RESEARCH PAPER

Evaluation of Antioxidant, Antibacterial and Photo catalytic Effect of Silver Nanoparticles from Methanolic Extract of Coleus Vettiveroids – an Endemic Species

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ABSTRACT

Biosynthesis of metal nanoparticles using plant extract has received much attention due to its eco-friendly nature. The present study elucidates the green synthesize of Silver nanoparticles (AgNPs) from methanolic extract of Coleus Vettiveroids –an endemic species. The synthesis of AgNPs was confirmed by UV-visible spectrometry at 416 nm. Further, biosynthesized nanoparticles were characterized by FTIR for the confirmation of biomolecules acting as reducing agent. Average size and presence of elemental silver were characterized by scanning electron microscopy (SEM) and Transmission electron microscopy (TEM).Average size of nanoparticles was found to be 5 nm. The antioxidant ability of AgNPs was analyzed using DPPH. In vitro antibacterial effect of various concentrations of AgNPs was investigated against both Gram positive (S.Aureus) and Gram negative (*E.Coli*) bacterial strains. The result shows that biosynthesized AgNPs have significant antibacterial activity. Synthesized silver nanoparticles were also used effectively as photo catalyst in degradation of Organic Dyes and can be concluded that synthesized silver nanoparticles are also promising photo catalyst.

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INTRODUCTION

Nanomaterials are the cornerstone for the emerging nano science and technology and heading towards getting things smaller. Nanostructures have attracted extensive research interest due to the beneficial influence of their dimensionality on electronic and optical material properties [1]. At the nanoscale, properties differ significantly from their bulk material counterparts, and materials can self-assemble spontaneously into ordered structure. These emergent properties have profound impact in mechanical, electrical, optical and other fields. This is due to the fact that many important chemical and electrical reactions occur only at surfaces and are sensitive to the shape and texture of a surface as well as, its chemical composition [2-3]. Therefore, nanoparticles are nurtured as building blocks of the next generation of optoelectronics, electronics and various chemical and biochemical sensors [4-5]. The unique properties of different types of nanoparticles have resulted in wide variety of novel applications. For example, compounds known to be generally inert materials may become

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catalysts. The extremely small size of nanoparticles allows them to penetrate cells and interact with cellular molecules. Because of these potentials, the science of nanotechnology has taken off in recent years, particularly in nano medicine.

Colloidal nanoparticles are usually used as carriers to deliver drugs and genes into cells for various therapeutic applications [6]. In many cases, the surface of their carriers is functionalized with antibodies [7]. Delivering of nanoparticles into cells involve their attachment to the cell surface. Location of nanoparticles, relative to cell membrane can influence behavior of a cell and the efficiency of therapeutic agent [8]. In many living organisms, oxidation is an essential biological process for the production of energy. Reactive oxygen species (ROS) cause damage of complex cellular molecules such as carbohydrates, proteins, lipids and DNA [9]. Appearance of many health problems like cancer, cardiovascular diseases, liver diseases, renal failure, inflammatory problems, and aging are on the rise [10]. Antioxidants are agents that, in one way or another, restrict the deleterious effects of these oxidant reactions. These restrictions can involve scavenging free radicals and therefore, can enhance the immune defense and lower the possibility of diseases occurrence [11]. The search for new antioxidants is of great importance to avoid the side effects. Fruits, vegetables and medicinal herbs have proven to be the richest sources of antioxidant compounds [12]. Natural products, mainly obtained from dietary sources provide a large number of antioxidants. Phytoconstituents are staging a comeback as an important source of antioxidant, and are capable of terminating the free radical chain reactions [13]. This green method of silver nanoparticle formation opens a new window for the treatment of various infectious diseases and tumors.

In textile, dyes are used as raw material which generates tons of waste water. By nanotechnology, it has now become possible to obtain new materials with specific properties. Among them, the photo catalytic activity is a feasible option for the management of organic pollutants [14]. Photo catalyst is the activation of a photochemical reaction, using UV or visible light as chemical energy in the presence of a catalyst substrate [15]. AgNPs has photo catalytic activity, which make it an attractive solution for water treatment [16]. Presence of silver improves its photo catalytic properties by promoting interfacial electron-hole and silver may trap photo generated electrons [17]. Nanomaterials have proved to be efficient catalyst. But the main challenge in applying nanoparticles is to stabilize and control their size [18].

The enhancement of a greener route to nanoparticles synthesis has been a boon to research platform with strong impetus for the future. The various resources available naturally for the green synthesis of nanoparticles are plants, plant products, bacteria, fungi, algae, yeast and viruses [19]. Though, there is a large platform for a greener synthesis of nanoparticles, the most commonly preferred way is plant extract as it is easy to handle, less biohazard and does not require maintenance of cell culture [20]. Another advantage is that the size of the nanoparticles synthesized can also be controlled easily by altering parameters, like pH and temperature [21]. The use of stabilizers to prevent aggregation is not required as the proteins in the plant extract act as stabilizers [22].

