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RESEARCH ARTICLE

Biosynthesis and Characterisation of Silver Nanoparticles using Leaf Extract of *Gardenia resinifera* and its Antioxidant and Photocatalytic Degradation Activity

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Abstract

Background: Green synthesis of nanoparticles and their bioactive applications have always been significant in the present scenario. Nanomedicine is a promising alternative approach for the development of sensitive and selective drugs for fast-progressing ailments like inflammation and cancer. **Aim:** The purpose of this study is to synthesise and characterise silver nanoparticles from the leaf extract of *Gardenia resinifera* and to test its application as an antioxidant agent. **Methods:** The green synthesised silver nanoparticle was authenticated and validated by Ultraviolet-Visible spectrophotometer (UV-Vis Spec), Fourier-transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopic (SEM) analyses. **Results:** In UV-Vis spectrophotometric analysis a change in colour of the mixture of plant extract and AgNPs (Silver nanoparticles) from green to yellowish-brown confirms the production of AgNPs. The presence of 12 peaks in FTIR spectra represents the respective bands and stretches of the bonds. SEM image shows the spherical shape of the AgNPs and an average size ranging from 70 nm to 100 nm. The most frequently used methods for determining antioxidant activity are the ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays and they are based on the reaction of coloured radicle measured by spectrophotometric methods. The antioxidant capacity of synthesised AgNPs was evaluated by these assays and compared with leaf extract and a standard. These findings strongly suggest that AgNPs may play a therapeutic role in oxidative stress-associated disorders. A 100% photocatalytic degradation of methylene blue a synthetic dye resulted in the presence of synthesised AgNPS. Based on the result it is evident that a gradual decrease in absorption peak was observed by an increase in reaction time which indicates the degradation of methylene blue dye. **Conclusion:** The present study explains the possibility of silver nanoparticles synthesised using *G. resinifera* leaf extract as an antioxidant agent due to its efficiency in radical scavenging.

Keywords: Antioxidant Analysis, Characterisation, Photocatalytic Degradation, Silver Nanoparticles

1. Introduction

Increased rates of pollution and frequent exposure to pollutants can cause alteration in the physiological metabolism of living organisms due to the generation of oxidative free radicles. The cell and tissue damage created by these free radicles are major concerns in the health care industry which is to be addressed effectively with necessary background research in phytochemical bioactive compounds with potential antioxidant properties. The advancement in biomolecular screening increased the identification of unique compounds from plants. Therefore, in recent years plant-based screening of bioactive compounds increased, which poses a

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threat to the conservation of rare and endangered plants. A large amount of plant tissues was required for the effective extraction of a very small quantity of bioactive molecules. An alternate approach with the use of the reduced quantity of biomolecules for advanced treatment systems can harness the large-scale utilization of plants and plant parts.

Application of various biosynthesised nanoparticles from diverse sources is common in recent pharmaceutical and drug development industries[1-4]. In the past two decades, different types of nanoparticles were synthesised and application-based studies on this nanoparticle increased on an exponential basis. Advanced green chemistry approaches enhanced and established the green synthesis of nanoparticles and that method seems to be less toxic, rapid and simple[5]. Nanomedicine is an emerging medical technology which focuses on targeted drug delivery in nano quantity with high efficiency through medical imaging at the molecular level. Comparatively high electrical conductivity, thermal stability and an array of biomedical applications like antimicrobial and anti-cancerous agents of silver fulfilled the requirement of an effective metal for nanoparticle synthesis with affordable cost[6,7]. Therefore green synthesis of silver nanoparticles using silver and plant extract will enhance the possibility of finding an effective and efficient bioactive compound.

Gardenia resinifera Roth commonly called *Dikamali*, belongs to the family *Rubiaceae*[8]. This plant was used to treat various ailments like toothache, skin diseases and stomach ache[9]. The main objectives of the present study were to use leaf extract of *Gardenia resinifera* for reducing silver ions to stable nanoparticles. Characterisation of synthesised AgNPs was done using different methods like FT-IR, SEM, *etc*. The synthesised nanoparticle was assessed for its antioxidant and photocatalytic activity.

