The formation of AgNPs in the fresh leaf extract of *Aconitum heterophyllum* was confirmed through a color change from pale yellow to dark brown. The color change was recorded under the UV-visible Spectroscopy. The UV-visible spectra of the synthesized AgNP3 by *Aconitum heterophyllum* in the 300-700nm wavelength range using a Shimadzu spectrophotometer, the technique measures the absorption of light across the desired optical range. A sample is dispensed into a cuvette and placed in the path between the optical light source and a detector.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) detailed micro structural observations and the surface morphologies of AgNPs are carried out using high-resolution FEI Q250 Thermo-Fisher environmental scanning electron microscopy ESEM with a resolution better than 2.9 nm at 30 kV 1 working voltages. In this technique, an electron beam strikes on the surface of the test sample. Scanning electron microscopy (SEM) is another technique where only milligram quantities of material may be used to determine particle size, shape, and texture. In SEM a fine beam of electrons scans across the prepared sample in a series of parallel tracks. The electrons interact with the sample, and produce several different signals which can be detected and displayed on the screen of a cathode ray tube. Particles less than 1 nm can be viewed.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was performed by a Leo 912 AB instruments. In brief, a drop of appropriately diluted sample of AgNPs was poured on carbon-coated copper grids and allowed to stand for 2 minutes. The excess solution was removed using a blotting paper and allowed to be dried at room temperature.

X-ray diffraction (XRD) technique.

The crystalline structure and particle size of Silver Nanoparticles were analysis in an X-ray diffractometer with an operating voltage of 45 KV and current of 0.8 mA. This test method is performed by directing an x-ray beam at a sample and measuring the scattered intensity as a function of the outgoing direction. Once the beam is separated, the scatter, also called a diffraction pattern, indicates the sample's crystalline structure.

Fourier transformed infrared spectroscopy (FTIR)

The aqueous leaf extract of *Aconitum heterophyllum* and AgNPs were subjected to Fourier transform infrared (FTIR) Spectroscopy in order to analyses their spectra. The chemical compositions of AgNPs 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 aqueous leaf extract responsible for the reduction of silver ions. The Infrared spectra were noted in the range of 4000–500 cm 1 at 4 cm 1 resolutions and assigned peak numbers. FT-IR spectroscopy allows valuable insight into the different functional groups that are present in a system by measuring the vibrational frequencies of the chemical bonds involved. This means that IR spectroscopy can be used to observe the vibrational transitions of self-assembled functional groups coordinated to nanoparticle surfaces, and both quantitative and qualitative analysis can be performed.

RESULTS AND DISCUSSION

Biosynthesis of Silver Nanoparticles using *Aconitum heterophyllum* Extract

Aconitum heterophyllum extract was used for the synthesis of silver nanoparticles. Apart from metal nanoparticles, some of the metal oxides such as iron oxide, indium oxide and hydroxyapatite have been synthesized in a greener approach using *Aconitum heterophyllum* plant extract. In all these method *Aconitum heterophyllum* plant was used as reducing agent as well as protecting agent. The synthetic experimental condition first optimized by controlling the concentration of extract, concentration of metal salt, temperature of the reaction medium and pH. The growth of the silver nanoparticles was monitored by continuously measuring the appearance of the Plasmon band intensity. Usually silver nanoparticles exhibit a strong Plasmon band at 420 nm. The UV-Visible spectra of silver nanoparticles grown at different time intervals at room temperature and reaction in alkaline pH medium. The surface plasmon resonance peak appeared at 425 nm which indicates the formation of silver nanoparticles with spherical shape and the resulting TEM images are shown. The particles are narrow size distributed with average size of 10 nm. Color changes were monitored by using UV-Visible spectroscopy at regular time intervals from 30 min to 24 h. Maximum absorbance attained after 24 h most of the silver ions converted to Nano sized particles. The variation of *Aconitum heterophyllum* extract concentration to silver ions, the maximum absorbance attained at 5 mL which is show in Figure the different concentration of *Aconitum heterophyllum* extract are tabulated in Table no -1.

Table-1: Synthesis of silver nanoparticles with varying concentration of *Aconitum heterophyllum* extract.

