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GREEN SYNTHESIS OF SILVER NANOPARTICLES (AGNPS) USING *MANILKARA ZAPOTA* FRUIT EXTRACT: POTENTIAL ROLE IN CATALYTIC DEGRADATION OF METHYLENE BLUE DYE

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Keywords:

Manilkara zapota, Catalytic degradation, Green synthesis, Antioxidant, Silver nanoparticles

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ABSTRACT: Present study reported a simple, rapid, eco-friendly and economical method for efficient production of AgNPs utilizing *Manilkara zapota* (*M. zapota*) extract. UV-Vis spectroscopic analysis was employedto ascertain the formation and stability of the synthesized AgNPs. The AgNPs were further characterized using TEM (Transmission Electron Microscopy), DLS, FT-IR, and XRD. The AgNPs exhibited a clear SPR band around 420 nm and were stable upto 6 months as confirmed during UV-Vis spectroscopic analysis. The nanoparticles were spherical in shape with a 10-15 nm size range, as evidenced by TEM results. FT-IR spectral studies demonstrated the presence of biomolecules such as phenols, amino acids, carboxylic acids, and aldehyde in the fruit extract, which might be responsible as reducing and capping agents during the formation of nanoparticles. From the DLS studies, the particles were found to have an average diameter of 90.28 nm, zeta potential value of -24.8 mV, and polydispersity index (PDI) of 0.147. The intense XRD pattern clearly revealed the face center cubic (FCC) structure as crystalline silver. The AgNPs efficiently and completely degraded methylene blue (MB) dye within 10 minutes. The AgNPs demonstrated good antioxidant activity in scavenging DPPH (2,2-diphenyl-1-picryl-hydrazyl) and H_2O_2 (Hydrogen peroxide). Owing to the results, the biosynthesized AgNPs indicate potential future applications in pharmaceutical and biomedical areas.

INTRODUCTION: In the last few decades, considerable attention has been given to silver nanoparticles owing to their unique physico-chemical properties in different fields like drug delivery, biomolecular detection, biosensing, water treatment, catalysis, textile industry, imaging and many other biomedical and engineering applications [1].

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Various physical and chemical methods are employed for the synthesis of AgNPs [2-3]. But they suffer from a number of drawbacks like the need for high temperature, energy, pressure and hazardous chemicals posing a great risk to both humans and the environment.

In recent times, the studies have become much focused on the green method of AgNP production, which comes with many advantages like freedom from toxic chemicals, cost effectiveness, simplicity, easy scalability, economic viability and eco-friendliness. AgNPs have been synthesized using different parts of plants, including fruit, seed, bark, peel, root, and leaf [4-6]. Secondary metabolites like polyphenols, terpenoids, flavonoids, saponins,

proteins, vitamins present in plant extract play an important role in the successful reduction of silver salt and stabilization/capping of the nanoparticles [7]. Artificial colorants, being used by various industries like pharmacy, paints, cosmetics, paper, textiles, plastics and food, pose great harm to human health and the environment. So, they need to be processed before being discharged into the water bodies. Various conventional methods like reverse osmosis, coagulation, adsorption, ultra-filtration, chlorination, and ozonation are being used to remove these dyes from water. But they fail to do so because these compounds are resistant to degradation due to their aromatic structural stability [8].

So, it is highly required to develop new techniques to degrade these dyes. Substantial efforts have been made in catalysis due to the increased issues of energy, resources, and the environment. Silver nanoparticles play an effective role in the catalysis of various chemical substances due to their high Fermi potential & size dependant rate constant. Hence, they could be exploited to degrade such toxic dyes [9]. Methylene blue, a thiazine dye, also known as methylthioninium chloride, is being used in numerous applications in biology, medicine, and chemistry. It is used for cell imaging as a staining agent, and also it is used for the analysis of sulphide ions in water. But it is toxic to the central nervous system. So, it is both biologically and environmentally important to degrade this dye before being discharged into the environment [10].