2. Materials and Methods

2. 1 Preparation of *Gardenia resinifera* Leaf Extract

Gardenia resinifera leaves were collected from the medicinal garden of Little Flower College, Guruvayur, Kerala, India. The collected fresh leaves were washed in running tap water to remove adhering dust particles. The leaves were thoroughly washed in double distilled water three to four times before air drying at room temperature. 50 gm of dried leaves were boiled by adding 500 ml of double distilled water for about 10 min and the water extract was cooled and decanted. The extract was filtered with filter paper (Whatman No.1) and stored at 4°C for silver nanoparticle synthesis and analysis.

2. 2 Preparation of AgNPs

0.017 g of $AgNO_3$ crystals were dissolved in 100 ml of deionized water for the preparation of 1M $AgNO_3$ solution. To 45 ml of freshly prepared IM $AgNO_3$ solution, 5 ml of *G. resinifera* leaf extract was added. The conical flask containing the reaction mixture was kept in the dark for 48 h, change in colour was observed. The reddish-brown coloured mixture was decanted and centrifuged. The residue was washed thrice with autoclaved distilled water.

2. 3 Characterization of Nanoparticle

Spectrometric analysis was done to confirm the presence of synthesised nanoparticles by using a Shimadzu UV-visible spectrophotometer (UV-1800, Japan). The characterization of silver nanoparticles was done by SEM and FTIR analysis. The size of the generated silver nanoparticle was analysed by SEM (JOEL - JSM 6390) at an accelerating voltage of 120kV.

2.4 Assessing Antioxidant Activity

The DPPH and ABTS assays were used to evaluate the antioxidant properties of synthesised silver nanoparticles.

2.4.1 DPPH Assay

The method of Brand-William *et al.,*[10] was used for assessing the antioxidant activity with slight modifications. The test sample's radical scavenging activity was calculated against stable 2, 2-diphenyl-2-picrylhydrazylhydrate (DPPH). The UV-visible spectrophotometer was used to quantify the colour shift (from deep violet to light yellow) at the optical density of 515 nm. Ascorbic acid was employed as a reference standard for the DPPH test.

% of inhibition = A0 – A1/A0 X100

where, A0 is the absorbance of the control, and A1 is the absorbance of the test compound.

2.4.2 ABTS Assay

2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) interacts with an antioxidant substance that could donate hydrogen to reduce ABTS. On a UV-visible spectrophotometer, the colour shift (from blue to colourless) was observed at the optical density of 734 nm. The reaction was started by adding 200µl of diluted ABTS to 1.56 - 800 g/ml of various sample concentrations. Methanol served as the blank. The absorbance was measured at 734 nm, and the percentage inhibition was calculated using the same formula used in the DPPH assay.

2.5 Photodegradation

The photocatalytic degradation property of synthesised silver nanoparticles was analysed using the synthetic dye methylene blue. Using UV-Vis Spec the mixture of dye and silver nanoparticles was analysed for measuring the photocatalytic degradation of dye by the silver nanoparticles in 30 min time gap.

3. Results and Discussions

3.1 Characterization of Synthesised Silver Nanoparticles

Silver ions when come in contact with the plant extract exhibit a colour change as a result of their reduction to silver nanoparticles. A dark yellowish-brown colour developed due to the Surface Plasmon Resonance (SPR)[11]. UV-Vis spectrophotometric analysis measured this gradual change in colour of the mixture from green to yellowish-brown and it confirms the production of AgNPs. The maximum peak of the synthesised nanoparticle was attained at 430 nm indicating various phytochemicals acting as reducing agents in *G. resinifera* leaf extract[12] (Figure 1).