Sr.no.	Volume of Ativisha Ext (mL)	Volume of 10 mM AgNO3 (mL)	Volume of Water (mL)
S1	1.0	5.0	44.0
S2	2.5	5.0	42.5
S3	5.0	5.0	40.0

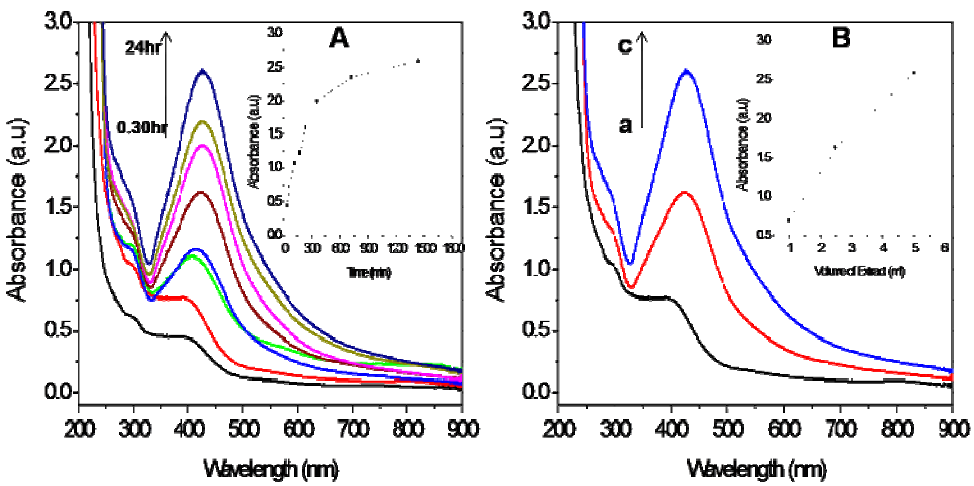

Figure - 1: UV-Visible spectrum of continuous growth of Ag nanoparticles using Ativisha [A] Different time intervals of AgNPs (30 min, 1, 2, 3, 4, 6, 12 & 24 h); [B] Different concentration of Ativisha extract (1.0, 2.5 and 5.0 mL at pH 11.25).

TEM Studies

The size and shape of the biologically synthesized nanoparticles was confirmed by TEM measurements. Figure shows the TEM images of silver nanoparticles with different magnifications. All are spherical in shape and the average size of the particles was found to be 10 nm ranges. Most of the particles are falling within range. From the selected area diffraction studies it is confirmed that silver nanoparticles are uniform in size and the diffraction pattern clearly indicates the different planes.

