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Optimization of Green Synthesis of Silver Nanoparticles from Leaf Extracts of *Tabernaemontana heyneana* and evaluation of their catalytic activity on reduction of methylene blue

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Abstract: The aim of the study was to optimize the process parameters involved in the green synthesis of silver nanoparticles (AgNPs) by aqueous leaf extract of *Tabernaemontana heyneana* and to evaluate the catalytic reduction of methylene blue (MB) perhaps for the first time. The various optimum parameters include boiling time for preparation of extract, concentration of extract, silver nitrate concentration, effect of pH, reaction time and temperature were analysed using UV-vis spectrophotometer. The optimized AgNPs were characterized by AFM and evaluated the catalytic activity on reduction of methylene blue. The optimized factors for synthesis of AgNPs were 10 min boiled leaf extract, 3 ml leaf extract addition to reaction mixture, 1 mM $AgNO_3$ concentration, pH 9 of reaction mixture, reaction time 2 h and temperature 80°C. AFM characterization confirmed the oval shaped AgNPs with size 5-20 nm. The synthesized AgNPs showed a good catalytic activity on the reduction of methylene blue. Therefore, it is proposed that, the optimized parameters helps in getting hold of applications in environmental and biomedical sciences.

Keywords: *Tabernaemontana heyneana*, AgNPs, AFM, Methylene blue.

INTRODUCTION

Today's era of research in 'Nanotechnology', fascinating to researchers and scientist because of its ample applications like Nanomedicine, nanoelectronics energy production and consumer merchandise. The associated application is due to its macroscopic physical and chemical properties in relation to the bulk macroscopic properties [1].

Synthesis of nanoparticles from novel metals of different size, shape and composition, appealing immense interest in getting hold of applications in environmental and biomedical sciences [2, 3]. Especially, the noble metal silver nanoparticles are studying more for their potential applications in both industrial and medical spheres [4, 5]. Therefore, the synthesis of stable and efficient silver nanoparticles offers an advanced prospective in the sphere of scientific research community. Currently, many methods are being reported along the establishment of silver nanoparticles, such as chemical, physical and photochemical routes. Each method uses toxic chemicals as a precursor or reducing or stabilizing agents, to obtain different particle sizes and configurations, but they are environmental catastrophe. Consequently, studies began under the green chemistry for the search of environmental benign approaches for the development of nanoparticles [6] and hence green synthesis process focused much as a practicable alternative for the shaping of metal nanoparticles without any chemical mixture. Biological resources such as bacteria, fungi, algae and plant material are employed in the formation of silver nanoparticles. Plant material, specifically leaf extract is considered as an advantage than microbial system, because the reduction rate of metal salts is rapid and the procedure is simple unlike of culturing and maintaining the cell and pathogenicity [7]. Subjects reported that leaf extracts found to be a suitable for the biosynthesis of AgNPs [8]. *Tabernaemontana heyneana* the medicinal herb belongs to Apocynaceae family and is known as Bilikodsalu, Halmeti, Maddarassa gida, Madle mara, Nagarkuda in Kannada. It is indigenous to India (Goa, Karnataka, Maharashtra, Kerala, Tamil Nadu). *T. heyneana* was considered a cure for skin diseases and oxidative damage. Contradictorily, *T. heyneana* was also used for antioxidant and renoprotective activities [9].Therefore, *T. heyneana* leaf extract was considered for the synthesis of AgNPs.

Dyes are the major category of man-made organic compounds applied in a broad scope of merchandise such as clothes, leather accessories, cosmetic, pharmaceuticals and textile manufactures. Study demonstrated that only 12% of these dyes are used in the dyeing process remaining 20% wastages enter the environment primarily through water [10]. These effluents are resistant to microbial system which was unmanageable for them to reduction by conventional biological and physical-chemical treatments [11]. For that reason, newer treatments are employed using nanoparticles in recent years. Suvith and Philip reported that the biosynthesized silver nanoparticles can act as a good catalyst in the reduction of methylene blue due to high surface area and energy with potential enhanced activity [12].