So the present study elucidates synthesis and characterization of silver nanoparticles (AgNPs) by methanolic extract of *Coleus Vettiveroids* – an endemic species. Thorough review of the literature revealed that C. Vettiveroids is probably native and endemic to Kerala and Tamil Nadu [23-24]. However, the wild source of species is still not known. It is grown under cultivation and extinct in the wild. Therefore *C. Vettiveroids* is not recorded from natural habitats, and has been assessed and considered as possibly extinct in the wild [25]. So species of *Coleus Vettiveroids* are not explored and particularly, in the green synthesis of AgNPs. In order to fill this gap, the present work was undertaken for the first time with the aim of biosynthesis of AgNPs and optimization of parameters for the best production of AgNPs and their characterization by *Coleus Vettiveroids* methanolic leaf extract. Synthesized AgNPs were evaluated for antibacterial, antioxidant and photo catalytic activities.

MATERIALS AND METHODS

Instrumentation Technique

UV-Visible spectral analysis was done by using Double beam UV-Visible spectrophotometer (specrtoscan UV-2600), with the revolution of 1nm between 300-700 nm. FT-IR spectra were recorded by using Bruker FTIR spectrophotometer, the range of 500-4000 cm^{-1}. Scanning Electron

Microscopy (SEM) was done using Quanta 200 FEG, Particle size and crystalline nature of nanoparticles were analyzed using Transmission electron microscopy (TEM –JEO2100).Cyclic Voltammetric study was performed by using CH silicone Voltammetric analyzer model 604 E work station with three electrode system. The fluorescence measurements were carried out on F-7000 FL spectrophotometer with the scan speed of 1200 nm/min.

Preparation of plant extract

Fresh leaves of *Coleus Vettiveroids* under cultivation were collected from Munnar, Idukki district of Kerala. For the preparation of extracts, successive solvent extraction method was followed using Soxhlet apparatus. The dried and powered leaves of Coleus Vettiveriods (10 grams) were packed in the body of Soxhlet extractor. The concentrated extract was filtered through Whatmann's filter paper no.41 and used for further experiments.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, $AgNO_3$ was treated with methanolic plant extract in the ratio of 1:9. To study the optimum factors for the synthesis of silver nanoparticles, the experiments were carried out at different conditions of varying temperature, pH and the concentrations of $AgNO_3$ solution. The effect of these parameters on the synthesis of silver nanoparticles was monitored by UV-Visible spectrophotometer.

Cyclic Voltammetry study

Multiple scan cyclic Voltammogram was performed using conventional three electrode system. Platinum wire as counter electrode, Carbon electrode as working electrode and silver/silver Chloride as reference electrode were used. 5mL colloidal solution of metal nanoparticles was mixed with 1 mL of 0.1 M KNO_3 supporting electrolyte.

Photo catalytic degradation of organic dyes

Photo catalytic degradation of Methyl Orange (MO) and Methylene Blue (MB) were carried out by using synthesized silver nanoparticles in the sunlight and identified spectrometrically by using double beam UV-visible Spectrophotometer. The absorbance spectrum of the supernatant was subsequently measured using UV-Vis spectrophotometer at different times.

Antibacterial activity

Gram negative Escherichia coli (MTCC 443) and Gram positive Staphylococcus Aureus (MTCC 96) bacterial pathogens were used for antimicrobial activity. These selected pathogenic strains were obtained from Microbial Type Culture Collection (MTCC), Chandigarh Punjab, India. The antibacterial activity was determined by well diffusion methods [26].

Antioxidant activity

The ability of the samples to annihilate the DPPH radical (1, 1-diphenyl-2-picrylhydrazyl) was investigated by the method described by Blois 1958 [27]. Stock solution of compound was prepared to the concentration of 10 mg/ml. Different concentration of the extract (200, 400, 600, 800, 1000 µg) of sample were added, at an equal volume to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard control. The annihilation activity of free radicals was calculated in percentage inhibition according to the following formula,

% of Inhibition = (Absorbance of control – Absorbance of Test)/Absorbance of control * 100

RESULTS AND DISCUSSIONS

UV-visible analysis

When methanolic extract was mixed with $AgNO_3$ and incubated, its color changed from greenish yellow to dark brown, indicating the formation of AgNPs. Silver nanoparticles suspension exhibits an intense dark brown color due to the surface Plasmon resonance (SPR), which form collective oscillations of their conduction band electrons in response to electromagnetic waves [28]. The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles [29]. Therefore, absorbance peaks can be used as tools to predict size and stability. A strong broad peak at 416 nm was observed for the silver nanoparticles prepared using methanolic extract of C.vettiveroids (Fig. 1). There was no peak for plant extract and silver nitrate solution. Observation of this peak, assigned to a surface Plasmon, is well documented for various metal nanoparticles with sizes ranging from 2 to 100 nm. It is an efficient and rapid method, which was very well explained by other researchers who worked with different plant systems [30-31].

