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Antibacterial and photocatalytic activity of *Verbascum thapsus* leaves extract mediated synthesized silver nanoparticles

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Abstract
In the present study, silver nanoparticles were synthesized using *Verbascum thapsus* leaves extract as reducing and capping agents. The addition of plant leaves extract to silver nitrate solution results in the colour change of the solution, indicating the formation of silver nanoparticles. The synthesized nanoparticles were characterized by UV-Visible spectroscopy, XRD, DLS, FTIR and TEM. The XRD analysis showed that the AgNPs are of face centred cubic (FCC) structure. DLS revealed that nanoparticles show polydispersity at 0.363 indexing. TEM analysis showed that the synthesized AgNPs have spherical shape. Green synthesized silver nanoparticles were effectively degrading the dye nearly 95% at 72h of exposure time. The synthesized silver nanoparticles showed significant antibacterial potential against gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative bacteria (*Salmonella typhi)*. The photocatalytic activity of the synthesized silver nanoparticles was examined by degradation of methylene blue under sunlight irradiation.

Keywords: *Verbascum thapsus*, silver nanoparticles, XRD, TEM, Antimicrobial activity

1. Introduction

Nanotechnology is an emerging technology, which can lead to a new revolution in every field of science [1]. It has wide applications ranging from biomedical [2-5], waste treatment [6], electronics [7], catalysis [8], sensors [9] etc. With the growing use of nanoparticles in various fields, the current situation demands an environmentally clean and economically viable way for the synthesis of nanoparticles [10]. For nontoxic synthesis of nanoparticles, many biological approaches which are free from any toxicity are used. This results in huge demand of green nanotechnology [11]. Bacteria, fungi and plants are used in biological synthesis of nanoparticles. Among these the plants were used widely for the synthesis of nanoparticles. Silver has an inhibitory effect toward many bacterial strains and microorganism [10]. Silver nanoparticles have various applications including skin ointments and creams, which are used for the treatment of burns and open wounds [12], silver-impregnated polymers used for preparation of medical devices and implants [13], anticancer agents [14]. Silver-embedded fabrics are now used in textile industry which mainly produces sporting equipment [15].

Verbascum thapsus, a member of the family Scrophulariaceae, is one of the important medicinal plant found in all over Europe, in temperate Asia and in North America. This medicinal herb contains various chemical constituents like saponins, iridoid and phenylethanoid glycosides, flavonoids, vitamin-C and minerals. A number of pharmacological activities such as anti-inflammatory, antioxidant, anticancer, antimicrobial, antiviral, antihepatotoxic and anti-hyperlipidemic activity have been ascribed to this plant. The plant is used to treat tuberculosis, ear ache and bronchitis [16]. In the present study, an attempt has been made for the simple, cost-effective and environmental friendly synthesis of silver nanoparticles using aqueous leaves extract of *Verbascum thapsus.* Hence, the main aim of the present study is to develop a novel approach for green synthesis of silver nanoparticles, so as to evaluate their antibacterial efficacy.

2. Materials and Methods

2.1 Materials

The healthy leaves of plants were collected from Srinagar Garhwal, Uttarakhand and identified (Acc. No. 117050) by Botanical Survey of India, Dehradun. All the analytical reagents were purchased from Fisher chemicals. The deionized water is used throughout the experiment.

2.2. Preparation of Plant leaves extract

The leaves extract was prepared by taking 10gm leaves thoroughly washed with running water, followed by deionized water. Dried and powered plant material was boiled with 100ml of deionized water at 60º for 30 min. This extract was cooled at room temperature and filtered by Whatmanfilter paper No.1 and finally, stored at 4ºC for further experiments as the reducing agent.

2.3. Preparation of silver nanoparticles

For the synthesis of silver nanoparticles, silver nitrate solution (2mM) solution was reacted with *Verbascum thapsus* leaves extract as the reducing and stabilizing agent. The solution was kept in dark for 48 hours. The colour changed from green to brown indicating the formation of AgNPs. The reaction mixture was centrifuged at 10,000 rpm for 15 min. A pellet was collected followed by redispersion of pellets of AgNPs in deionized water to get rid of any uncoordinated biological material.

