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ORIGINAL ARTICLE

Antibacterial and photocatalytic activities of biologically synthesized silver nanoparticles using aqueous extract of *Terminalia bellirica* fruit

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ABSTRACT
In this work, silver nanoparticles are synthesized by using Terminalia bellirica (Bahera)fruit aqueous extract as a reducing and capping agent. Synthesized silver nanoparticles were characterized by UV-Vis. Spectroscopy and Dynamic Light Scattering. An absorbance peak at 440 nm was observed for silver nanoparticles synthesized by Terminalia bellirica fruit extract. The average size is in the range of 10-25 nm. Nanoparticles show good photocatalytic activity against methylene blue dye. Silver nanoparticles (AgNPs)of Terminalia bellirica fruit aqueousextract showa good bactericidal effect against E.coli.
Keywords: *Biological synthesis, Silver nanoparticles (AgNPs), Antibacterial activity, Photocatalytic activity.*

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INTRODUCTION

Different optical devices have been developed by synthesized nanoparticles of noble metal like silver,copper, and gold[1].Because of great surface area and unique physical, optical, electromagnetic properties, nanoparticles have much importance than bulk materials. As the size of nanoparticles decreases, surface area increases which plays an important role in catalytic and biological properties of silver nanoparticles. Chemical method although the most popular method for synthesis of nanoparticles [2]but there are so many side effects because of harmful chemicals used. There are so many different uses of noble metal nanoparticles in daily life like cosmetics, soaps,shampoo, etc. Similarly, these nanoparticles are also useful in medical fields[3].

So there is a great requirement of non-chemical i.e. green synthesis of nanoparticles which has no harmful effects[4–6].The plant extracts are much more beneficial than other biological agents as they do not require complex reactions and can be easily used for large scale synthesis as they are easily available[7].The reduction of metal ions to nanoparticles takes place by the phytochemicals like phenols and flavonoids etc. present in the plants and these phytochemicals protect the plants from various pathological conditions.The aqueous extract of *Terminalia bellirica*fruitis used for the synthesis of zinc, iron and copper oxides nanoparticles which are good biological and pharmaceutical agents to struggle against different pathogens[8].

T. bellirica belongs to the Combretaceae family, a huge deciduous tree discoveredin the course of India. This plant generally grows in Nepal, Sri Lanka, Malaysia and South East Asia[9]. It is normallyregarded as "Bahera" or Beleric or myrobalan. This fruit is used in the ayurvedic remedy triphala. *T.bellirica* is used to shield the liver, to lower cholesterol levels in the body and also used in the treatment of digestive as well as respiratory disorders [10]. It is a good antioxidant and lowers sugar like glucose etc. due to the presence of polyphenolic compounds like gallic acid, tannins and flavones, etc. [11]. Hence, this plant becomes selected for the synthesis of AgNPs.

Because of the hazardous effect of chemical synthesis of nanoparticles on environment green synthesis of nanoparticles is used as an alternative approach[12–15].Biological synthesis of nanoparticles using plant, algae, fungi, and bacteria are in trend nowadays[16–20]. Biological synthesis by using plants has advantages over the microbial method because of less biohazardous, eco-friendly and non-toxicity[21,22]. Phytochemicals present in plant extract are responsible for the reduction of metal ion to its zero-valent form[23,24].

MATERIALS AND METHODS

Collection of the material

The *T. bellirica*fruits were collected from the local market, Mathura. All the chemicals used were of AR grade,purchased from CDHand used without further purification.

Plant extract preparation

Sample of *T. bellirica*fruit was first washed with water then sterilized with alcohol.This sample was dried in shade and grind to prepare a smooth powder. 2 grams of *T. bellirica* fruit powder was dissolved in 200 ml of triple distilled water and kept overnight. After filtering the aqueous extracts through the Whatman filter paper, the solvent was evaporated. The extract was collected and stored at 4°C for further use.

Synthesis of silver nanoparticle using *T. bellirica*

10ml of the *T. bellirica*fruit extract put into the conical flask and 100 ml 1mM of the silver nitrate is added to the extract.This reaction mixture was stirred for 30 minutes at 80°C for the synthesis of silver nanoparticles. The synthesis of silver nanoparticles (AgNPs) was confirmed by the change in color of the reaction mixture (pale yellow to light brown) as shown in Figures 1 and 2.The surface plasma resonance vibration is responsible for this color change.