The study of methanolic extract of *Coleus Vettiveroids* leaf showed the presence of alkaloids, phenolic compounds, reducing sugar, starch, proteins, aminoacids and tannis[32]. Flavonoids act as an oxidizing agent and is oxidized by $AgNO_3$ resulting in the formation of AgNPs. The phenolic compound has hydroxyl and ketonic groups which are able to bind to metal ions and reduce the metal salt. The enzyme released from the plant extract act on the silver ions and there is a release of AgNPs from enzyme. The AgNPs probably combine with protein released from the plant

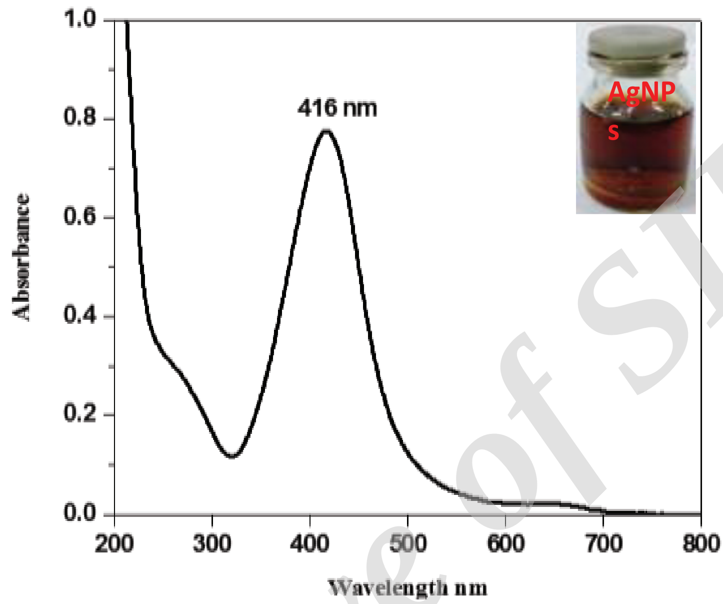


Fig. 1. UV-Visible absorbance spectrum of synthesized AgNPs from Methanolic extract of C.Vettiveroids. Inset: Photo of synthesized AgNPs.

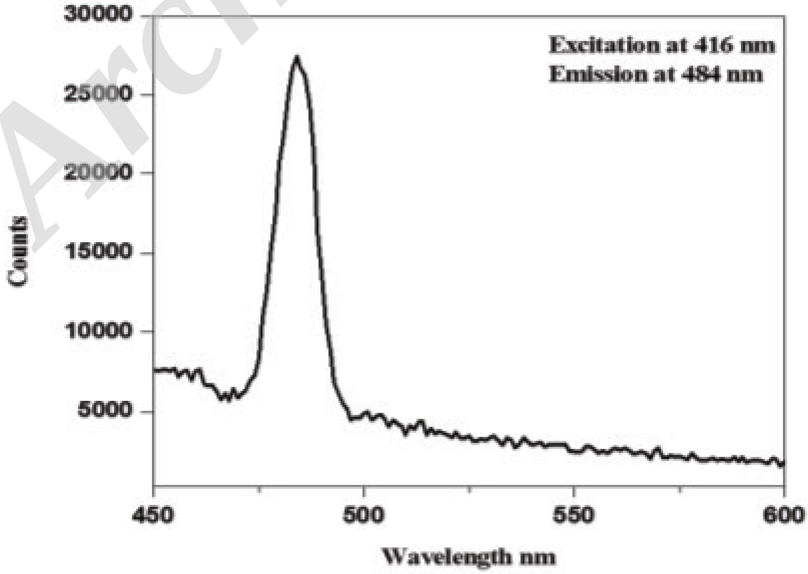


Fig.2. Fluorescence emission spectrum of AgNPs from Methanolic leaf extract of Coleus Vettiveroids

extract which may lead to the formation of protein capped silver nanoparticles.

Fluorescence spectroscopy

Photoluminescence (PL) of the synthesized AgNPs is studied by fluorescence emission spectroscopy to estimate the optical property of the AgNPs as photonic materials [32]. The synthesized AgNPs were excited at 416 nm, and the emission peak was obtained at 484 nm [33]. have reported similar emission band. The origin of the fluorescence could be attributed to the promotion of d-band electrons of the silver nanoparticles on absorption of the incident photon energy to higher electronic states in the sp-band (Fig. 2).

FTIR Analysis

FTIR measurements were carried out to determine the biomolecules of leaf extract responsible for the reduction of silver ions and capping of AgNPs (Fig. 3). The broad band appearing at 3410.59 cm^{-1} is assigned for O-H stretching vibrations indicating the presence of hydroxyl group in the reducing agents [34]. Peak at 2341.05 cm-1 is attributed to C-H (aliphatic) stretching. The strong peaks at 1630.08 cm^{-1} is due to N-H bending and 1355.99 cm^{-1} corresponds to C-N stretching vibrations as well as amide I bands of proteins in the C.vettiveroids extract. Peak observed at 1018.02 cm^{-1} corresponds to secondary –OH stretching, indicating that the secondary –OH also participates in nanoparticles synthesis. From FTIR, it can be concluded that, the biological components are known to interact with metal ions (Ag^{+}) via these functional groups and mediate their reduction to nanoparticles (Ag^{0}).