2.9. Antibacterial property of synthesized AgNPs

The antibacterial property of the silver nanoparticles was estimated against the pathogenic bacteria viz. grams positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* and gram's negative bacteria such as *Salmonella typhi*. The antimicrobial activity of synthesized AgNPs and standard were tested by disk diffusion method. All the plates were incubated at 37ºC for 24hours and the zone of inhibition of bacteria was measured.

2.10. Photocatalytic Degradation of Methylene blue

1mg of methylene blue was added to100mL of deionized water used as stock solution. About 20mg of biosynthesized silver nanoparticles was added to 50mL of methylene blue solution [20]. The resultant solution was mixed in magnetic stirrer for 30 min. Finally the solution was kept in sunlight. At specific time intervals, aliquot (2 ml-3ml) was withdrawn. The concentration of MB during degradation was determined by UV-Vis spectrophotometer. Percentage of dye degradation was estimated by the following formula:

$$\%\text{Decolourization} = 100 \times (C_0 - C)/C_0$$

Where C_0 is the initial concentration of dye solution and C is the concentration of dye solution after photocatalytic degradation.

3. Results and Discussion

3.1 UV-VIS spectroscopic analysis

The addition of plant leaves extract to silver nitrate solution result in colour change of the solution from greenish brown to reddish brown exhibited the reduction of the silver nitrate in aqueous solution. This is due to the excitation of surface Plasmon vibration in silver nanoparticles [20].

After 48hours of incubation of the aliquot, the sample was analysed by UV-VIS spectroscopy which showed that SPR occurred at 430nm. The SPR absorbance is sensitive to the nature, size and shape of particles presents in the solution. Fig.1 shows SPR occurred at the wavelength of 430nm which confirmed the synthesis of silver nano particles. All the figures reveals that AgNPs synthesized in the ratio of 1:9 (plant extract and $AgNO_3$, 2mM) gave the best results.

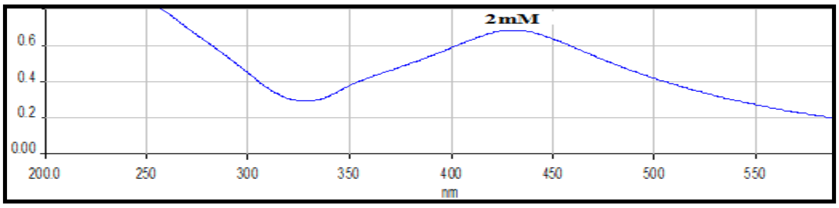


Fig 1: (a) UV –Vis absorption spectra of synthesizedAgNPs using 2mM $AgNO_3$.

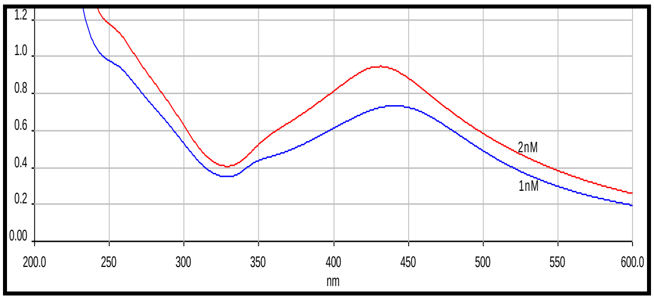


Fig 1(b): UV –Vis absorption spectra of synthesizedAgNPs using 1mM $AgNO_3$ and 2mM $AgNO_3$.

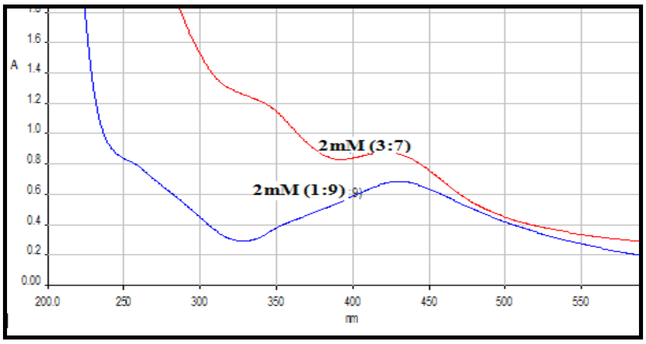


Fig 1(c): UV –Vis absorption spectra of synthesizedAgNPs using 3:7 and 1:9 ratio of Plant leaves extract and $AgNO_3$ (2mM) solution.