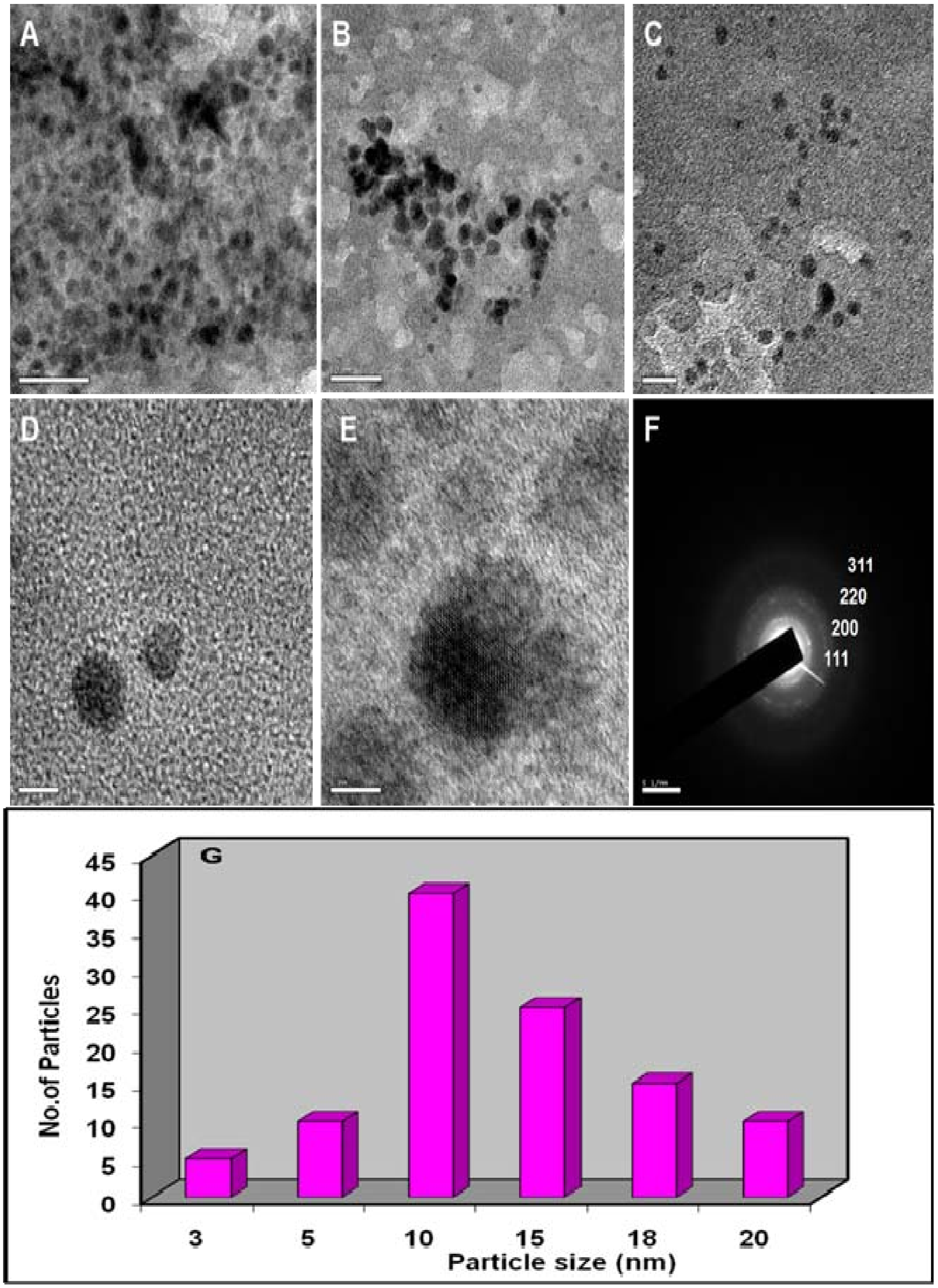

Figure - 2: HR-TEM images for Silver nanoparticles grown using Aconitum *heterophyllum* extract (different magnification images [A] 50 nm, [B] 50 nm, [C] 20 nm, [D] 10 nm, [E] 5 nm, [F] SAED pattern and [G] histogram of particle size.

XRD Analysis

The XRD pattern of the silver nanoparticles synthesized from *Aconitum heterophyllum* extract. The XRD peaks were indexed at (111), (200), (220), and (311) planes. The broad peak indicates the

Nanocrystalline nature of silver particles. Thus XRD pattern indicates a strongly support for the formation of silver nanoparticles apart from the UV-Visible analysis. The particles size and shape were further confirmed from SEM and TEM studies.

FT-IR Spectroscopy Study

The FT-IR spectrum of silver nanoparticles protected with *Aconitum heterophyllum* plant extract. The major peaks at 1587.6 cm-1 (C=C groups or from aromatic rings), 1386.4 cm-1 and 1076 cm-1 were obtained which indicates the formation of flavanones and terpenoids stabilized metal nanoparticles.

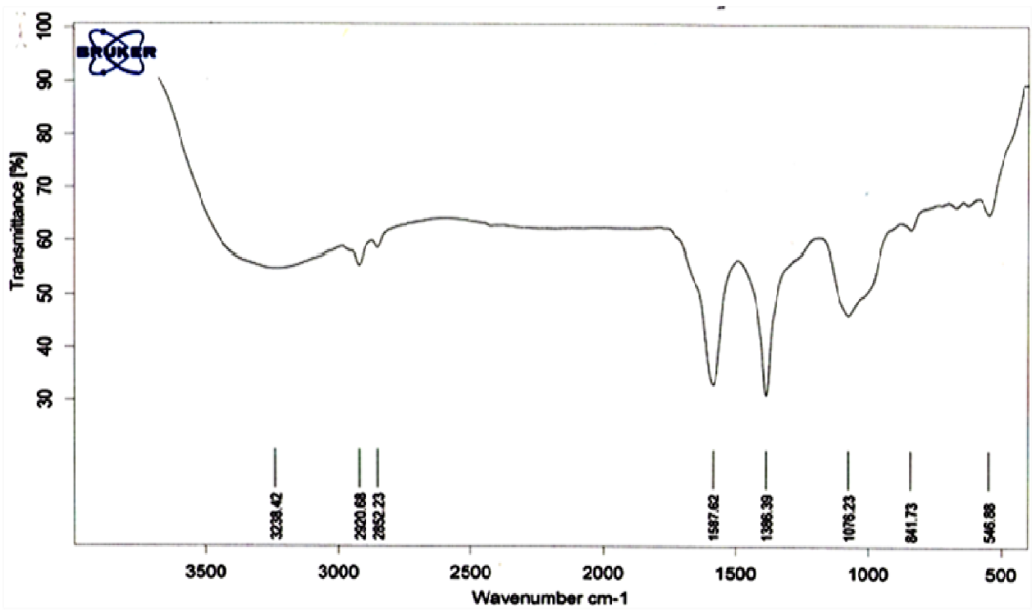

Figure - 3: FT-IR spectrum of *Aconitum heterophyllum* extract stabilized AgNPs.