Figure 1*: T. bellirica* fruit extract

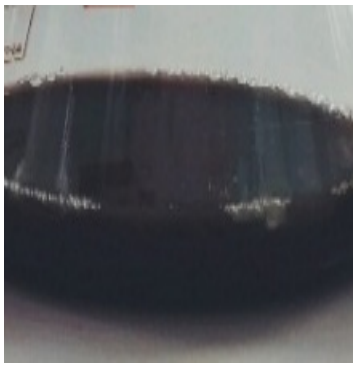

Figure 2: T. bellirica mediated silver nanoparticles

Preparation of media

The media was prepared as per the guidelines given in the Bacteriology Manual [25].All the dry ingredients given in the manual were taken in beaker and dissolved in distilled water.The so prepared medium was sterilized by keeping this in autoclave at 121°C for 30 minutes. 15 ml of this medium was poured in petri plate and this plate was incubated for 24-48 hours at 37°C.

Collection of Bacteria

E. coli was procured from CSIR-IMTECH (MTCC-40), Chandigarh

Characterization

Initially, the synthesis of silver nanoparticles (AgNPs)was confirmed by color change of solution and by absorption spectrum produced by UV-Vis spectrophotometer at 200-700 nm wavelength. The reduction of silver ions to the nanoparticle was confirmed by the UV-Visible spectra of the solutions [26]. Dynamic Light Scattering was employed to determine the size using Zeta sizer Nano ZS (Malvern Instruments, UK). All the analysis was carried out in an automatic mode.

Antibacterial assay

Antibacterial activity of different nanoparticles was tested by the disc diffusion method against gram-negative (*E. coli*) bacterial cultures, prepared by the standard process. Before the use, petri plate and media were autoclaved.10µL of pure bacterial culturewas uniformly spread on nutrient agar media in petri plate using L-rod. A 10µL sample of AgNPs was poured on a sterile disc. Three sterile discs (1 disc

for AgNPs,1 for $AgNO_3$ and 1 for an antibiotic) were placed on the bacterial culture in the petri plate.This plate was incubated for 48 hours at 37°C.After 48 hours 'results were observed. The zone of inhibition was measured in mm.

Photo catalysis

The photocatalytic activity of the AgNPs was tested against methylene blue (10 mg/l) aqueous solution.For this activity reaction mixtures were prepared by mixing 1ml of fruit extract in 10 ml of methylene aqueous solution and another solution was prepared by mixing 1 ml of nanoparticle suspension in 10 ml of methylene blue and fruit extract solution. This mixture was stirred in dark conditions for 30 minutes to establish the equilibrium in the mixture. After equilibrium, the mixture was placed under sunlight for 5-6 hours. The reaction mixture was monitored by UV-Vis. Spectrophotometer at regular time intervals.

RESULTS AND DISCUSSIONS

Physicochemical characterization

The free electrons are responsible to produce an SPR absorption [27–30] band. These free electronsin metal nanoparticles jump freely between the conduction and valence band which are close to each other. Bio reduction of silver ions present in their solution into respective nanoparticles by the phytocompounds present in the *T. bellirica* fruit extract was studied using UV-Vis Spectrophotometer[31–33]. The highest absorbance peak was observed at 440 nm (Figure 3).The size of the obtained silver nanoparticles was in the range of 10-25nm (Figure 4).