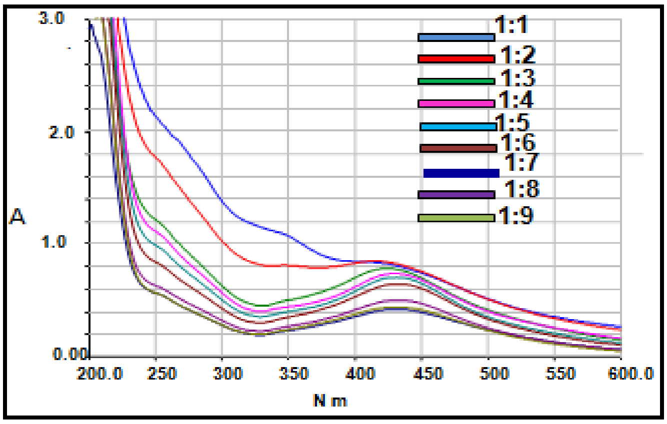


Fig 1(d): Different ratio of Plant leaves extract and $AgNO_3$ (2mM) solution range from 1:1 to 1:9.

3.2 X-ray diffraction analysis of synthesized AgNPs

Intense peak occurring at 27.70, 32.25, 38.18, 46.21, 54.60, 57.55, and 77.11 which indexed the lattice planes (111), (200), (211), (220), (311), (222) and (420) respectively of a pure cubic face centred crystalline (fcc) structure of silver. These Bragg's reflections are corresponding to the planes which are in good agreement with the database of Joint Committee on Powder Diffraction Standards(JCPDS) file no.04-0783.

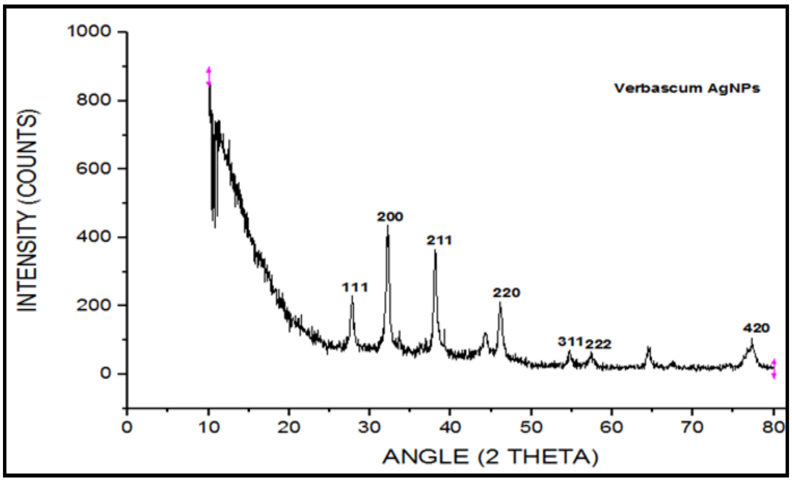


Fig 2: XRD pattern of AgNPs using leaves extract of *Verbascum Thapsus*

3.3. TEM analysis of synthesized AgNPs

To determine the morphology and particles size of silver nanoparticles synthesized by *Verbascum thapsus*, transmission electron microscopy was carried out. TEM micrograph figure 3 revealed that the particles are spherical and well dispersed without agglomeration. The particles size of synthesized silver nanoparticles from *Verbascum thapsus* leaves extract is in the range of 5-40 nm. It has been noted that biomolecules present in the leaves extract is responsible for reduction of silver salts to nanoparticles and also act as a capping agent, which inhibiting the nanoparticles from aggregation [21].

