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Characterization and Evaluation of the Antibacterial and Dye-degrading Potential of Silver Nanoparticles from *Ayapana triplinervis* Vahl

Sona S. Dev[1], Chikku Santhosh[1], Greeshma James[1]

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ABSTRACT

Background: Over the last decades, silver has been engineered into nanoparticles that attracted much attention and found applications in diverse areas. The small size of the nanoparticles has maximized the total surface area leading to increased activity-to-weight ratio. The present study aims to synthesize silver nanoparticles (AgNPs) from the methanolic leaf extract of a medicinal plant *Ayapana triplinervis* Vahl. and assess their antibacterial and dye-degrading properties.

Methods: The AgNPs produced were characterized by using UV-Vis spectroscopy, FTIR, TEM and SEM. The dye degradation property of these AgNPs was also evaluated.

Results: UV-Vis spectroscopy of prepared silver colloidal solution showed an absorption maximum at 410 nm. The FTIR analysis confirmed the presence of different functional groups which were responsible for the reduction and stabilization of silver ions. The SEM analysis revealed that AgNPs are clustered and exhibit both cubical and spherical shape. TEM analysis helped to investigate the effect of capping agent and precursor concentration on the size and shape of the nanoparticles. The nanoparticles synthesized also exhibit antibacterial activity against *Staphylococcus aureus* and *Salmonella* sp. Catalytic degradation of dyes by these silver nanoparticles in the presence of sunlight was in the order Methyl Red (MR) > Eosin > Methylene Blue (MB).

Key words: Antibacterial activity, *Ayapana triplinervis*, Catalytic degradation, Silver nanoparticles.

INTRODUCTION

Nanotechnology is a multi-disciplinary science dealing with synthesis and manipulation of particles with ultra-fine dimensions. The metallic nanoparticles are considered as the most promising as they exhibit numerous properties due to their large surface area to volume ratio. Compared to other noble metal nanoparticles, silver nanoparticles because of their unique properties such as chemical stability, good conductivity, catalytic antimicrobial and anti-inflammatory activities have attained a special focus.

The high cost and environmental hazards, limits the use of nanoparticles prepared by different physical and chemical methods. Plant-based experimental processes for the synthesis of nanoparticles popularly called the “green synthesis” is evolving into an important branch of nanotechnology mostly due to its low cost, less toxicity and easy scaling up for large scale synthesis. Even other biological methods like synthesizing nanoparticles from microbe, involve the additional cost of microorganism isolation and their culture media. Recent studies on synthesis of silver nanoparticles from plant extracts of *Gymnema sylvestre* leaves (Gomathi *et al*., 2020), *Murraya koneigii* leaf extract (Chahande *et al*., 2020), *Myristica fragrans* fruit extract (Sasidharan *et al*., 2020), *Aegle marmelos* fruit extract (Devi *et al*., 2020), *Moringa oleifera* (Bindhu et al., 2020), *Citrus limon* peel extract (Alkhulafi *et al*., 2020), *Centella asiatica* (Raina *et al*., 2020) has been reported by many researchers.

[1]Department of Biotechnology, St. Peter’s College, Kolenchery, Ernakulam-682 311, Kerala, India.

Corresponding Author: Sona S. Dev, Department of Biotechnology, St. Peter’s College, Kolenchery, Ernakulam- 682 311, Kerala, India. Email: sona.dev@stpeterscollege.ac.in.

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Ayapana triplinervis Vahl (syn.: *Eupatorium ayapana* Vent; *Eupatorium triplinerve* Vahl) is a tropical water hemp belonging to Asteraceae family, used in traditional medicines (Fig 1). The herb is a stimulant, tonic in small doses, laxative when taken in large quantity, antiseptic, haemostatic (the methylene ether of esculetin), contains vitamin C and carotene (Unnikrishnan *et al*., 2014). Not much research has been carried out to exploit the immense potential of this plant.

In this study, the methanolic extract of *Ayapana triplinervis* was used for silver nanoparticle (AgNP) synthesis followed by characterization studies. The antibacterial activity of these nanoparticles as well as its photocatalytic activity on dye degradation was also analyzed.

Fig 1: *Ayapana triplinervis* plant in its natural habitat.

