Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 8, 2014

Original Article

REMOVAL OF MALACHITE GREEN USING SILVER NANOPARTICLES VIA ADSORPTION AND CATALYTIC DEGRADATION

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Received: 23 Jun 2014 Revised and Accepted: 06 Aug 2014

ABSTRACT

Objectives: The objectives of the present study were a) to optimize the parameters for the AgNPs synthesis using aqueous leaf extract of *Aegle marmelos* b) to evaluate the performance of AgNPs as nanosorbents of synthetic dye Malachite Green c) to investigate the performance of AgNPs as nanocatalysts in the reduction of Malachite Green.

Methods: The effects of parameters such as leaf extract concentration and pH were studied by varying the leaf extract concentration from 5% to 20 % and reaction pH from 3 to 8 respectively. Under optimized leaf extract concentration and pH, AgNPs were synthesized and subjected to biosorption of Malachite Green from aqueous environment. Influence of pH, sorbent dosage and contact time on sorption of dye was investigated. In addition, the Catalytic activity of AgNPs in reduction of the synthetic dye using aqueous leaf extracts of *Aegle marmelos* was also investigated.

Results: The UV -visible absorption spectra of the AgNPs exhibited distinct band around 400- 460 nm. 20% leaf extract concentration and pH 7 were found to be the optimum conditions for synthesis of AgNPs. Sorption studies on influence of pH, sorbent dosage and contact time showed maximum adsorption at pH 5, 0.3 g and 4 h respectively. The UV visible spectra of the reaction mixture containing aqueous leaf extract of *Aegle marmelos*, Malachite Green and AgNPs confirmed the catalytic degradation of Malachite Green.

Conclusion: Our study revealed that AgNPs synthesized using aqueous leaf extract of *Aegle marmelos* can be used as nanosorbents and nanocatalysts in treatment of dye containing wastewater.

Keywords: Silver Nanoparticles, Aqueous *Aegle marmelos* Leaf Extract, Sorption, Catalytic activity, Malachite Green.

INTRODUCTION

In recent years, there has been an increasing demand for Silver Nanoparticles (AgNPs) due to their unique physico-chemical properties such as catalysis [1, 2] magnetic and optical polarizability [3] electrical conductivity [4] Surface Enhanced Raman Scattering [5]. These properties have been widely utilized in photography [6] catalysis [1] biological labeling [7] photonics [8] optoelectronics [9] and information storage [10].

AgNPs can be synthesized using a number of production techniques such as reduction in solution, Chemical and photochemical reaction, electrochemical reaction, sonochemical, microwave and laser technology [11, 12, 13, 14]. However these techniques yield low concentration of metallic nanoparticles. Furthermore these methods are costly and mainly involve the use of toxic, hazardous chemicals which pose potential environmental and biological risks.

The reduction of an aqueous solution of silver nitrate is a most widely used method for the synthesis of silver nanocolloids [15]. A search for sustainable process to reduce silver ions has led to the development of biomimetic approaches where biological principles are exploited for the growth of advanced nanoparticles. Many microorganisms including bacteria [16], fungi [17] and algae [18] have been reported to synthesize inorganic nanoparticles. Though the microbial synthesis of AgNPs is cost effective and eco-friendly, the Green synthesis of AgNPs using plant sources is gaining more interest in recent years. Plant extracts are known to contain several small molecules which have the potential to carry out reduction of silver ions to AgNPs followed by stabilization [19].

The synthesis of AgNPs using plant extracts is simple, economical and requires less reaction time. Moreover, the microorganisms generate nanoparticles at a much slower rate than the plant extracts. Leaf extracts of *Ficus benghalensis* [20], *Crossandra infundibuliformis* [21] have been shown to produce AgNPs with good stability. Other parts of plants such as tuber [22], bark [23] and buds [24, 25] have also been reported in AgNPs synthesis.

Aegle marmelos is one of the useful medicinal trees popularly known from pre-historic time for its nutritional, environmental and commercial importance [26]. The leaves contain broadly alkaloids phenylpropanoids, terpenoids and other polyphenols which were well recognized for their healing power towards wide variety of bacterial and fungal infections [27, 28]. Roa and Paria [29] reported the inexpensive one pot synthesis of silver nanoparticles by green route at room temperature, stabilized in situ using *A. marmelos* leaves extract. They also revealed that polyphenols present in leaves extract are crucial for AgNPs formation and capping by adsorbing on the surface of the formed nanoparticles.