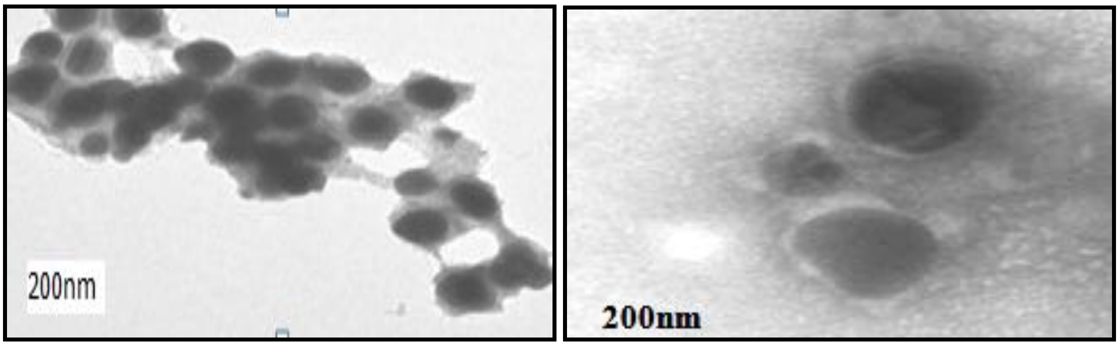


Fig 3: TEM micrograph of AgNPs using leaves extract of *Verbascum thapsus*

3.4. Particle Size Analysis

Dynamic light scattering (DLS) is a technique used to determine the size distribution profile and polydispersity index of particles in a colloidal suspension. Polydispersity index (PDI) is a measurement for distribution of AgNPs from 0.000 to 0.5. PDI greater than 0.5 values indicates the aggregation of particles. In this study, PDI was found to be 0.363 and various sizes of nanoparticles with effective diameter around 195.3nm.From this, it is clear that AgNPs synthesized from *Verbascum thapsus* leaves extracts does not aggregate at all.

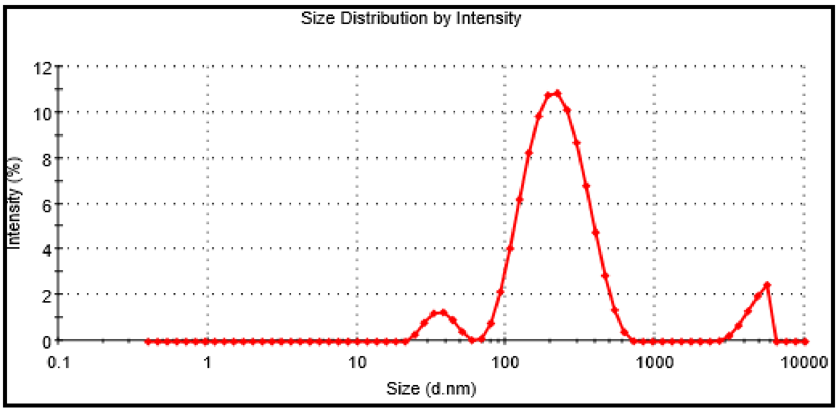


Fig 4: Particle size distribution profile of AgNPs using leaves extract of *Verbascum thapsus*

3.5. FTIR

FTIR spectrum of the leaves extract shows peaks at about 3435,2915,2328,1614,1384 and 1108cm^{-1}. Peak at 3435cm^{-1} is indicative of O-H group due to presence of alcohols, phenols, carbohydrates etc. C-H bond of alkyl group showed peak at 2915cm^{-1}. Peaks at 2328cm^{-1} and 1614cm^{-1} is due to N-H bond of amino acids. Peaks at 1384cm^{-1} corresponded to C-N stretching vibration of amide group. C-O stretch assigned to alcohols represented by peak at 1108cm^{-1}