FTIR enables the identification of small functionally active residues and indicates the presence of biomolecules which were involved in the synthesis of specific nanoparticles[13,14]. Furthermore, identification of nanoscale materials, confirmation of functional groups, recognising binding molecules and interaction pattern of biomolecules involved during the reduction of silver nitrate to silver can be elucidated by FTIR[15,16]. The FTIR graph of silver nanoparticles shows 12 peaks (Figure 2) which indicates the complexity of the sample.

In FTIR spectra, the peak of synthesized AgNps shows different stretches of bond at 3288.97, 2918.93, 2382.30, 1634.62, 1283.08, 1069.77, 1039.39, 819.29, 799.52, 730.93, 529.37 and 416.67 cm^{-1}. During the formation of metal nanoparticles, the stabilization of bonds occurs to manage the tendency for coagulation. The stabilization is acquired by stretching different bonds like N-H, C-N, C-O, O-H, *etc.*[17]

The broad absorption band present at the region of 3288.97 cm^{-1} is due to the presence of O-H which could be present in carbohydrates or phenolics. The absorption peak at 2918.93 cm^{-1} may be the presence of C-H stretching of the methylene group or aliphatic group. 2000-2500 cm^{-1} indicates a triple bond region. A

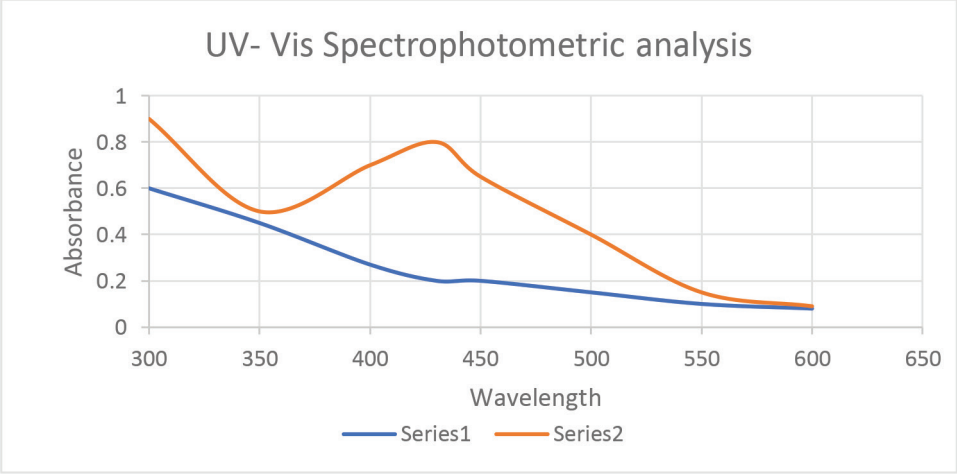


Figure 1. UV-Vis absorption spectrum of Series (1) *Gardenia resinifera* leaf extract and Series (2) biosynthesized AgNPs using *Gardenia resinifera* leaf extract.