Due to rapid industrialization, dyes have become one of the main sources of severe water pollution as it can be widely used [30, 31]. The release of dyes into our surrounding water bodies has toxic effect on human health and marine life. Malachite green is an organic compound that is used as a dyestuff and has emerged as a controversial agent in aquaculture. Malachite green is traditionally used as a dye for materials such as silk, leather, and paper. It is highly toxic to mammalian cells, carcinogenic and can cause skin irritation. Therefore, removal of Malachite Green from effluent is essential to protect the environment. Conventional biological treatment in removing dyes from wastewaters is generally ineffective as the dyes are resistant to microorganisms. Moreover, the physico-chemical treatment methods are ineffective at higher effluent concentrations. In recent years, Nanotechnology has been extended to the wastewater treatments. Due to high surface area AgNPs exhibits an enhanced reactivity [32]. To the best of our knowledge till date there is no study available on catalytic activity of AgNPs in reduction of Malachite Green dye using leaf extract of *A. marmelos.* In the present study, the influence of various parameters such as leaf extract concentration and pH on synthesis of AgNPs using aqueous *A. marmelos* leaf eaxtract was investigated. The performance of AgNPs as nanosorbents of synthetic dye Malachite green was also investigated. Moreover, the catalytic degradation of synthetic dye Malachite green by aqueous *A. marmelos* leaf extract in presence of AgNPs was investigated.

MATERIALS AND METHODS

Preparation of *Aegle marmelos* leaf extract

Fresh *Aegle marmelos* leaves were collected and washed with distilled water. Known amount of leaves (5 g) were added to 100 ml of distilled water and boiled for five minutes. The mixture was then filtered. The extract was immediately used for the study.

Synthesis of AgNPs

For the synthesis of silver nanoparticals (AgNPs), 5 ml of *Aegle marmelos* leaf broth was added to 45 ml of 0.01M $AgNO_3$ solution. The experiments were performed in 250 ml Erlenmeyer flask. The flasks were incubated in rotary shaker for desired time at 28ºC. The synthesis of AgNPs was monitored by visual observation and UV-visible spectral analysis. The broth was centrifuged for 10 min at 8000 rpm. The AgNPs pellet was collected, washed and dried at RT. The dried pellet was finely powdered using mortar and pestle.

Influence of *Aegle marmelos* leaf extract concentration on bioreduction of Ag^+

Different concentration (5%,10%, 15%, and 20%) of *Aegle marmelos* aqueous extract were prepared by boiling different amounts of fresh leaves (5g, 10g, 15g, 20g) in 100 ml of distilled water for 5 minutes respectively. The freshly prepared extract of different concentration were added to $AgNO_3$ solution and incubated for 3 hours in rotary shaker at 100 rpm.

Influence of pH on bioreduction of Ag^+

pH play an important role in the nanoparticles synthesis, this factor induce the reactivity of leaf extract with silver ions. The influence of pH in the synthesis of nanoparticles was evaluated under different pH (3, 4, 5, 6, 7, and 8) of the reaction mixture.

FT-IR spectroscopic studies

Nanoparticles formed in presence of leaf extract were separated, washed properly with water, dried and mixed with KBr. FT-IR spectral scanning of green synthesized AgNps was carried out using Bruker FT-IR spectrometer (SPECTRUM 1000) from 500 to 4000 cm^{-1}.

Batch adsorption studies

Batch adsorption studies were carried out using AgNPs as adsorbents of synthetic dye Malachite Green. To 100 ml of 10 ppm of Malachite green solution, 0.1 g of AgNPs was added and agitated for 6 h. Effect of pH was studied by varying the pH of dye solution from 3 to 8. Effect of Sorbent dosage was studied by varying the dosage from 0.1 to 0.5 g/ 100 ml. Effect of contact time was studied by collecting samples at regular intervals and determining the final concentration of dye using UV visible spectrophotometer at λmax 618 nm. Dye removal percentage was calculated as follows

$$\text{Dye removal percentage} = \frac{C_i - C_f}{C_i} \times 100$$

Where, C_i= initial concentration of dye before sorption and C_f= final concentration of dye after sorption,

Evaluation of effect of synthesized AgNPs on the reduction of Malachite Green by *aegle marmelos* leaf extract

In order to assess the catalytic activity of synthesized AgNPs, two reactions were carried out as described by Edison and Sethuraman [33]. In the first reaction, 1 ml of Malachite Green (1×10^{-4} M) was mixed with 0.2 ml of aqueous leaf extract of *Aegle marmelos* and 1. 8 ml of water and the reaction was monitored after 30 min. In second reaction, 1 ml of Malachite Green (1×10^{-4} M) was mixed with 0.2 ml of aqueous leaf extract of *Aegle marmelos* and 1.8 ml of synthesized AgNPs (100 mg/ml) and the reaction was monitored after 30 min. The values of absorption maxima of the reaction mixtures were compared with that of Malachite Green.