SEM analysis

SEM micrographs of the synthesized silver nanoparticles using *Coleus Vettiveroids* methanolic extract is shown in Fig. 4. The biosynthesized silver nanoparticles were uniformly spherical in shape, well dispersed and homogeneous. Homogeneity of these properties is important in many applications [35].

TEM Analysis

Transmission electron microscopy has been used further to identify the size of synthesized silver nanoparticles (Fig. 5). The graphical representation of the formation of AgNPs is shown in (Fig. 6a). The size of the AgNPs was found to be 5 nm with distinct and spherical in shape. Selected area electron diffraction (SAED) pattern

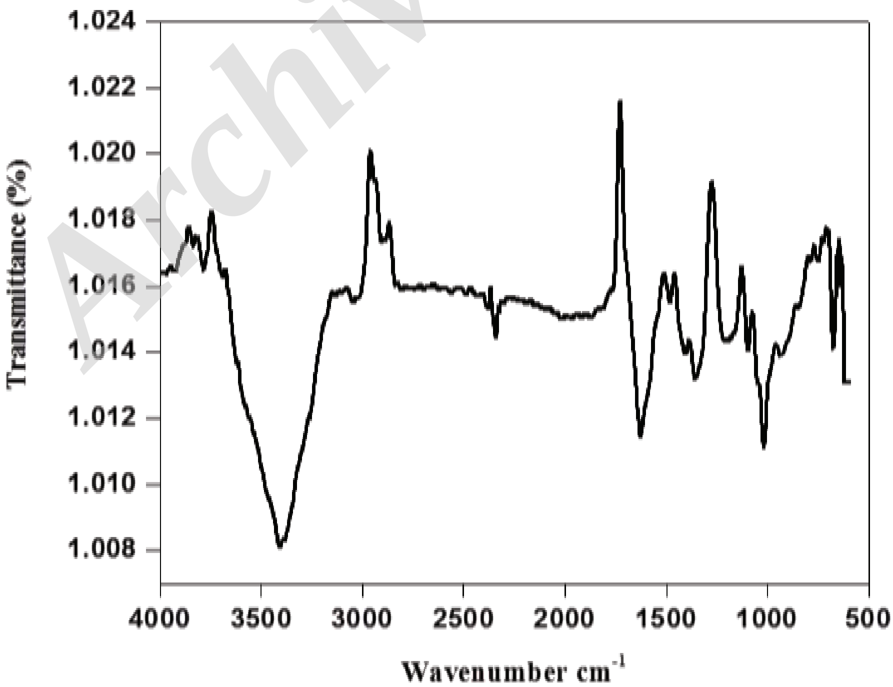


Fig. 3. FTIR spectrum of synthesized silver nanoparticles from methanolic extract of Coleus Vettiveroids

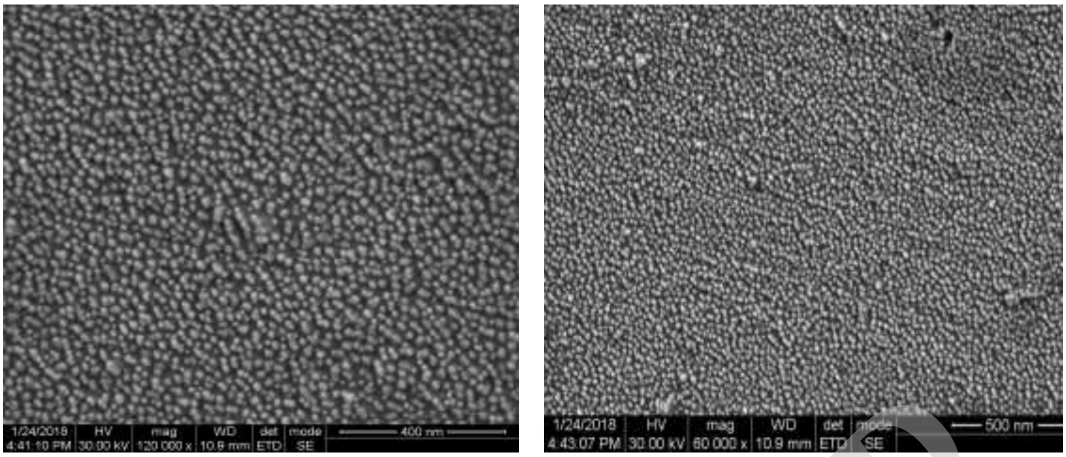


Fig. 4. SEM images of synthesized AgNPs from methanolic extract of C.vettiveroids