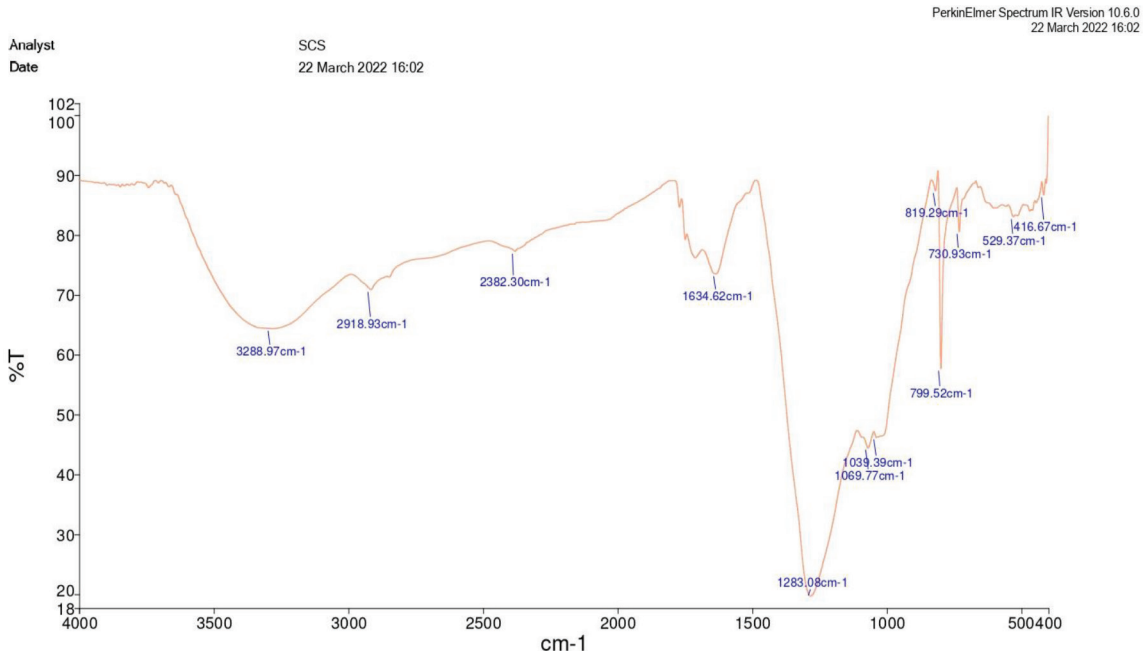


Figure 2. FTIR-spectra of synthesised silver nanoparticles using *Gardenia resinifera* leaf extract.

strong triple bond was detected at the region of 2382.30 cm^{-1}. Absorption spectrum at 2382.30 cm^{-1} due to the presence of C=-N stretching of alkene group. 1500-2000 cm^{-1} denotes the double bond region. A peak at 1634.62 cm^{-1} suggests C=O is associated with the Carboxyl group or esters. An absorption band at 1283.08 cm^{-1} corresponds to the vibration of amines C-N. The absorption spectrum at 1069.77 cm^{-1} and 1039.39 cm^{-1} regards the presence of Aliphatic Fluro compounds (C-F). The band at 819.29 cm^{-1} was because of the C-O stretch and C-S stretch. The sharp band present at 799.52 cm^{-1} and 730.93 cm^{-1}, indicates the presence of Aliphatic chloro compound (C-Cl) and Aliphatic bromo compound (C-Br). The peak at 529.37 cm^{-1} and 416.67 cm^{-1} may be due to the presence of O-H from phenols. The result indicates that carbohydrate, hydrocarbons, and aliphatic compounds present in plant extract are responsible for the synthesis of silver nanoparticles and they act as reducing, stabilizing and capping agents.

The morphology of the green synthesized silver nanoparticle was examined by using SEM on JEOL JSM 6390 on an accelerating voltage of 20 kV[18]. The scanned silver nanoparticle shows a spherical shape and the average size ranged from 70 nm to 100 nm. The SEM analysis of silver nanoparticles showed the non-uniform distribution. The morphology of silver nanoparticles depends on the association of organic molecules with the Ag (Figure 3).

3.2 Antioxidant Activity

The antioxidant activity of synthesized silver nanoparticles was analysed by DPPH and ABTS assay. DPPH is a stable organic free radicle usually used for the study of free radicle scavenging activities of various natural products. Figure 4 shows the response for the DPPH activity of leaf extract, synthesized silver nanoparticle and a standard drug ascorbic acid. The IC 50 values of Ascorbic acid were 0.19 µg/mL while that of leaf extract and AgNPs was 0.89 µg/mL and 0.31 µg/mL, respectively. IC50 values represent the concentration of sample that can scavenge 50% of the DPPH free radical present in the mixture. IC50 values are inversely proportional to the antioxidant activity. The AgNPs gave a slightly higher IC50 value than the standard but it was much lower than the plant extract. Earlier reports on the synthesis and analysis of antioxidant properties of AgNPs from different plant extracts were comparable with the current result[19]. The reduction in absorbance indicates an increase in percentage inhibition and higher radicle scavenging activity[20].