Here, we explore the optimization parameters as one of the fruitful inputs for synthesis of AgNPs using leaf extract of *T. heyneana* and investigated its catalytic activity in the reduction of methylene blue perhaps for the first time. The procedure for the development of stable AgNPs is simple, rapid and safe. The efficiency of AgNPs as a hopeful prospect for the reduction of methylene blue.

MATERIALS AND METHODS

T. heyneana leaf extract Preparation

T. heyneana leaves (Fig.1a) were collected from Karnataka Institute of DNA Research (KIDNAR) garden, Karnatak University, Dharwad, India. The herbarium (GK/SMH 42) was deposited in the Department of Botany, Karnatak University, Dharwad, India. The leaves were washed quite a few times with distilled water to remove the grime's and blotted using blotting paper. The blotted leaves were cut into small pieces and boiled in a 250 ml glass beaker along with 200 ml of sterile distilled water. After churning, the vividness of the aqueous solution changed from watery to yellow colour. The aqueous extract was separated by Whatman No.1 filter paper and then centrifuged at 1,200 RPM for 5 minute to remove the heavy biomolecules. The *T. heyneana* aqueous leaf extract (TE) was stored in a sterile condition to be used for synthesis of silver nanoparticles from silver nitrate. All glassware's were washed with distilled water and dried in hot air oven, prior to use.

Optimization study of AgNPs

Extract preparation and optimization of different parameters was followed as described previously [13]. To find the optimum boiling time of the infusion, the leaf extract was boiled for 5min, 10min and 15min. and then 2ml of leaf extract was added to 50ml of 1mM $AgNO_3$. Further, the extract concentration was optimized by the addition of an extract concentration (10. ml, 2.0ml and 3.0ml) to 50ml of 1mM $AgNO_3$. The different concentration of silver nitrate (0.5mM, 1.0mM and 1.5mM) was reacted to the reaction mixture to study the tremendous effect on the size of synthesized AgNPs. The effect of pH in the reaction mixture was adjusted to different pH (6, 7, 8, and 9) to study the smaller size and number of particle formations. The effect of reaction time on the formation of AgNPs was decided at various time intervals (10min, 30min, 60min, 2hr and 24hr) and the effect of reaction temperature was evaluated from10°C to 100°C to find the characteristic maximum absorbance peak of AgNPs. The silver nitrate ($AgNO_3$), A.R., used in this study was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India and was processed without further purifications.

AFM Characterization

The morphology, particle size, and distribution of the biogenic nanoparticles (air dried) were supported using an Atomic Force Microscope (Model-Nanosurf easyscan 2 AFM, Switzerland). AFM images were taken with silicon cantilevers of Tap190Al-G. A nanosurf easy scan 2 software tools was used for the roughness and particle analysis. The technique was standardised according to the need of the study.

Effect of synthesized AgNPs on the reduction of methylene blue

The catalytic activity of synthesized AgNPs was assessed by following Jebakumar and Sethuraman [14]. Briefly, in one set 1 ml of methylene blue (1×10^{-4} M) was mixed with 0.2 ml leaf extract and 1.8 ml of water, this reaction was observed after 30 min using UV–visible spectrophotometer. Another set, 1 ml of methylene blue (1×10^{-4} M) was mixed with 0.2 ml leaf extract and 2 ml synthesized AgNPs and this reaction was checked at different time intervals (30 and 45 min). In all the sets total volume of the reaction mixture was made up to 3 ml. The values of the absorption maxima (λ_{max}) were compared, with that of methylene blue.