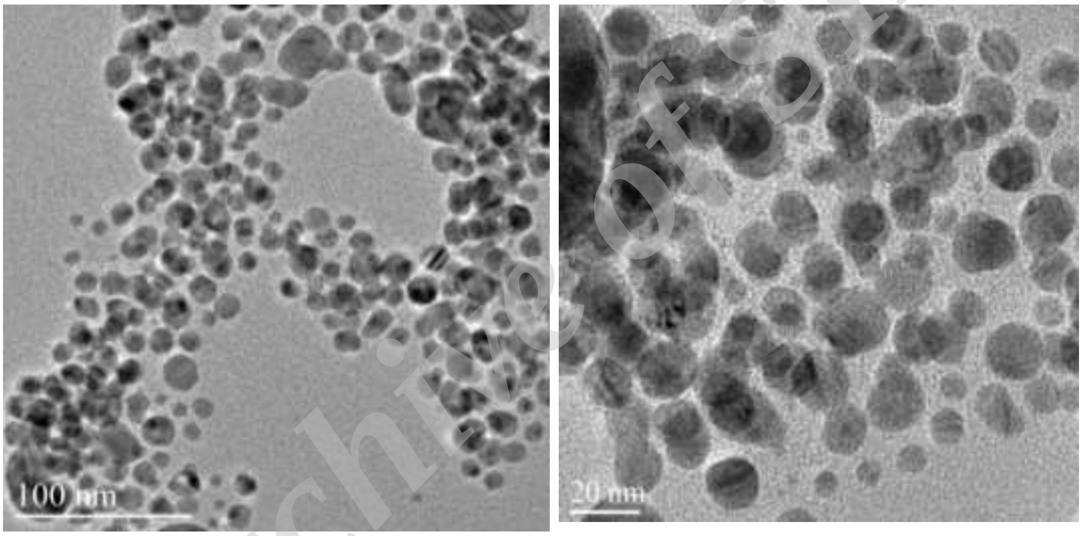


Fig. 5. TEM images of synthesized AgNPs at the magnifiactions of 100nm and 20 nm

showed that the synthesized silver nanoparticles were crystalline in nature. (Fig. 6b)

Cyclic Voltammetry study

Cyclic Voltammetry measurements were carried out at a scan rate of 50mV/s in aqueous solution. The CV curve shows prominent anodic peak. The anodic peak is due to the oxidation of silver nanoparticles in the size of quantum dots at the carbon electrode, which is also evidence for the formation of AgNPs. Double scan of CV indicates the decrease in concentration of diffusible particles. Thus AgNPs show irreversible oxidation peak (Fig. 7).

Photo-catalytic degradation studies

Photo-catalytic degradation of Methyl Orange and Methylene Blue dyes were investigated using, biometrically synthesized AgNPs by solar irradiation technique at different time intervals of 1,2,3,4 and 5 hours. The characteristic absorption peak of pure MO and MB dyes were found to be 464.5 nm and 667 nm respectively. The catalyzed reaction with AgNPs spectrum shows a sudden fall in absorbance value indicating catalytic effect of Ag nanoparticles under exposure to sunlight (Fig. 8a). There was no much change in absorbance peak without AgNPs indicating very slow reduction rate of methyl orange and Methylene Blue (Fig. 8b).