Antimicrobial Activity of the Silver Nanoparticles Synthesized using *Aconitum heterophyllum* Plant Extract

The antifungal activity of the synthesized silver nanoparticles was tested against clinically isolated multi drug resistant human pathogens such as gram positive bacteria Staphylococcus aureus, Bacillus cereus, Micrococcus lutes and gram negative bacteria Escherichia coli and Klebsiella pneumonia. Since Ativisha plant extract have been widely used to treat various diseases, we are interested to test its antimicrobial activity against varies types of microorganism. The antibacterial activity of Aloe Vera extract, silver nanoparticles containing Ativisha extract and EDTA capped silver nanoparticles was compared and the zone inhibition values (mm) was calculated. Among all the samples AgNPs Ativisha exits highest zone of inhibition values which indicates an enhanced antibacterial activity of the silver nanoparticles synthesized by using *Aconitum heterophyllum* plant exact. Though, the mechanism of antibacterial activity of silver nanoparticles is not yet clearly understood, but the generalized mechanism is that the interaction of silver ion with the phosphorus moieties present in DNA which is responsible for inactivate of DNA replication. The summary of antimicrobial activity of silver nanoparticles synthesized using *Aconitum heterophyllum* plant extract, EDTA capped silver nanoparticles synthesized by chemical reduction and *Aconitum heterophyllum* plant extract are shown. The ciprofloxacin antibiotic drug was used as internal standard. From the overall observation it is clear that the combination of *Aconitum heterophyllum* extract and silver nanoparticles exhibit enhanced antimicrobial activity than the pure silver nanoparticles (synthesized by chemical reduction method) and *Aconitum* *heterophyllum* plant extract. Hence such a kind of combined forms of herbal medicine could be used to treat the resistant antibacterial strains All tested samples are placed. It has been reported in the literature on preparation of silver nanoparticles impregnated house hold items to prevent the spread of bacterial infection.

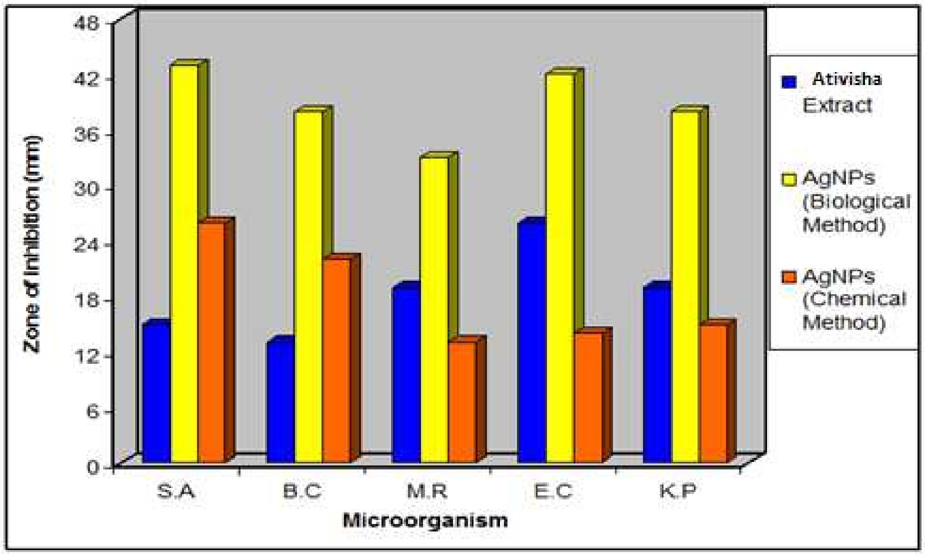

Figure - 4: Histogram for zone of inhibition of *Ativisha* extract, *Ativisha*

CONCLUSION

The potency of *Aconitum heterophyllum* in Biosynthesizing AgNPs, the present study reports the biosynthesis of AgNPs by *Aconitum heterophyllum* leaf extract confirmed by XRD, TEM, UV – Visible spectroscopy and FTIR Analysis. Antifungal activity of the biosynthesized AgNPs were then evaluated towards plant. Mushroom and Human pathogenic fungi. There is a body of information on antimicrobial activities of AgNPs biosynthesized by a broad range of plant species. In conclusion the data presented here demonstrate that Aconitum heterophyllum may be considered a green tool for synthesizing AgNPs with efficient antifungal activity. Thus the finding of this study could be adopted for several applications in the plant protection field. Further studies are underway to investigate treatments of pathogenic fungi by the Aconitum heterophyllum derived AgNPs, as it has been well known that nanoparticles have potent antibacterial activity. Other biological activity namely antibacterial and other activities of Aconitum heterophyllum nanoparticles could be also studies in the future.

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