RESULTS AND DISCUSSION

Optimization of different parameters

Synthesis of AgNPs comprises the addition of a leaf extract to silver nitrate solution and incubating the infusion at room temperature. The biomolecules present in the leaf extract reduces silver ions to silver nanoparticles. In order to enhance the reproducibility of the experimental results and to obtain good AgNPs, it is crucial to optimize the different process parameters, such as boiling time of leaf extract preparation, leaf extract and $AgNO_3$ concentration, Effect of pH, reaction time and temperature of reaction mixture. The factors may differ with the plant extract and plant part used, therefore optimizing these parameters are important as also reported by other researchers [15-17]. The formation of AgNPs was noticed by colour change that occurs when leaf extract added to silver nitrate solution. The change in colour from pale yellow to brown colour was due to surface plasmon resonance. Initially, 2 ml of leaf extract was added to 50 ml of 1mM $AgNO_3$, the development of brown colour at room temperature indicating the synthesis of AgNPs (Fig-1b). Moteriya and Chand recorded such colour change response for different plants [13].

UV-vis spectroscopy study of AgNPs

UV-vis spectroscopy is a versatile tool to study the synthesis of AgNPs in infusion. The synthesized silver nanoparticles showed the characteristic absorbance band in the visible region in the range of 350 – 600 nm. Further, the number and size of nanoparticles formed depends on the peak size and peak intensity. Zayed et al reported that broader peak indicates larger particles and narrow peak indicates smaller particles [18]. Additionally, the absorption peak intensity indicates the number of particles formed suggests that both are directly proportional to each other. Thus the optimizing of various parameters was analysed in the present [19] work.

Initially, the boiling time for leaf extract preparation was optimized. The leaf extract was boiled for 5, 10 and 15 min. and then 2 ml of leaf extract was added to 50 ml 1mM $AgNO_3$.Leaf extract boiled for 10 min (Fig.1c) showed higher absorbance intensity and could be taken for the extract preparation than 5 and 15min.The results are in accord with previous works on different plants [13]. The next factor optimized was with the addition of extract concentration in the reaction medium. Different concentration (1.0, 2.0, 3.0 ml) of 10 min boiled extract was added to 50 ml 1mM $AgNO_3$. Extract concentration 3 ml (Fig.1d) showed higher absorption intensity. It also noticed that, the absorbance intensity increased with increasing extract concentration indicating the possibility of biomolecules was required in reduction of silver ions to silver nanoparticles is more and results in the production of more AgNPs. Earlier studies used 15 ml and 10 ml extract concentration for the synthesis of AgNPs, therefore 2 ml leaf extract concentration was decided for the synthesis of AgNPs from the leaf extract of *T. heyneana* [19, 20].The size of synthesized AgNPs depended on the concentration of silver nitrate solution. Therefore, 10 min boiled 3 ml leaf extract concentration was added to 50 ml different concentrations (0.5, 1.0 and 1.5 mM) of $AgNO_3$. $AgNO_3$ 0.5mM did not show any peak resulting no synthesis of AgNPs; whereas 1.0 and 1.5 mM $AgNO_3$ (Fig.1e) showed a characteristic peak at 435 nm illustrating the synthesis of AgNPs. But studies demonstrated that higher concentration of $AgNO_3$ forms larger particle size and the present study is in agree, where the broad spectrum with higher absorbance intensity, hence 1 mM $AgNO_3$ was decided for the synthesis of AgNPs [21]. pH of the reaction mixture plays a significant part in the synthesis of AgNPs. Three ml 10 min boiled leaf extract was added to 50 ml 1 mM $AgNO_3$ and the infusion was mutated with different pH (6, 7, 8, 9).At pH 6 the absorbance peak was broader and less intensity (Fig.1f), illustrating less number of particle production with larger size. As the pH increased, from pH 7 to pH 9, the peak becomes narrowed and steadily increased in intensity; clearly illustrating smaller size and more number of particles produced. Finest particles materialized at pH 9 and was advisable for the synthesis of AgNPs as also reported by [22]. Certainly, the effect of reaction time was evaluated at different time intervals (10min, 30min, 60min, 2h and 24h).The characteristic maximum absorbance peak of synthesized AgNPs was noticed at 435 nm (Fig.1g). No significant variation was found either in absorption peak or intensity after 2h and 24 h reaction time illustrates the completion of reaction. Earlier studies reported the reaction time for the synthesis of AgNPs was completed within 24 h by different plant systems and our results are found to be in agreement [23 24]. Hence, 2 hr reaction time was decided for the synthesis of AgNPs. Finally, the effect of temperature on the synthesis of AgNPs was assessed by heating the reaction mixture at different temperatures (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100°C). Initially, from 10°C to 50°C no peak intensity was noticed illustrates reaction process not started (Fig.1h). As the temperature increasing from 60°C to 100°C, an increase in peak intensity was found could be increase in reaction rate for nanoparticle formation. Mock et al., and Fayaz et al., demonstrated the intensity of peak depends on the size of the synthesized AgNPs as with higher temperature, particle size may be smaller, results into intensity of surface plasmon band of silver nanoparticles and also reported by [8]. Hence, temperature at 80°C was decided for the synthesis of AgNPs with smaller size particles.