RESULTS AND DISCUSSION

Synthesis of AgNPs : The formation of nanoparticles was easily detected and characterized by UV-Visible spectroscopy owing to the surface plasmon resonance (SPR) i.e. the interaction of electromagnetic radiation and the electrons in the conduction band around the nanoparticles [34]. AgNPs, are observed strongly in the range 400-460 nm in visible region. In the present work, the AgNPs, were formed after the addition of aqueous leaf extract of *Aegle marmelos* which was evident from the appearance of brown color (Fig. 1). The color development was directly proportional to the time of incubation and was due to excitation of surface plasmon vibrations in AgNPs.

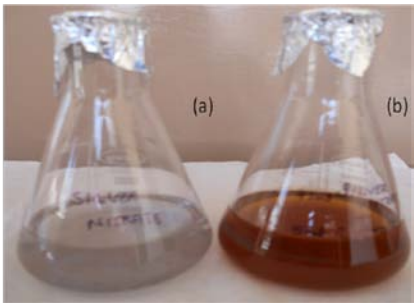

Fig. 1: conical flasks a) containing Ag^+ ions b) containing AgNPs formed after addition of leaf extract of *Aegle marmelos* which was indicated by the appearance of brown color.

The reduction of pure Ag^+ ions to Ag^0 was monitored by measuring UV-Visible spectrum of the reaction media at regular time intervals. After 5 min, Surface Plasmon Resonance of Ag occurred at 460 nm and steadily increased with the time of reaction without much change in the peak wavelength (Fig. 2).

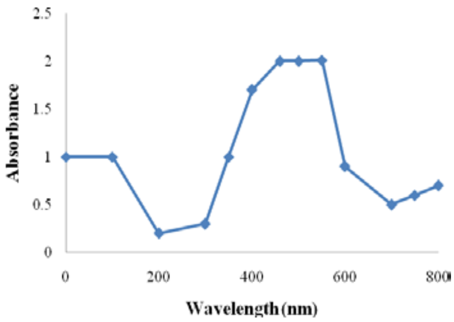

Fig. 2: UV-Visible spectral analysis of reaction mixture containing Silver Nitrate solution and aqueous leaf extract of *Aegle marmelos*. Leaf extract concentration: 5 ml of 5%; pH: 7; Silver Nitrate Solution: 45 ml of 0.01 M.

The SPR bands are influenced by the size, shape, morphology, composition, and dielectric environment of the prepared nanoparticles. Our results are in agreement with Roa and Paria [29] findings who reported the inexpensive one pot synthesis and stabilization of silver nanoparticles by green route at room temperature, stabilized in situ using *A. marmelos* leaves extract. They reported that the polyphenols present in the leaf extract are crucial for AgNPs formation and protocatechuic acid type phenolic compound was involved in capping by adsorbing on the surface of the formed nanoparticles.

Influence of *Aegle marmelos* leaf extract concentration on bioreduction of Ag^+

The influence on leaf extract concentration on formation AgNPs was evaluated by UV-Visible spectroscopic studies. The color of the reaction mixture containing Ag+ ions and aqueous leaf extract of *Aegle marmelos* increased with increasing concentration of leaf extract. Zhang et al. [35] observed that the quantity of *Aloe* leaf extract affected the AgNPs synthesis. When they increased the quantity of the biological material mediating nanoparticle synthesis, higher content of the biomolecules involved in the metal reductive process were available and thereby resulted in a more intense color of the reaction mixture. Similar effect had been observed with the bark extract of *Cinnamon zeylanicum* and with the leaves of *C. camphora* [23, 36].