MATERIALS AND METHODS

The experiments were conducted at the laboratory of Department of Biotechnology, St. Peter's College, Kolenchery during the period 2020-22.

Purified silver nitrate salt ($AgNO_3$), *Ayapana triplinervis* plant methanolic extract, bacterial strains such as *E. coli, Salmonella, Pseudomonas, Staphylococcus* and *Bac illus* to check the antibacterial property and deionised distilled water was used in all the experimental work.

Preparation of leaf extract

The leaves of *Ayapana triplinervis* was collected, brought to the laboratory and thoroughly washed in running tap water to remove any debris. It was then rinsed with distilled water and finally, air- dried and powdered using a mechanical blender. This sample weighing 50 grams was taken in a thimble of the Soxhlet apparatus for extraction using methanol as solvent. The mixture was filtered using Whatman filter paper No.1, poured into a round bottom flask, and concentrated using Rotavapor® R-300 (BUCHI). The concentrate was weighed and diluted with methanol (1 mg/ml) and stored at 4°C until further use.

Synthesis of silver nanoparticle

Nanoparticle synthesis was tested using each of the five different concentration of silver nitrate (1 mM, 2 mM, 3 mM, 4 mM and 5 mM), with different proportions of the methanolic leaf extract (1:1, 1: 3, 1: 5, 1: 9, 1: 10 and 3: 10). $AgNO_3$ served as control. These solutions were heated in a water bath, set at 60°C for 3 hours and the colour change was noted. A colour change from yellow to brown indicates the formation of colloidal AgNPs (Fig 2).

The synthesized silver nanoparticles were separated by centrifugation using a REMI centrifuge at 12,000 rpm for 20 min. The supernatant liquid was re-suspended in sterile double distilled water. The process was carried out thrice to get rid of any uncoordinated biomolecules. After the desired reaction period, the supernatant liquid was discarded and the pellets were collected, dried and stored at 4°C until further use.

Characterization of AgNPs

The formation of silver nanoparticles was further confirmed by UV-Visible spectroscopy (ELICO UV-Visible spectrophotometer, Model 1800). The bio-reduction of the Ag^+ ions in solution was monitored by recording the absorption spectra of sample aliquots against water at periodical intervals.

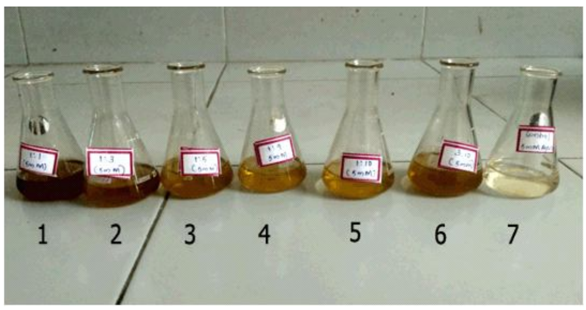
Silver nanoparticles associated biomolecules were identified using the Thermo Avtar 370 FTIR (Fourier transform infrared) spectrometer with the spectral range of 4000–400 cm^{-1} at 4 cm^{-1} resolution and an interferogram of 32 scans using KBr pellets. A small quantity of the solid sample is added to KBr in the ratio 1:100 approximately. The fine powder obtained on grinding the matrix for 3-4 minutes using mortar and pestle, is transferred into 13 mm diameter die and made into a pellet using a hydraulic press by applying a pressure of 7 tonnes. The fine pellet is subjected to FTIR analysis using universal pellet holder.

Surface morphology of AgNPs were analysed using SEM (TESCAN VEGA3) operated at 20kV. The test samples were smeared on the adhesive carbon tape which is fixed on a brass stub, followed by subjecting to gold coating using the sputtering unit for 10 sec at 10 mA of current. The gold coated sample placed in the chamber and secondary electron/back scattered electron images are recorded.

Transmission Electron Microscope (Model: JEM-2100) analysis of the sample was carried out by dropping small amounts on a carbon-coated copper grid and allowing to dry overnight in vacuum, under a lamp. When the sample is subjected to TEM, an image is formed due to the interaction of the electrons transmitted through it. The image is finally magnified and focused on an imaging device (Kumara Swamy *et al.*, 2015).