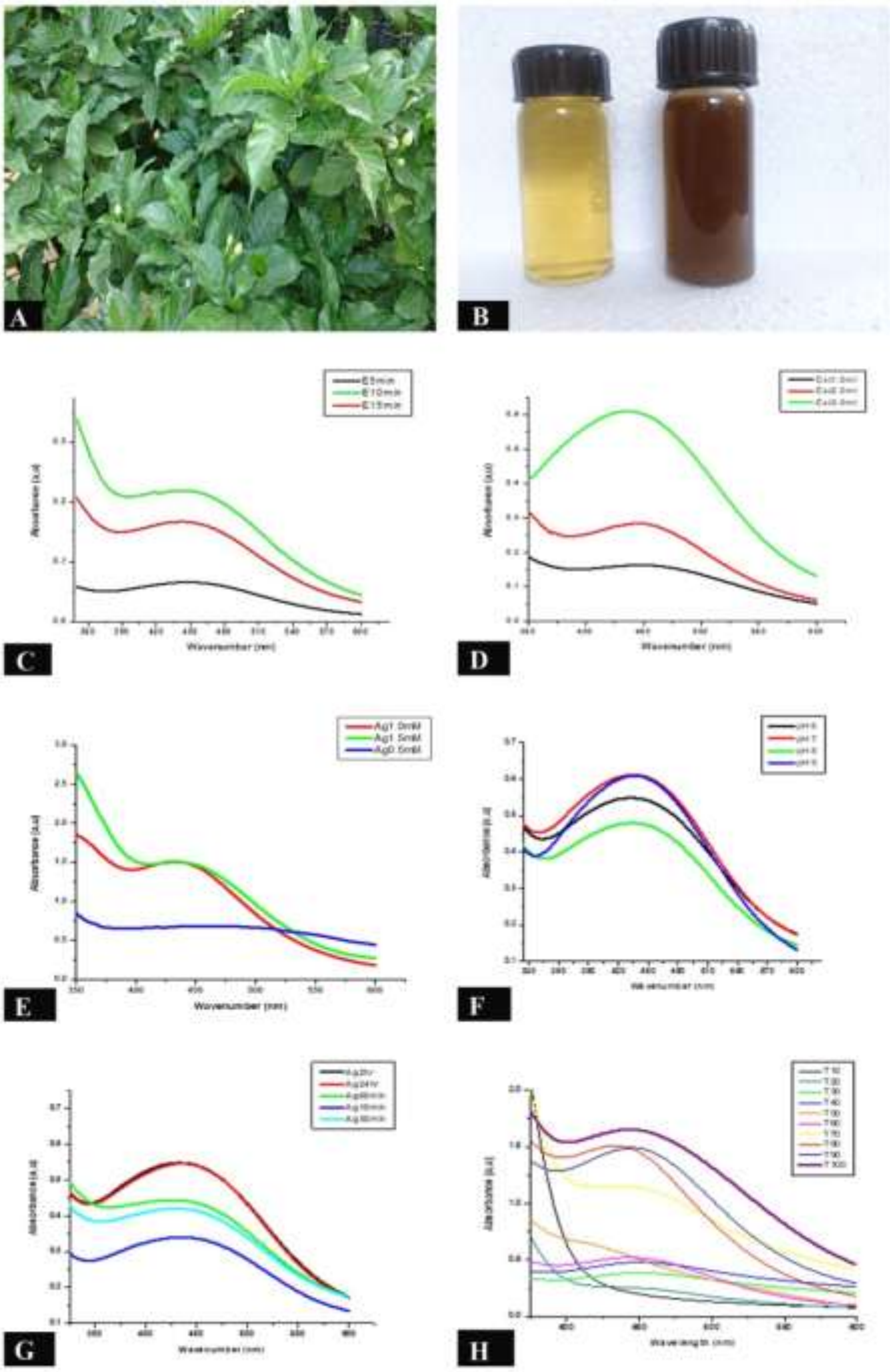

Fig-1: (A) *T.heyneana* leaves, (B) Colour change image, (C) Effect of boiling time, (D) Effect of extract quantity, (E) Effect of silver nitrate concentration, (F) Effect of pH, (G) Effect of reaction time and (H) Effect of temperature.

Conclusively, optimum factors for synthesis of AgNPs by *T.heyneana* leaf extract was 10 min boiling time for leaf extract preparation, 3 ml leaf extract addition to reaction mixture, 1 mM $AgNO_3$ solution, pH 9 of reaction mixture, reaction time 2 h and temperature 80°C for the synthesis of AgNPs.

AFM characterization of synthesised AgNPs

The AFM is magnificent tool to examine the biophysical properties of nanoparticles. Tapping mode was employed to investigate the surface topography of optimized AgNPs. It presents the effectiveness of 3D visualization and provides information on many physical attributes including size, shape, and roughness in terms of qualitative and quantitative. The AFM images (Fig-2a) were obtained under optimized conditions and all the scans were 10 x 10 µm2 in size. The perspective view of the synthesised AgNP possesses oval shape with a size in the range of 5-20 nm and is monodispersed. Previous studies reported uneven shapes and sizes from different plant systems [27, 28, 6]. The roughness of the surface were determined by measuring the root-mean-square (RMS) roughness parameter, defined as the root-mean-square average of the height (z) taken from mean data plane. Table 1 showed the roughness properties, which gives the information about the statistical average properties and the physical body of the height distribution. Two parameters were set, which are indicative of the roughness of the surface topography maps (Sa and Sq). Sq is the standard deviation of the surface topography and has a value of 28.00 nm. The Sa was 18.77 nm which detects decrease in variation frequency of the surface area, indicates a definite change in surface topography [29]. Overall, the roughness parameters, Sa and Sq, suggest a surface distributed with large but shallow bags of varying sizes and in compliance with a qualitative assessment of the image in 3D view (Fig.2b). This case of quantitative topographic information suited for the analysis of nanoparticles and other kind of atom system.