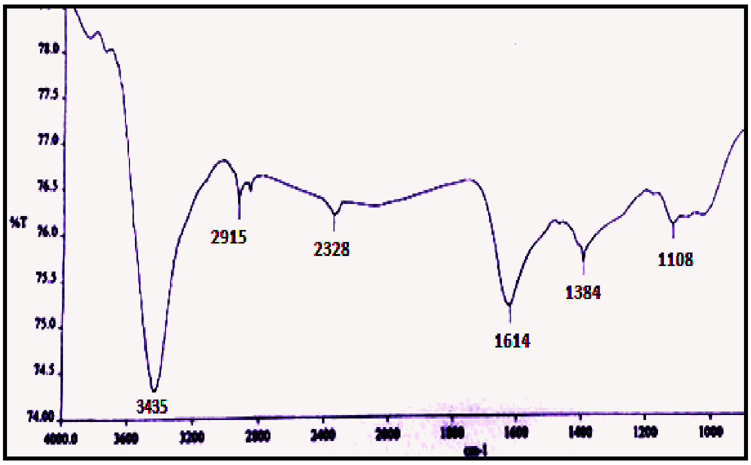


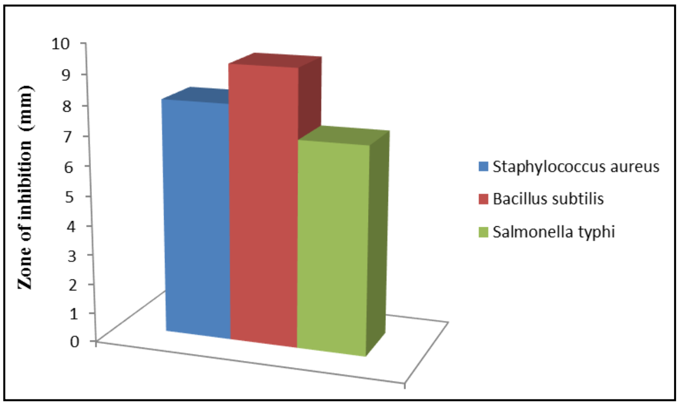
Fig 5: FTIR spectrum of AgNPs using leaves extract of *Verbascum Thapsus*

3.6. Antibacterial property of synthesized AgNPs

The synthesized AgNPs showed the maximum antibacterial potential against grams positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative (*Salmonella typhi*) and also found to be inactive against gram negative Escherichia coli

Table 1: Zone of inhibition (mm) of silver nanoparticles synthesized from *Verbascum thapsus* against pathogenic bacterial strains

Bacteria	Zone of inhibition (mm)	Positive Control (Streptomycin- 25 µg)
Staphylococcus aureus	8.0	24.3mm
Bacillus subtilis	9.3	23mm
Salmonella typhi	7.0	18.3mm



Histogram1. Zone of inhibition (mm) of silver nanoparticles synthesized from *Verbascum thapsus* against pathogenic bacterial strains.

3.7. Photocatalytic Degradation of Methylene blue

Photocatalytic activity of silver nanoparticles was evaluated by the degradation of methylene blue, which was carried out in the presence of sunlight. The absorption spectrum peaks for methylene blue was decreased at different time intervals. The intensity of the absorption peaks at 660 nm for methylene blue was decreased gradually with the increase of the exposure time which shows the photocatalytic degradation reaction of methylene blue. The degradation percentage was increased as increasing the exposure time of dye silver nanoparticles solution in sunlight (Figure 6). Absorption peak for methylene blue was found at 660 nm in visible region which diminished and finally, it disappeared while increasing the reaction time. The percentage of degradation efficiency of silver nanoparticles was calculated as 87% at 48 h.

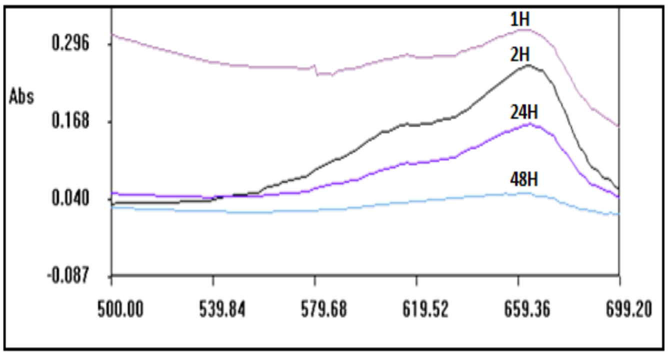


Fig 6: The absorption spectra of aqueous solution of methylene blue treated with 20mg of synthesized silver nanoparticles using *Verbascum thapsus* at different time intervals

4. Conclusion

Silver nanoparticles were successfully obtained by bioreduction of silver nitrate solution using *Verbascum thapsus* leaves extract. The reduction of silver ions and stabilization of the AgNPs were supposed to occur due to the Surface Plasmon Resonance with the biochemicals present in the *Verbascum thapsus* leaves extract which were characterized by UV-Vis, XRD, TEM, DLS and FTIR techniques. The results show that synthesized AgNPs comprised of significant antibacterial potential against gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative (*Salmonella typhi*). And the results of the photocatalytic study reveal that these biosynthesized silver nanoparticles have efficiency to degrade methylene blue under sunlight irradiation. Therefore, these can find applications in pharmaceutical, textile industry and water treatment plants.

5. Acknowledgement

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