Another method employed for the calculation of the antioxidant activity of biosynthesised AgNPs is mediated by a redox reaction indicating the radical scavenging potential of silver in that against ABTS (Figure 5).

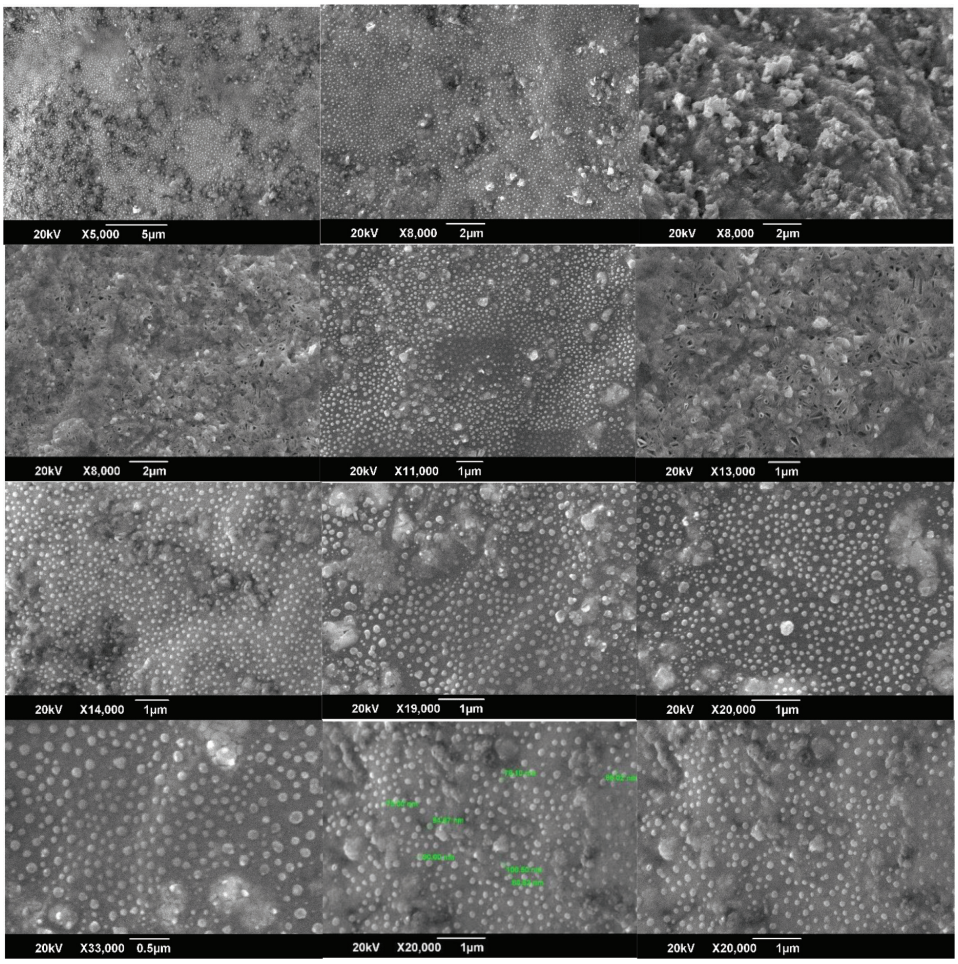


Figure 3. SEM images of silver nanoparticles at 2µm and 1µm in different magnifications like 8000x,11000x, 19000x and 20000x.