As the concentration of the leaf extract increased from 5% to 20%, the sharpness of absorption peak increased and blue shift was observed from 460 nm to 420 nm. This blue shift indicates a reduction in the mean diameter of the silver nanoparticles. The blue shifted and sharp narrow shaped SPR band indicating the formation of spherical and homogeneous distribution of silver nanoparticles was observed (Fig. 3). Similar results were observed by Khalil et al. [37] in green synthesis of silver nanoparticles using olive leaf extract. Aggregation of nanoparticles is dependent on the biomolecules present in the leaf extract which act as reductants and stabilizing agent or capping agents. At higher extract concentrations the biomolecules act as reducing agent and cap the nanoparticles surfaces and protecting them from aggregation. Aggregation of nanoparticles occurred at lower concentration of leaf broth due to deficiency of biomolecules.

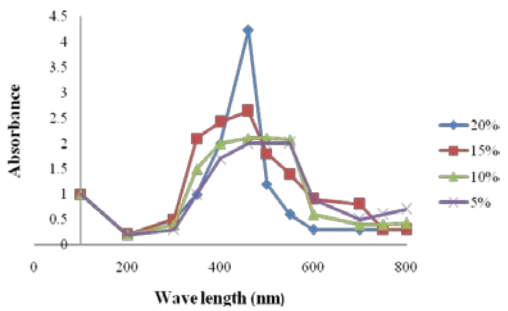

Fig. 3: Influence of leaf extract concentration on synthesis of AgNPs. Leaf extract concentration: 5%, 10%, 15 % and 20%; pH: 7; $AgNO_3$ Solution: 45 ml of 0.01 M.

Influence of pH on bioreduction of Ag^+

pH play an important role in the nanoparticles synthesis, this factor induce the reactivity of leaf extract with silver ions. The influence of pH in the nanoparticles was evaluated under different pH (3, 4, 5, 6, 7, 8) of the reaction mixture. From the Fig. 4, it is evident that the formation of AgNPs mainly depends on the pH of the reaction medium. The absorbance value was increased gradually with increasing of pH range from 3 to 8, suggesting the rate of formations of AgNPs is high in neutral and basic pH than in acidic pH. In pH 7, a narrow peak at 460 nm was observed.

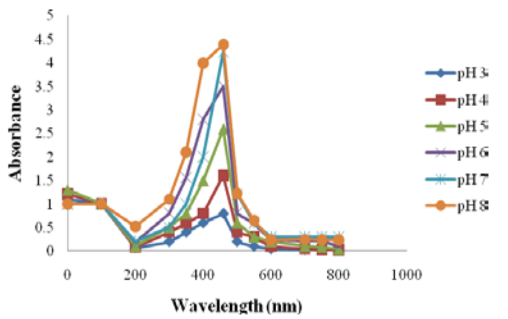

Fig. 4: Influence of pH on bioreduction of Ag^+. pH: 3,4,5,6,7 and 8. Silver nitrate solution: 45 ml of 0.01M. Leaf Extract concentration: 20%.

The slow rate of formation and aggregation of AgNPs at acidic pH could be related to electrostatic repulsion of anion present in the solution [38]. At the basic pH range there is a possibility of Ag+ precipitating as AgOH also. On the basis of the results, it could be concluded that the optimum condition for the preparations of AgNPs using aqueous extract of *Aegle marmelos* can be taken as the neutral pH. The results are in good agreement with reported literatures on biosynthesis of AgNPs using *Annona squamosa* leaf extract [39] and *Terminalia chebula* fruit extract [33]. The authors observed the formation of AgNPs occurred rapidly in neutral pH and basic pH which might be due to ionization of the phenolic groups present in the leaf extract and fruit extract.

FT-IR spectral analysis

FT-IR characterization was done to identify the functional groups involved in capping and stabilization of the synthesized AgNPs. Fig. 5 shows significant peak at 1595.65 cm^{-1} which corresponds to C=C stretching in aromatic ring. Subsequently the peak at 3385.20 cm^{-1} corresponds to O-H stretch of phenol group. Moreover, the peaks at 1383.18 cm^{-1}, 1265.99 cm^{-1}, 1163. 12 cm^{-1}, 1112.50 cm^{-1} and 1064.73 cm^{-1}correspond to O-H bend of poly phenols. The short peaks at 2922.00 cm^{-1} and 2665.59 cm^{-1} are because of C-H stretching vibrations in the aromatic compound.