Determination of antimicrobial activity

The effect of AgNPs on pathogenic bacterial strains (*E. coli, Salmonella, Pseudomonas, Staphylococcus* and *Bacillus*) was tested by the standard agar well diffusion method. Fresh culture of each bacterial strain is spread on the agar plate using sterile cotton swabs and incubated for 5 minutes in an inverted position. Four wells were made using gel



1. 1:1; 2. 1: 3; 3. 1: 5; 4. 1: 9; 5. 1: 10; 6. 3: 10; 7. 5 mM $AgNO_3$ (Control).

Fig 2: Different ratios of 5mM $AgNO_3$ + leaf extract.

puncture on the agar plates and to each well, equal volume (20 µl) of double - distilled water, methanolic extract, silver nanoparticle solution and 5 mM $AgNO_3$, were added. It was incubated overnight at 37°C and the antibacterial activity was measured on the basis of the inhibition zone around the wells.

Photocatalytic dye degradation

Experiments to carry out dye degradation was carried out by adding 1 ml of 1 mM Methyl red (MR), Eosin and Methylene Blue (MB) to 1 ml of silver nanoparticle (1 mg/ml) synthesized from 5mM silver nitrate solution. The volume was made up to 5 ml by adding distilled water to the mixture. Each of the solutions was mixed well for 2 h using a magnetic stirrer to achieve equilibrium between dye and nanoparticles. Control sets of pure dyes without $AgNO_3$ was also maintained. This was followed by incubation, in sunlight for 0"48 h. Small amounts of the above mixtures were used to evaluate the progress of dye degradation by measuring the absorption maxima of dyes at different time intervals.

Percentage of dye degradation was estimated by the following formula:

$$\% \text{ Decolourization} = 100 \times \frac{A_0 - A_t}{A_0}$$

Where

A_0 =Initial concentration of dye solution.

A_t = Concentration of dye solution at time *t* (Vizuete *et al*., 2017).

Experiments were repeated thrice, and the mean percentage value was recorded.

RESULTS AND DISCUSSION

The formation of silver nanoparticles was first confirmed by visual observation. The colour changes of leaf extract of *A. triplinervis* to brown on the addition of $AgNO_3$ was due to the reduction of Ag^+ to Ag_0 by various biomolecules present in the leaf extract. In the study conducted, incubation of 1:1 ratio of leaf extract and 5 mM $AgNO_3$ showed large quantity of silver nanoparticle formation (dark brown colour).

The formation of colour occurred due to the excitation of surface plasmon resonance of the silver nanoparticles (Mulvaney, 1996). The formation of silver nanoparticles indicates the potential of *A. triplinervis* for synthesizing AgNPs. Gomathi *et al.* (2020) reported the surface plasmon resonance band at 442 nm in the UV-visible absorption spectra which strongly affirms the development of AgNPs in the aqueous *Gymnema sylvestre* leaf extract.

Characterization of synthesized AgNPs

Characterization of synthesized silver nanoparticles was carried out using UV visible spectroscopy, SEM, TEM and FTIR.

UV visible spectroscopy

The characterization studies of the silver nanoparticles formed was primarily carried out by UV-Vis Spectroscopy. The study indicated that the sample with 5 mM of the silver nitrate, exhibited a strong absorption at 410 nm as shown in Fig 3.

UV visible spectrum of silver nanoparticles

Most of the green synthesized nanoparticles exhibited maximum UV–vis absorption spectra peak within the range of 350-480 nm (Roy and Bharadvaja, 2018). A typical peak was obtained due to Surface Plasmon Resonance (SPR) of silver nanoparticles. The resonance condition is established when the frequency of light photons matches the natural frequency of surface electrons oscillating against the restoring force of positive nuclei. SPR in nanometer-sized structures is called localized surface plasmon resonance (Zeng *et al*., 2011). Similar kind of results was also observed by Gomathi *et al*. (2017) for the AgNPs synthesized using the leaf extract of *Datura stramonium*.