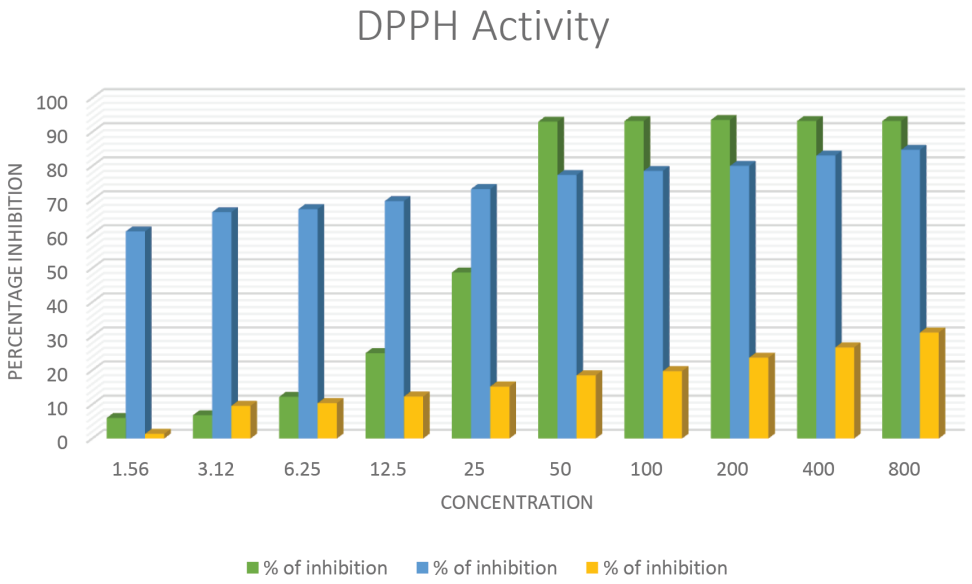


Figure 4. DPPH assay for antioxidant activity of AgNPs synthesised from *Gardenia resinifera* leaf extract compared with the leaf extract and a standard drug.

The IC50 value of the standard was 19.69 µg/mL, whereas that of the leaf extract and AgNPs were 76.12 µg/mL and 26.12 µg/mL, respectively. These results indicated the better efficiency of silver nanoparticles synthesised from *Gardenia resinifera* leaf extract as antioxidant agents by the previous reports of AgNPs using other green sources[21,22].

3.3 Photocatalytic Degradation of Synthetic Dyes

Photocatalytic activity of synthesised silver nanoparticles from *Gardenia resinifera* leaf extract was carried out by evaluating the efficiency of degrading the methylene blue. The degradation was measured by the change in colour at a visible range

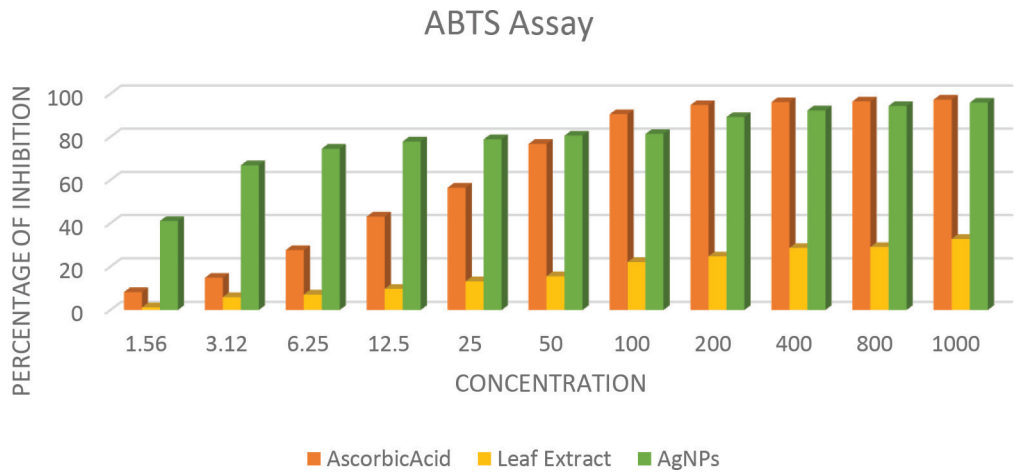


Figure 5. ABTS assay for antioxidant activity of AgNPs synthesised from *Gardenia resinifera* leaf extract compared with the leaf extract and a standard drug.