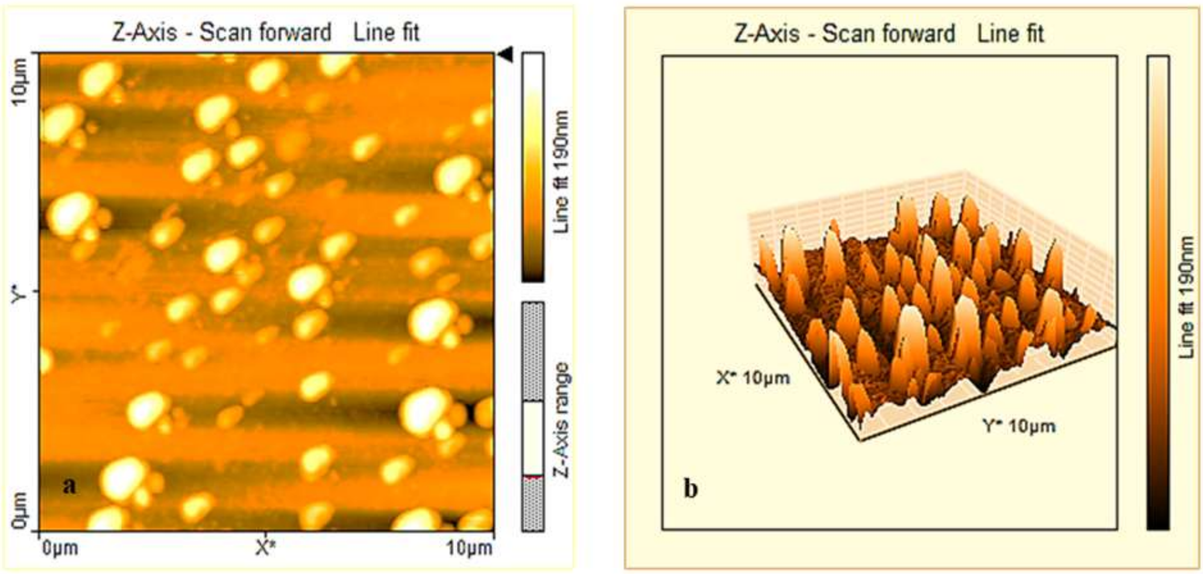

Fig-2: AFM images; (a) Prospective view, (b) 3D view

Table-1: Roughness properties of synthesied AgNPs.

Area	100.8pm^2
Sa	18.77nm
Sq	28.00nm
Sy	197.19nm
Sp	132.19nm
Sv	-64.99nm
Sm	-5.576fm

Effect of synthesized AgNPs on the reduction of methylene blue It is known that AgNPs are good catalyst in the area of dye reduction. The plot of relative absorbance intensity with wavelength (Fig.3) after 30 min interval of time reveals that the complete reduction of dye. Pure methylene blue has a λ_{max} value at 664nm. Thirty minutes after the addition of extract to dye the absorbance was steadily decreased and shifted to lower wavelength illustrating the ability of leaf extract to degrade the dye. Mixture containing dye, extract and AgNPs after 30min and 45min of time interval showed a significant decrease in absorbance of methylene blue suggesting AgNPs an efficient catalyst. The efficient catalytic activity of the AgNPs was due to electron relay effect as also reported by [11].

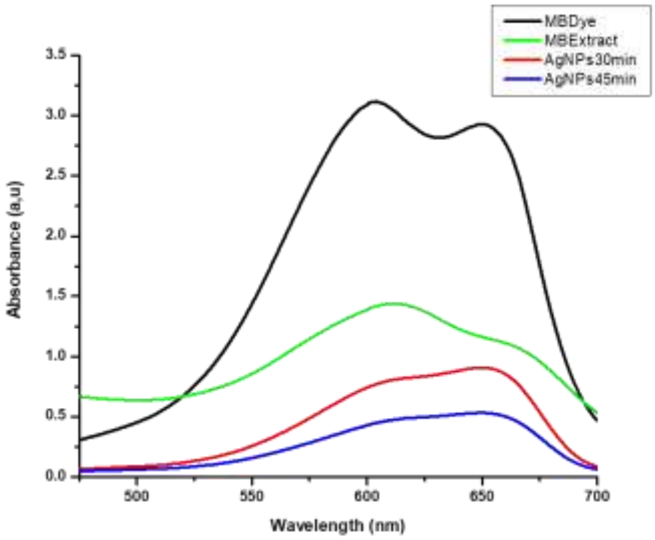

Fig-3: Effect of silver nanoparticles on the degradation of methylene blue

CONCLUSION

We have demonstrated that aqueous leaf extract of *T.heyneana* is suitable for the synthesis of AgNPs. Various optimum parameters were analysed for better yield of AgNPs. AFM characterization confirmed the oval shaped AgNPs with smaller in size. The biosynthesized AgNPs exhibited efficient catalytic activity in a reduction of methylene blue.

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