FTIR analysis

FTIR studies were carried out to get an insight regarding the various biomolecules in plant extract and how the various functional groups are responsible for the reduction of silver nitrate to silver nanoparticles. The FTIR spectrum obtained for *Ayapana* leaf extract (Fig 4) revealed strong absorption peaks at 3436.74 cm^{-1} resulting from stretching of the –NH band of amino groups or is indicative of carboxylic groups. The absorption peaks at about 2855.93 cm^{-1} could be assigned to stretching vibrations of -CH2 and CH3 functional groups. The peaks at 1742.56 and 1623.39 cm^{-1} indicate amino acids containing NH2 and amine bond of type *I* which are capable of forming bonds with metals. The spectrum also showed different peaks at 1455.44, 1380.70, 1160.05, 1034.11 cm^{-1} *etc.* These narrow bands were formed due to the $-NO_2$ aliphatic nitro groups and C-N stretching of aliphatic amines and C-O stretching of carboxylic groups.

The extensive analysis of the FTIR spectra of the silver nanoparticles under study, confirmed that the proteins and the carbonyl group of amino acid present in *Ayapana* leaf extract had a strong binding ability with metal, acted as a reducing agent, imparted stability and prevented agglomeration. The biomolecules present in the leaf extract

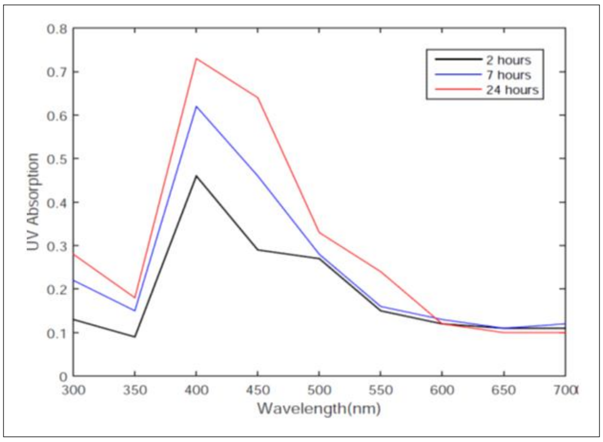


Fig 3: UV visible spectrum of silver nanoparticles.

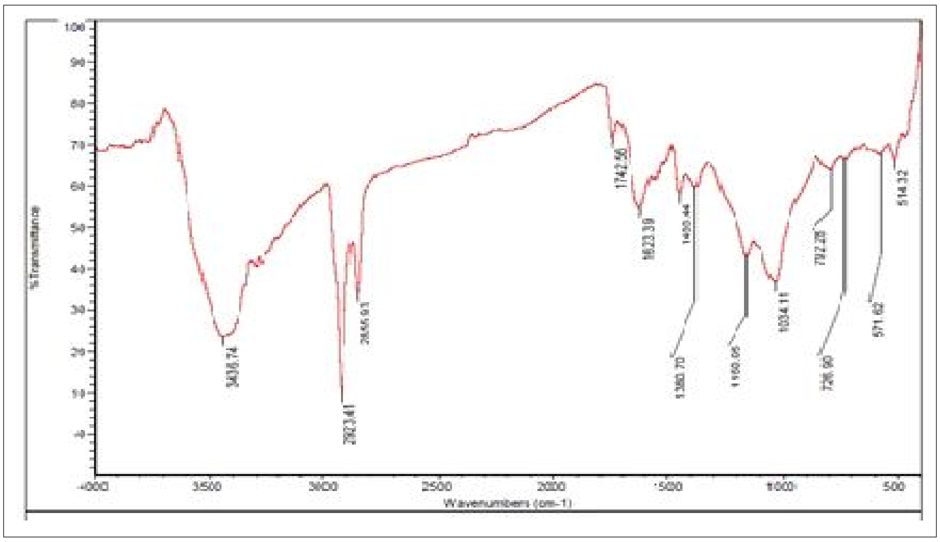


Fig 4: FTIR spectra of biosynthesized silver nanoparticles using methanolic leaf extract *of Ayapana*.

of *A. triplinervis* formed a strong coating/capping on the nanoparticles.

Vanaja *et al*., (2014) had also made similar observations by FTIR spectrum on the occurrence of the functional biomolecules - hydroxyl, carboxylic, phenol and amine groups in *M. tinctoria* leaf extract involved in the reduction of silver ions.