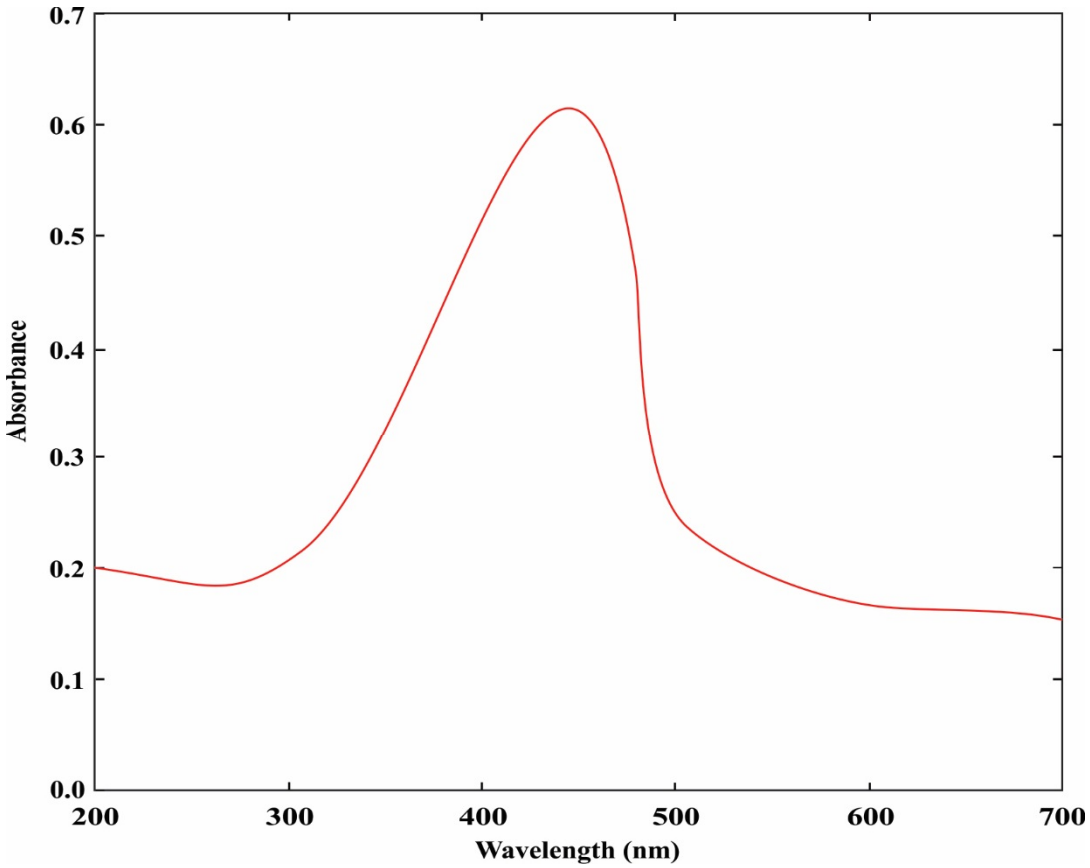

Figure 3: UV-Vis spectra of *T.bellirica* mediated silver nanoparticles

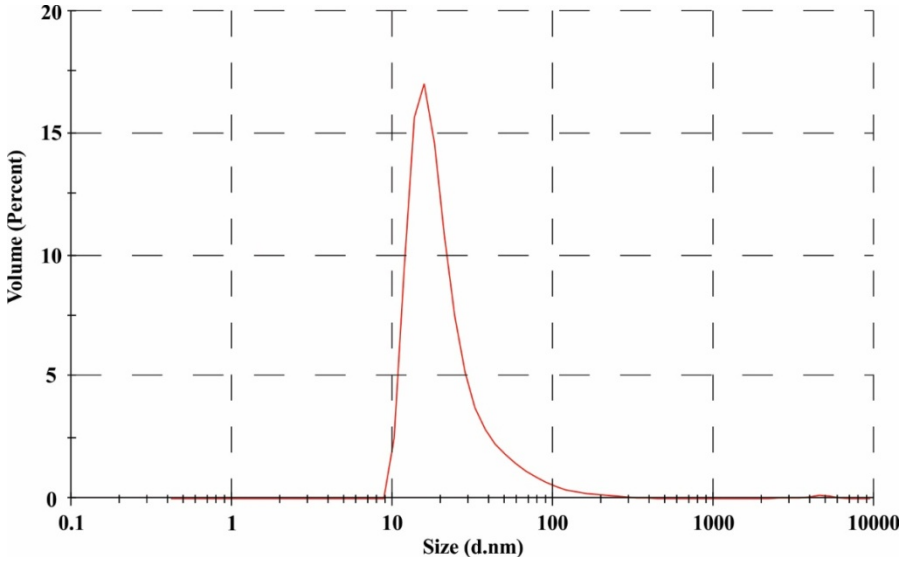

Figure 4: Size analysis of *T.bellirica* mediated silver nanoparticles

Photocatalysis

Methylene blue is an aromatic dye which is used in textile industries for various purposes. But the effluent from these industries contain some amount of MB. The contact with this contaminated water may irritate eyes, skin and gastrointestinal tract[34].When this contaminated water was treated for a particular period with AgNPs synthesized by *T.bellirica* fruit extract, the color of the reaction mixture decreased gradually from dark blue to light blue because of the photocatalytic degradation reaction of AgNPs under sunlight. These results were confirmed by the absorption spectra of dye solution compared with the reaction mixture with fruit extract and with AgNPs. The absorption band for MB dye is observed at 665 nm due to the n-π* transition of electrons in MB dye[35,36].The photocatalytic degradation of reaction mixture was clear from the decreasing absorption intensity as time passes under sunlight. This decrease in absorption intensity is due to the surface plasma resonance of AgNPs. The maximum degradation of methylene blue dye was noticed at 72 hours[37].In the present work, degradation was followed up to 10 hours, a noticeable decrease in the peak intensity was observed (Figure 5).So it is clear from the results that AgNPs synthesized by *T.bellirica* fruit extract are good efficient photocatalytic agents for the degradation of methylene blue dye under sunlight.This method can be used to purify water eliminated from textile industries.