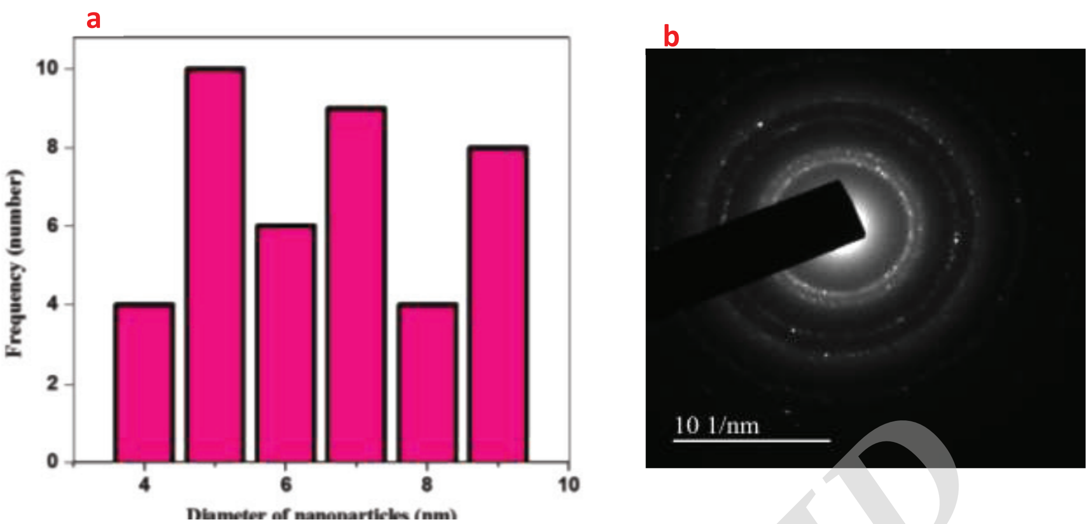


Fig. 6. (a)Histogram showing particle size (b) SAED pattern of synthesized AgNPs

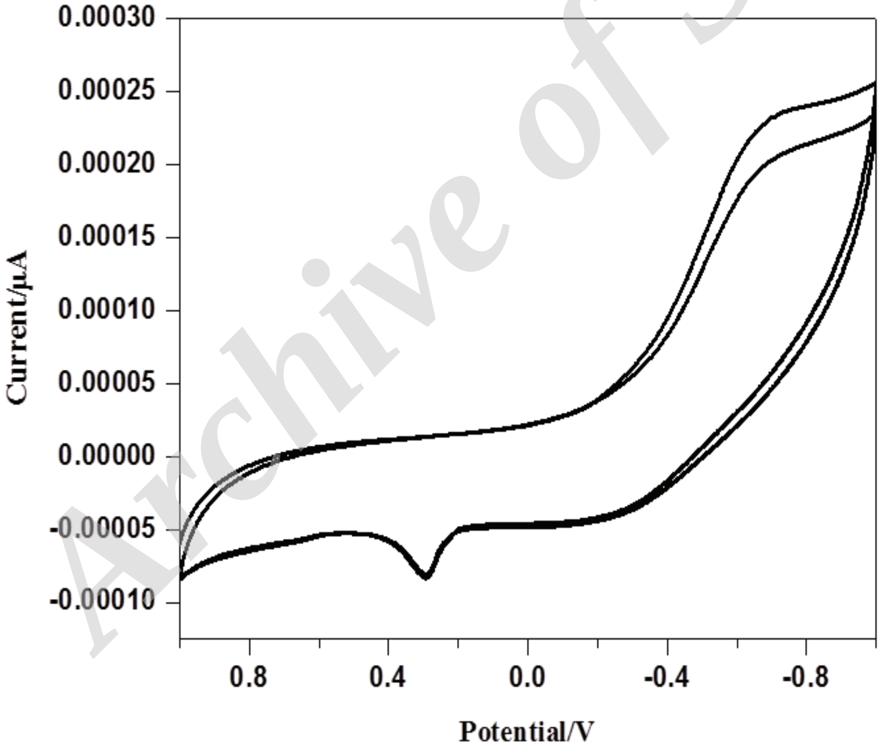


Fig. 7. Double scan of cyclic voltammogram for synthesized Silver nanoparticles from methanolic leaf extract of Coleus Vettiveroids.

Degradation of dyes was faster in the presence of synthesized silver nanoparticles of methanolic extract of *C. Vettiveroids*.

The probable mechanism of degradation could be attributed to the SPR effect where the excited surface electrons might interact with the dissolved oxygen molecules and ultimately produce hydroxyl radicals while allowing Ag^+ ions to interact with the anionic dye [36-37]. The solar light was found to be more effective than other radiation techniques for degrading dyes as reported by the previous studies[38]. During exposure in sunlight, when the photons hit the nanoparticles present in the colloidal mixture, the electrons the particle surface are excited[39]. The dissolved oxygen molecules in the reacting medium, accepts excited electrons from particle surface and are converted into oxygen anion radicals. These radicals break the organic dye into simpler organic molecules, leading to the rapid degradation of the dye [40-41]. Therefore, the biosynthesized Ag nanoparticles may act as a stable and efficient photo catalyst for degradation of methyl Orange and Methylene blue under visible light irradiation [42].Hence, it is evident that AgNPs synthesized from C.vettiveroids methanolic extract is highly potential photo catalytic agent for the degradation dye in the presence of sunlight.

Antimicrobial activity

Metal and their nanoparticles exhibit potential antimicrobial property [43-45]]. The antimicrobial activity of *C. Vettiveroids* leaf extract, silver nitrate ($AgNO_3$) and synthesized AgNPs were determined by disc diffusion method to distinguish the antimicrobial activity of the tested samples.

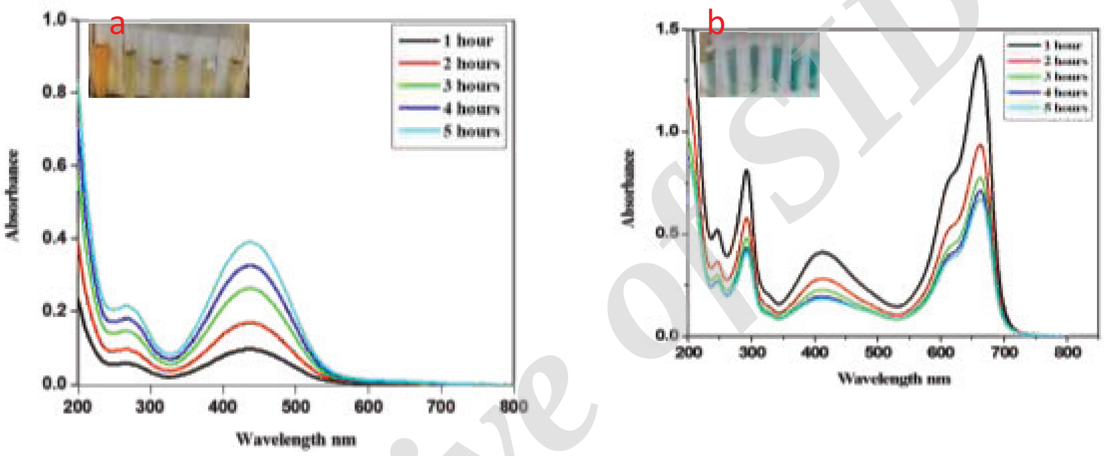
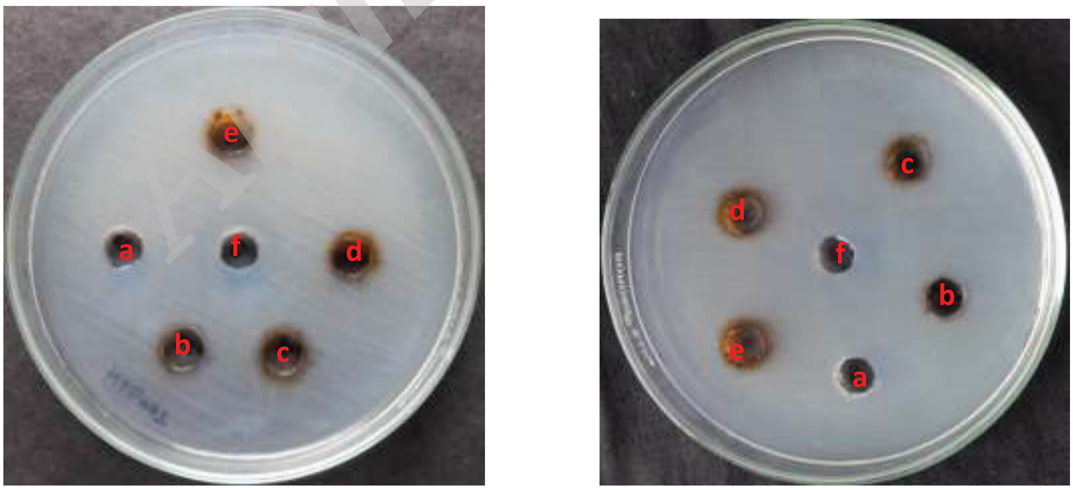