SEM analysis

Scanning Electron Microscopy (SEM) was used to study the surface morphology and topography of the AgNPs. SEM analysis revealed the size and shape of the AgNPs synthesized using *Ayapana* leaf extract. The silver nanoparticles were crystalline, spherical and cubical in shape with particle size ranging from 20 to 100 nm (Fig 5). SEM analysis of AgNPs synthesized from leaf extracts of *Carya illinoinensis* revealed that they were crystalline with face-centered cubic geometry and in different sizes ranged 12-30 nm (Javan *et al*., 2020). Roy and Bharadvaja (2018) reported spherical silver nanoparticles of size 55 nm. The larger size and cubic structure of silver nanoparticles may be due to the aggregation of the smaller particles.

Aggregation of the nanoparticles mainly occur due to the insufficiency of capping agent in the leaf extract. Moreover, the observed strong agglomeration of the nanoparticles prepared by this method may be interpreted in terms of the increase in the catalytic activity of the surface of the nanoparticles (Ghidan *et al*., 2016).

TEM analysis

The morphology of the synthesized silver nanoparticles was investigated using TEM. The structure of the synthesized nanoparticles at different magnifications are shown in Fig 6. The nanoparticles were mostly spherical and contain compounds which are seen as halos.

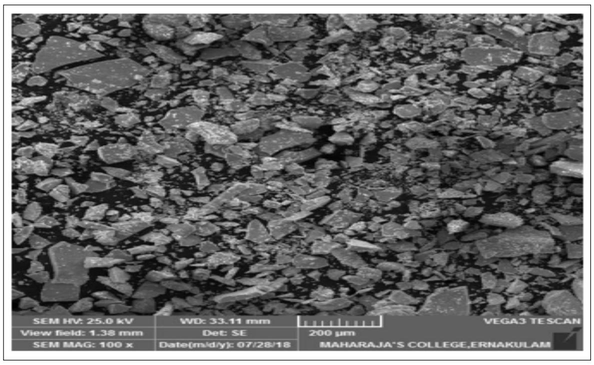


Fig 5: SEM image of silver nanoparticles (SEM HV: 20.0kV, View field: 1.38 mm).

Antibacterial Assay

The silver nanoparticles (AgNPs) synthesized using *Ayapana triplinervis* leaves extract were tested for antimicrobial activity by agar well diffusion method against different pathogenic bacteria *Escherichia coli (E. coli), Pseudomonas, Staphylococcus, Bacillus* and *Salmonella.* The synthesized AgNPs showed maximum antibacterial effect against the strains, *Staphylococcus aureus* (8.33±0.60 mm) and *Salmonella sp* (6.23±0.15 mm). The results showed that the antimicrobial activity of silver nanoparticles synthesized using *Ayapna triplinervis* extract was much higher than the activity of methanolic plant extract (Table 1).

The antibacterial activity of AgNPs should be associated with several mechanisms including (i) generation of Reactive Oxygen Species (ROS) like superoxide anions (O_2^-) and hydroxyl radicals (OH^-). ii) the presence of Ag^+ ions in AgNPs are making a bond with sulphhydryl groups which direct to denaturation of proteins in the bacteria and (iii) release of Ag^+ ions from the AgNPs which simply penetrate into the cell wall and cause the disorganization of plasma membrane

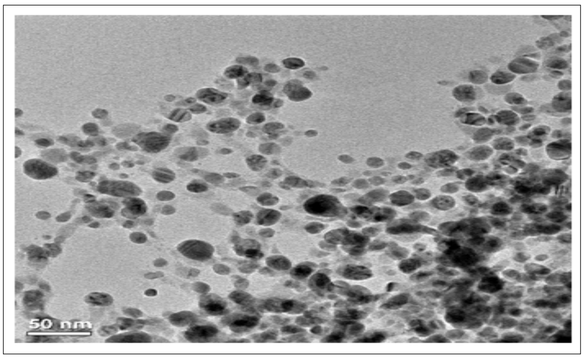


Fig 6: TEM image of synthesized silver nanoparticles.

potential leading to cell death of bacteria (Patil *et al.*, 2012). Weiming *et al.* (2023) has also reported in a study using silver nanoparticles from banana flower extract, that the antibacterial mechanism was likely a combination of cell membrane damage and ROS induction.