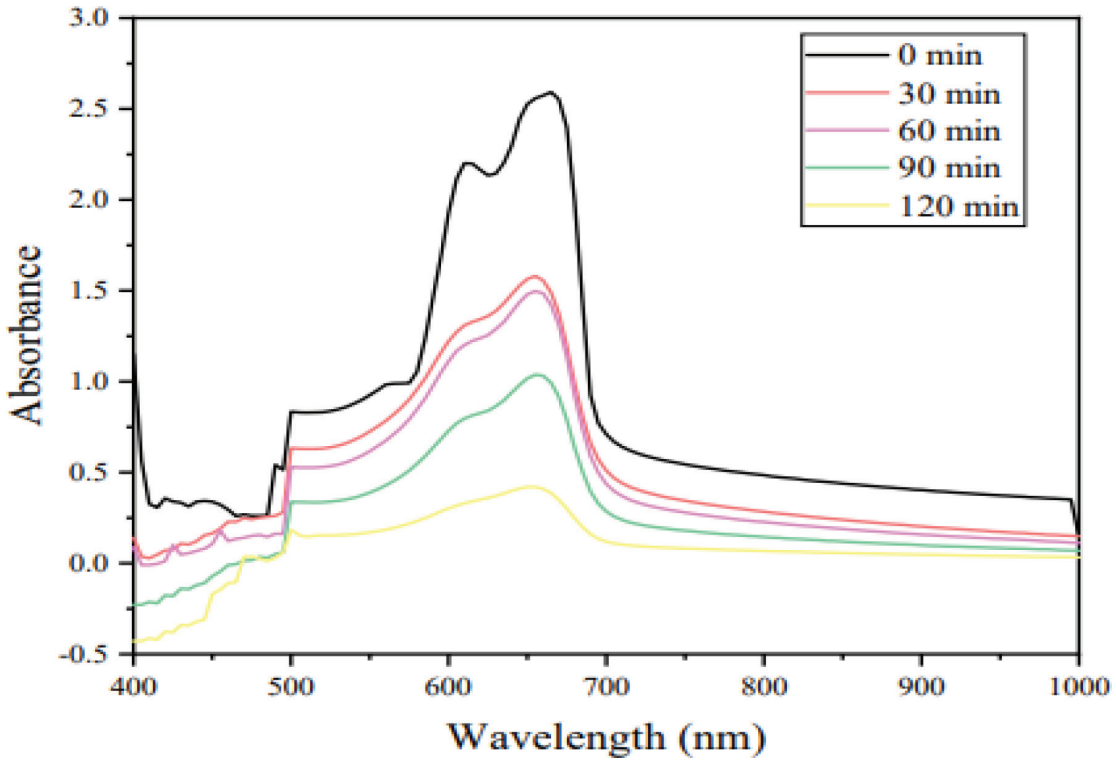


Figure 6. UV-Vis absorption spectra for the degradation of methylene blue by AgNPs synthesised from *Gardenia resinifera* leaf extract, under solar light.

of the spectrum using UV-Vis Spec[23] without the addition of any catalyst. Initially, a high-intensity absorption peak resulted in methylene blue at 660 nm.

A gradual decrease in absorption peak was observed by an increase in reaction time. The absorption peak generated at 660 nm nearly vanishes after 120 min indicating almost total degradation of methylene blue present in the solution (Figure 6).

4. Conclusion

This study provides an easy and environment-friendly approach for the synthesis of AgNPs using *Gardenia resinifera* leaf extract as a reducing and capping agent. The synthesised AgNPs were characterised by UV-Vis spectrophotometry, FT-IR and SEM analyses. Different peaks and the presence of bonds in FTIR spectra indicated the occurrence of several phytochemicals responsible for reduction and stabilization during the formation of AgNPs. Antioxidant assays like DPPH and ABTS revealed the antioxidant properties of AgNPs. The photocatalytic degradation of methylene blue mediated by AgNPs proved it as an inexpensive and green approach for the degradation of synthetic chemicals.

5. References

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