The small peaks between 900 cm^{-1} and 670 cm^{-1} are due to C-H out of plane bend because of substitutions in different positions of aromatic ring. From the FT-IR spectra of the biosynthesized AgNPs, it is confirmed that the presence of phenolic compounds in the leaf extract is responsible for capping and stabilization of nanoparticles. Rao and Paria [29] identified the functional groups from FT-IR spectra of *A. marmelos* leaf extract which corresponded to the polyphenolic compounds. They also reported the presence of phenolic compound such as tannic acid product on AgNPs surfaces which may be responsible for the capping and particle stabilization.

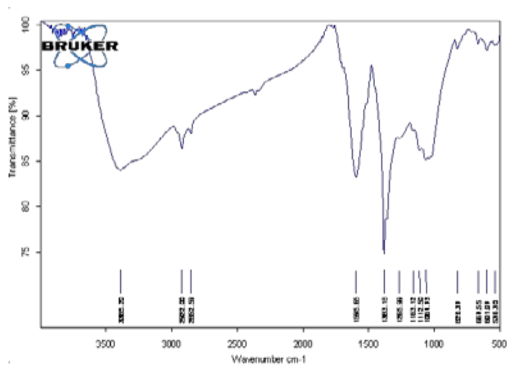

Fig. 5: FT-IR spectra of AgNPs synthesized using aqueous leaf extract of *Aegle marmelos.*

Batch adsorption studies

Effect of pH on adsorption of malachite green

The effect of initial solution pH on adsorption of Malachite Green using AgNps, is shown in Fig. 6. It was observed that dye removal percentage increased from 40% to 65% on increasing the pH from 3 to 5. Initial pH was selected as 5.0 for further adsorption experiments. Alishavandhi et al. [40] observed that when pH of the solution was increased from 1 to 8, the adsorption of Acid Yellow 199 on Silver nanoparticles loaded activated carbon decreased. At lower pH values the number of positively charged adsorbent sites on the surface of AgNPs loaded activated carbon increased.

As a consequence, the electrostatic interaction between the negatively charged dye molecules and the binding sites on the surface of AgNPs loaded activated carbon increased which favored the uptake of dye anions. In contrast, at higher pH values the AgNps loaded activated carbon surface became more negatively charged and so the electrostatic repulsion between the dye molecule and the AgNPs surface sites increased which led to decrease in uptake of dye anions.

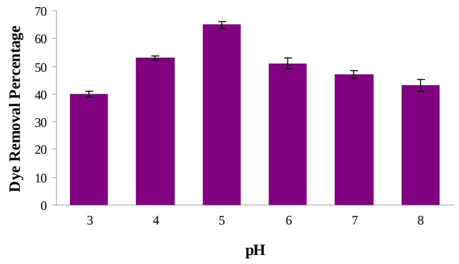

Fig. 6: Effect of pH on adsorption of Malachite Green using AgNPs. pH: 3, 4, 5, 6,7 and 8. Malachite Green Concentration: 10 mg/L. AgNPs Dosage: 0.1 g/100 ml.

Effect of sorbent dosage on adsorption of malachite green

Effect of Sorbent dosage was studied by varying the dosage from 0.1 to 0.5 g/ 100 ml. The amount of adsorbent has important influence on the adsorption and removal percentage and determines the capacity of adsorbent for given initial concentration. The effect of amount of AgNPs on the Malachite Green removal percentage showed that the initial removal percentage increased rapidly with the raising the AgNPs and after 0.3 g/ 100 ml, the removal percentage almost reached constant maximum value 99.8% (Fig. 7). The increase in dye removal percentage is due to increased available adsorption sites. Similar results were observed by Karimi et al. [41].

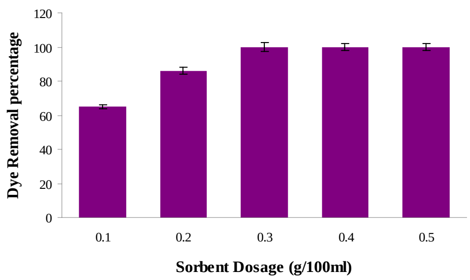

Fig. 7: Effect of Sorbent Dosage on adsorption of Malachite Green. pH: 5; AgNps Dosage: 0.1, 0.2, 0.3, 0.4, 0.5 g/100 ml. Malachite green Concentration: 10 mg/L.