Antibacterial Activities

Because of the enhanced surface area of nanoparticles than atomic size, nanoparticles can easily interact with bacterial cells.The interaction of AgNPs with bacterial cellskills the bacteria by attacking the respiratory chain and cell division as AgNPs get attached to sulfur and phosphorous constituents of the bacterial cells [38].This result is clear from Figure 6, that AgNPs synthesized by *T. bellirica* fruit aqueous extract are good bacteriostatic agents. Zone of inhibition for antibiotics 7 mm, for $AgNO_3$ 2 mm and AgNPs 3 mm was measured. AgNPs have better antibacterial activity than the free silver ions [39].

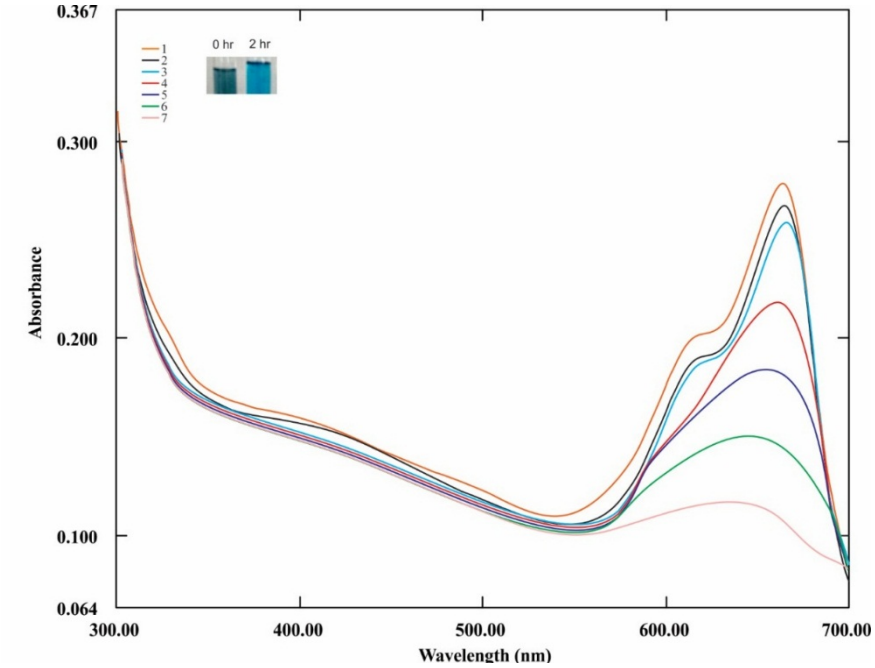

Figure 5: UV-Vis spectra for photocatalytic degradation of methylene blue

1. Methylene blue
2. Methylene blue with fruit extract
3. Methylene blue and fruit extract with silver nanoparticles – after 30 min.
4. Methylene blue and fruit extract with silver nanoparticles – after 01 hr.
5. Methylene blue and fruit extract with silver nanoparticles – after 02 hrs.
6. Methylene blue and fruit extract with silver nanoparticles – after 05 hrs.
7. Methylene blue and fruit extract with silver nanoparticles – after 10 hrs.

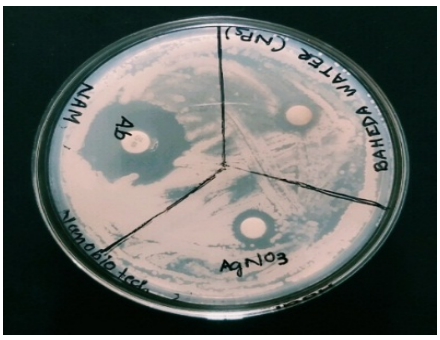

Figure 6: Zone of inhibition against *E.coli*

CONCLUSION

The green synthesis of nanoparticles has been successfully carried out using the fruit extract of *Terminalia bellirica*. The size of the obtained silver nanoparticles was in the range of 10-25 nm. The phytocompounds present in *T. bellirica* fruit extract are responsible for the bioreduction of metal ions into metal nanoparticles. AgNPs showed a good bacteriostatic effect against gram–ve bacteria *E.coli.* These nanoparticles also showed good photocatalytic activity against methylene blue under sunlight thus green approach of synthesizing nanoparticles can be utilized in antibacterial and catalytic action.

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CONFLICTS OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this article.

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