The small size and spherical shape of the nanoparticles is also another reason for the efficient antibacterial property as compared to the plant extract (Guzman *et al.*, 2012). The antibacterial effect of AgNps synthesized from plant extracts paves way for the development of new antibacterial drugs. Dev *et al.*, (2018) has reported the antibacterial property of AgNPs from *Ayapana triplinervis* aqueous extract. The silver nanoparticles synthesized using aqueous, ethanol and methanol extracts of *Achillea millefolium* showed good antibacterial potential against common human pathogens such as gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative bacteria (*Salmonella enterica*, *Escherichia coli* and *Pseudomonas aeruginosa*) (Yousaf *et al.*, 2020). Similarly, the Ag NPs synthesized from leaf extracts of *Gymnema sylvestre* (Gomathi *et al.*, 2020) and plant extracts of *Ziziphora clinopodioides* (Esmaile *et al.*, 2020) also showed good antibacterial activity on the growth of both *Staphylococcus aureus* and *Escherichi coli*. Bindhu *et al.* (2020) reported that AgNPs from *Moringa oleifera* flower extract inhibited the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus.* Zheng *et al.* (2021) have reported that green synthesized AgNPs exhibited long term antimicrobial properties which could be used as a potential nano-bactericidal agent. In a very recent study, green synthesis and characterization of silver nanoparticles using *Eugenia roxburghii* DC. extract exhibited activity against biofilm-producing bacteria (Giri et al, 2022).

Photocatalytic dye degradation

The photocatalytic degradation potential of Methyl Red (MR), Eosin and Methylene Blue (MB) using solar irradiation was studied in the presence and absence of synthesized silver nanoparticles. The relative absorption intensity at a wavelength of 410 nm in a regular interval of time (4h, 12h, 24h and 48h) revealed the complete reduction of MR in the presence of silver nanoparticles. The results indicated the photocatalytic degradation of dyes was in the order MR> Eosin > MB. Maximum degradation of 98.96 % was observed in MR followed by Eosin and MB *i.e.* 92.45% and 86.93% after 48 hours (Fig 7).

The majority of the organic dyes released from industries are carcinogenic and cause health problems, hence, it necessitates the development of simple environmentally friendly and cost- effective method to solve the dye effluent pollution problem (Wang *et al.*, 2017). Majority of the dyes in the effluent are xenobiotic and very difficult to remove by conventional waste treatment method. Nanoparticles due to their excellent surface properties and chemical reactivity have emerged as a better solution for dye removal and degradation (Marimuthu *et al.*, 2020). Typically, a bigger surface-to-volume ratio shows a higher catalytic activity. When the size of AgNPs decreases, there is an increase in the number of low coordinated Ag atoms which promote the adsorption of the reactant, dye on the catalyst surface and facilitates the reduction.

Photocatalytic degradation of Methyl orange using CuO nanoparticles synthesized from *Centella asiatica* (66.66% in 10 min) was reported by Devi and Singh (2014). Jyoti and Singh (2016) separated AgNPs from *Zanthoxylum armatum* leaves and established its catalytic property in the degradation of hazardous dyes and proved the degradation efficiency due to their very high surface area as well as the accelerated migration rate of electrons/hole to the surface of the nanoparticles. Catalytic degradation of methyl orange using silver nanoparticles was reported by Fatimah (2016). Although there have been reports of achieving degradation of dyes using the catalytic method in a shorter time, the photocatalytic method is more environmentally safe although

Table 1: Antibacterial activity of silver nanoparticles.

Name of the organism	Zone of inhibition (mm)[a]			
	Methanolic leaf extract	Silver nitrate	AgNPs	Double distilled water (Control)
Escherichia coli	-	4.65±0.35	1.25±0.22	-
Pseudomonas	-	3.57±0.43	-	-
Staphylococcus	1.33±0.78	4.96±0.25	8.33± 0.60	-
Bacillus	-	4.22±0.12	-	-
Salmonella	2.64±0.34	3.45±0.13	6.23 ± 0.15	-

[a]Mean values of three triplicates ± standard deviation.