Fig. 8. UV-Visible absorption spectra of photo degradation of (a) Methyl Orange and (b)methylene blue dye by synthesized silver nanoparticles from methanolic leaf extract of Coleus Vettiverioids at different times.



(a) ***Staphylococcus aureus*** ***(b) Escherichia coli***

Fig. 9. Antibacterial studies against (a) S.Aureus and (b) E. Coli (a: 0µg/well; b: 25µl/well; c:50µl/well; d:75µl/well; e:100µl/well; f: 30µg/well (Azithromycin)

The antibacterial activity of silver nanoparticles was studied against E. coli, and S. Aureus (Fig. 9). The silver nanoparticles demonstrated a zone of inhibition against all the test organisms with maximum inhibition against Gram positive *S.Aureus* (29 mm), followed by antimicrobial activity against Gram negative *E.Coli* (26mm). Antibacterial effect of $AgNO_3$ solution was found to be 10 mm and 12 mm for *S.Aureus* and *E.Coli* respectively (Table 1). The results indicated that the leaf extract exhibited very low antimicrobial effect against S. Aureus (5mm) and *E.Coli* (7mm). Silver nitrate showed an appreciable positive effect against the tested microorganisms (low clear zone). However, plant mediated AgNPs showed the greatest antimicrobial activity against the tested microorganism. Results showed that AgNPs has the ability to inhibit the bacterial growth of gram positive and gram negative bacteria. The inhibition zone was greater in gram positive than in gram negative bacteria (Fig. 10). Kala S have reported that Antimicrobial activity of methanolic leaf extract of Coleus Forskohlii by disc diffusion method was found to be 11mm for *E.Coli* and 13 mm for *S.Aureus* and 10 mm and 12 mm for *E.Coli* and *S.Aureus* respectively by agar well diffusion method [46].

Several mechanisms have been proposed for the mode of action of silver nanoparticles against bacteria. Silver nanoparticles have demonstrated bactericidal activity by inhibiting cellular respiration and membrane permeability [47]. The nanoparticles bind to the Sulphur containing proteins on bacteria and inactivate them. It is reported that the attachment of silver nanoparticles to the bacterial cell wall dissipates proton motive force, destabilizes the outer membrane and ruptures the cell causing depletion of intracellular ATP32. Besides the activity of the silver nanoparticles, the antimicrobial compounds and Phytoconstituents present in the plant extract may also contribute to the antimicrobial activity of

Table 1. Antibacterial activity of synthesized AgNPs, Methanolic plant extract and silver nitrate solution

Concentrations (µg/well)	Zone of inhibition (mm) Staphylococcus Aureus	Zone of inhibition (mm) *Escherichia coli*	Zone of inhibition (mm) Methanolic Plant extract	Zone of inhibition (mm) $AgNO_3$
25	25	21	05	11
50	26	23	07	13
75	27	24	08	15
100	29	26	11	17

Table 2. DPPH scavenging activity of Synthesized AgNPs from methanolic extract of C.vettiveroids

Concentrations (µg)	Percentage inhibition AgNPs	Percentage inhibition Control
200	22.89	82.36
400	33.54	89.65
600	46.98	91.82
800	52.63	94.47
1000	63.87	95.12

the bio stabilized silver nanoparticles as they also have bactericidal effect [48].