Effect of Contact time on adsorption of malachite green

The adsorption of Malachite Green onto AgNPs was studied as a function of contact time. Fig. 8 showed that the dye removal percentage increased on increasing the contact time and reached equilibrium after 4 hours for initial concentration of 10 mg/L. Karimi et al. [41] reported the adsorption of Methyl orange using Silver nanoparticle loaded on activated carbon. They observed that the contact time for Methyl Orange solutions to reach equilibrium were 15 and 18 min for 10 and 20 mg/L of concentration, respectively.

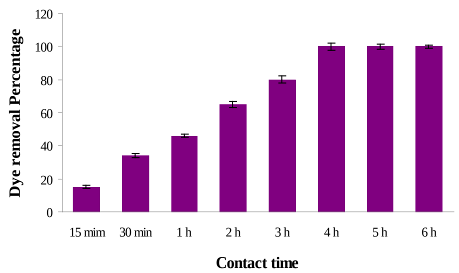

Fig. 8: Effect of Contact time on adsorption of Malachite Green. Contact time 15 min to 6 h. pH: 5; Concentratoion of Malachite green: 10 mg/L; sorbent dosage: 0.3 g/100 ml.

Catalytic activity of synthesized AgNPs on the reduction of malachite green by *Aegle marmelos* leaf extract

It is a well known fact that AgNPs and their composites show great catalytic activity in the area of dye reduction and removal. Pal et al. [42] studied the reduction of Methylene Blue by arsine in the presence of Silver Nanoparticles. Mallick et al. [43] studied the catalytic activity of AgNPs on the reaction of phenosaffranin dye. The present study aims at the reduction of Malachite Green by the natural green aqueous leaf extract of *Aegle marmelos* containing AgNPs. Pure Malachite Green has a λmax of 618 nm. Thirty minutes after the addition of the extract to the dye, the absorbance is gradually decreased in the first reaction (Fig. 9). The decrease of absorbance is indicative of the ability of phytoextract to degrade Malachite Green. In the second reaction system containing the dye, AgNPs and the extract, at the end of 30 min showed a marked decrease in the absorbance of Malachite Green and increase of SPR peak of AgNPs (Fig. 9). This reveals AgNPs act as an electron transfer mediator between the extract and Malachite green by acting as a redox catalyst, which is often termed as electron relay effect [44]. Catalytic activity of AgNPs on reduction of methylene blue by *Terminalia chebula* fruit extract was reported by Edison and Sethuraman [33].

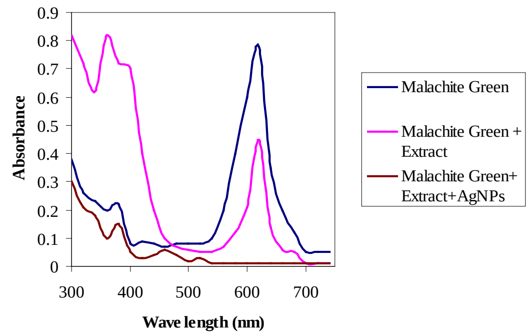

Fig. 9: UV-Visible Spectra of reaction mixtures containing Malachite Green alone; Malachite Green and aqueous leaf extract of *Aegle marmelos*; Malachite Green, aqueous leaf extract of *Aegle marmelos* and biosynthesized AgNPs.

CONCLUSION

We conclude that the AgNPs synthesized using aqueous leaf extract of *Aegle Marmelos* exhibit adsorption and catalytic activity in dye reduction process. Henceforth, Biosynthesized AgNPs could serve as both nanosorbent and nanocatalyst in treatment of Malachite Green bearing wastewaters. In technological perspective, Dye reduction by leaf extracts is a challenging alternative to chemical reduction and has high potential application. Further research is required to evaluate the mechanism of dye reduction by aqueous leaf extract of *Aegle Marmelos* using AgNPs as nanocatalysts.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENTS

The authors thank the Management of M. M. E. S Women's Arts and Science College, Melvisharam for providing the facilities to carry out this work. The authors are grateful to Dr. A. S. Sahul Hameed, HOD, Department of Biotechnology, C. Abdul Hakeem College of Arts and Science, Melvisharam and Mr. Vimal M.Sc., Research Scholar, Department of Biotechnology, C. Abdul Hakeem College of Arts and Science, Melvisharam for their support rendered in FT-IR spectral analysis.

Special Thanks to the General Secretary Mr. S. Ziauddin Ahmed Sahib, the Correspondent Mr. K. Anees Ahmed Sahib and the Principal Dr. Freda Gnanaselvam

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