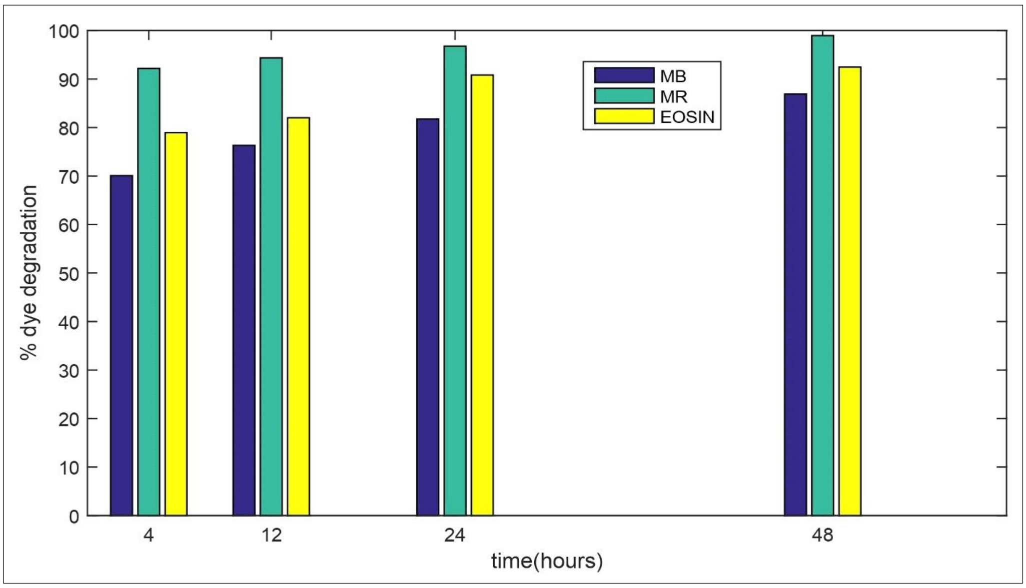


Fig 7: Percentage of photocatalytic degradation of dyes by silver nanoparticles.

we have to wait a bit longer to achieve complete degradation. There are also reports of photocatalysis of herbicide using Zinc oxide nano particles (Gaggara, 2020).

Silver and copper nanoparticles synthesized using *Centella asiatica* exhibited potential catalytic activity as compared to the photocatalytic degradation. This could be due to the ability of $NaBH_4$ to accelerate the degradation process in less time as compared to the photocatalytic method where $NaBH_4$ was absent (Raina et al., 2020). Moreover the size of silver nanoparticles also affects the degradation rate of hazardous dyes, methyl orange, methylene blue and eosin Y by $NaBH_4$ (Vidhu and Philip, 2014).

Conclusion

For a healthy environment, it is advocated to promote green technology and hence the adoption of the synthesis of nanoparticles from plant extracts gains much significance. In the present study, silver nanoparticles were synthesized from methanolic extracts of *Ayapana triplinervis,* as indicated by colour changes due to surface plasmon resonance. UV-Vis spectroscopy of prepared silver colloidal solution showed an absorption maximum of 410 nm. The biomolecules associated and the morphological characters were analysed by FTIR, SEM and TEM studies. This green synthesized nanoparticles exhibited inhibitory action against *Salmonella* sp and *Staphylococcus aureus.* In collaboration with plant metabolites having good antioxidant content, they can be used as a successful alternative for synthetic drugs in order to combat the issue of multi drug resistance. Catalytic degradation of the dyes in the presence of sunlight was also exhibited by these nanoparticles. This assumes significance to overcome the problem of environmental pollution due to highly stable organic dyes from industrial effluents. Although there are many reports about the synthesis of silver nanoparticles using plant extracts, further studies need to be carried out for devising a commercially viable, economic and environmentally friendly route to find the capacity of the natural reducing constituent to form silver nanoparticles. Significant variation in chemical compositions of plant extract of the same species when it is collected from different locations may lead to a difference in results obtained in different laboratories. Hence, this stands as a major obstacle in the synthesis of nanoparticles using plant extracts. Identification of biomolecules present in the plant which are responsible for mediating the nanoparticles production for rapid single-step protocol can help to overcome the above - said problem and give a new facelift for particles. The green synthesis method of silver nanoparticle production opens the possibility of exploring more areas of research in nanotechnology with wider applications to society.

Conflict of interest

The authors declare that they have no conflict of interest.

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