Antioxidant Assay

The methanolic extract of AgNPs from *Coleus Vettiveroids* exhibited a maximum DPPH scavenging activity with IC_{50} value as 719.109 µg/ml (Table 2). The DPPH activity of the AgNPs was found to increase in a dose dependent manner (Fig. 11). When adding AgNPs in the DPPH solution, color change occurred which is due to the scavenging of DPPH due to donation of hydrogen atom to stable the DPPH molecule which is responsible for the absorbance of 517 nm [49]. The methanolic extract of *Coleus Vettiveroids* exhibited a maximum DPPH scavenging activity of 55.08% at 1000 µg/ml have been reported by G. Gopalakrishnan et al.[50]. The study shows that AgNPs from plant extract possessed higher scavenging activity compared to the C.vettiveroids plant extract alone. This is the

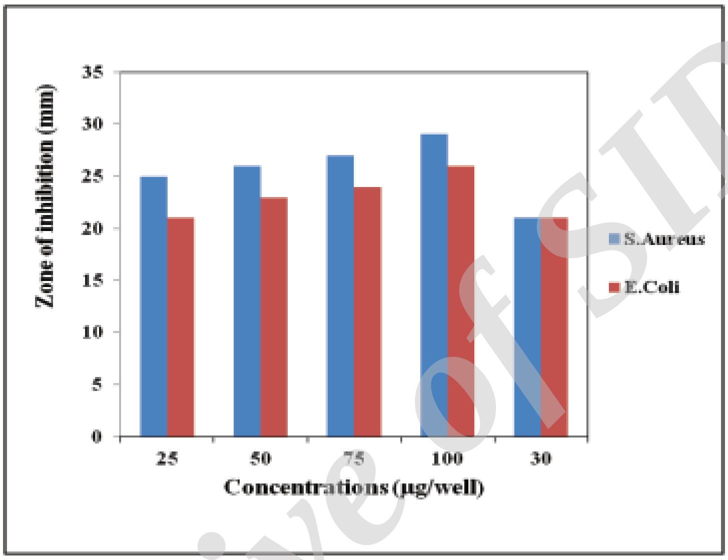


Fig. 10. Result showing zone of inhibition by synthesized AgNPs

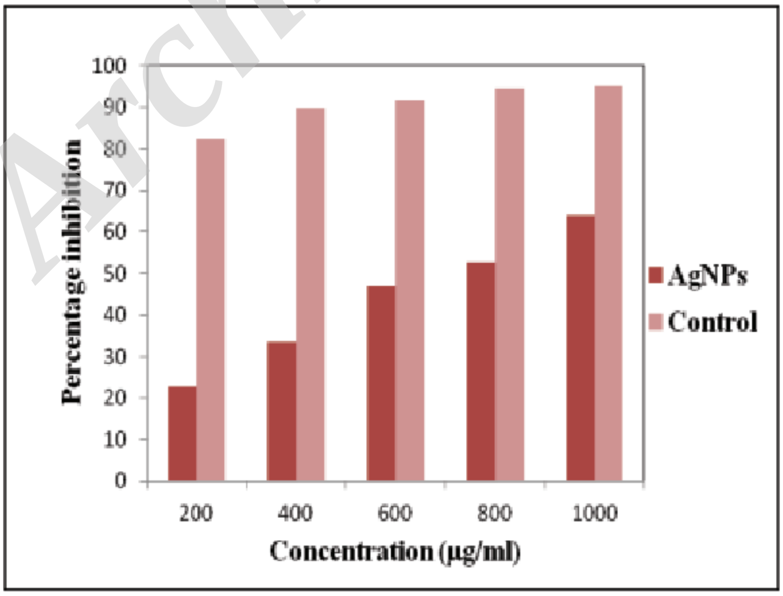


Fig. 11. Bar diagram indicating the Radical Scavenging Activity (Antioxidant activity) at increasing concentrations of AgNPs.

first report of antioxidant activity of synthesized AgNPs from *Coleus Vettiveroids* methanolic leaf extract.

CONCLUSIONS

The current study revealed that silver nanoparticles can be synthesized in a simple method using *C. Vettiveroids* methanolic leaf extract - an endemic species. Synthesis of Silver nanoparticles from *Coleus Vettiveroids* does not require addition of any stabilizing and accelerating agent. The TEM analysis showed that the sizes of the synthesized AgNPs to be 5 nm. Photo catalytic study concludes that synthesized AgNPs from *Coleus Vettiveroids* have high efficiency to degrade Methyl Orange and Methylene Blue dyes under solar irradiation, therefore paving its way in water purification and textile industries. The synthesized AgNPs can be employed as natural antioxidants for health preservation against different oxidative stress associated with degenerative diseases. Plants produces large amount of antioxidants to prevent the oxidative stress, they represent a potential source of new compounds with antioxidant activity. Therefore study confirms the application of AgNPs as a potential free radical scavenger. The biosynthesized AgNPs were found to have a pronounced antibacterial activity against E. coli, and S. Aureus compared to plant extract alone. Thus green synthesis of AgNPs has paved a better platform for medical approach and can be used as an effective bactericidal agent, against various microorganisms which can endanger human beings.

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CONFLICT OF INTERESTS

The author declares that there is no conflict of interests regarding the publication of this paper.

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