Manilkara zapota L., commonly known as sapodilla or naseberry, belonging to the family Sapotaceae is native to Mexico and Central America. The major phytoconstituents reported in the fruits include polyphenols (quercitrin, dihydromyricetin, methyl chlorogenate, myricitrin, (+)-gallocatechin, (-)-epicatechin, (+)-catechin, and gallic acid). The leaves have long been used in the treatment of diarrhea, cough, and cold. The bark is astringent, febrifuge, and antibiotic. Seeds are diuretic tonic, aperients, and febrifuge. Chicle from the bark is used to make chewing gums and in dental surgery. Fruit is used in the treatment of pulmonary diseases and diarrhea. The fruits and leaves of the plants have been demonstrated to possess strong antioxidant activity in previously conducted studies [11]. In the present work, AgNPs have been successfully synthesized using *Manilkara zapota* L. fruit extract, and their catalytic role in the degradation of the toxic dye; methylene blue, has been investigated.

MATERIALS AND METHODS:

Materials and Chemicals: *M. zapota* fruits were collected from the local market of Rohtak, Haryana (India). Silver nitrate was procured from Loba Chemie, India. Sodium borohydride was purchased from CDH, India, and Methylene blue from Rankem, India. All the aqueous solutions were prepared in Milli-Q water.

Preparation of Extract: *M. zapota* fruits were washed properly first with running tap water and afterward with Milli-Q water. The washed fruits were sliced into small pieces and crushed. About 40 g of the fruit paste was added to 200 ml of Milli-Q water. The resulting mixture was stirred on a magnetic stirrer for 20 minutes and filtered through Whatman filter paper no. 1; the resulting filtrate (yellowish brown in color) was used afresh to synthesize AgNPs.

Green Synthesis of AgNPs: 10 ml of the prepared extract was slowly added to 50 ml of 1mM aqueous $AgNO_3$ solution (optimal condition). The mixture was stirred on a magnetic stirrer for 10 minutes at 2,000 rpm at around 60ºC. The reaction mixture turned yellowish-brown in color within 5 min, and the color further changed to deep brown with time. The resulting AgNPs were centrifuged at 15000 rpm for 20 minutes, and the pellet thus obtained was given several washings with Milli-Q water. The purified nanoparticles were dried using lyophilizer and characterized further using different analytical techniques.

Characterization of as-synthesized AgNPs: The AgNPs were characterized using UV-vis Spectrophotometer (UV-1800 Shimadzu, Japan) within the wavelength range of 200-800 nm using quartz cuvettes of 1 cm path length. The morphological features (shape and size) of the synthesized AgNPs were investigated by TEM (Talos F200X TEM for Materials Science) using carbon-coated copper grids. FT-IR spectroscopic analysis was done to find the possible functional groups involved in reducing and capping AgNPs using KBr pellets using Bruker Alpha II FT-IR.

The particles' size and stability were evaluated using a zeta sizer instrument (Malvern Zeta sizer, Nano ZS90). X-ray diffractometer (XRD) was used to analyze the crystallographic structure of the nanoparticles.

Determination of *In-vitro* Antioxidant Activity of AgNPs: The synthesized nanoparticles were evaluated for their antioxidant activity by DPPH radical scavenging activity and Hydrogen peroxide scavenging activity using standard methods with slight modifications [12]. Different concentrations (20-100 µg/ml) of plant extract, AgNPs and standard were prepared. For the DPPH assay, 0.5 ml of different concentrations of plant extract, AgNPs, and standard were added separately to 5 ml of 0.1 mM methanolic solution of DPPH. The reaction mixtures were vortexed and incubated at room temperature in the dark for 30 min. The absorbance of each solution was noted at 517 nm. For the H_2O_2 assay, 1 ml of different concentrations of plant extract, AgNPs, and standard were added separately to 2 ml of 20 mM H_2O_2 solution prepared in phosphate buffer saline (pH 7.4). The reaction mixtures were incubated for 10 minutes at room temperature after shaking them vigorously. The absorbance of each solution was noted at 517 nm. Ascorbic acid was taken as standard in both assays. The control contains all the reagents except samples and standards. The decrease in absorbance was noted, and the percentage free radical scavenging activity was calculated using below mentioned formula. The absorbance of the resulting solutions against the corresponding blank solutions was measured using UV-Vis spectrophotometer.

$$\% \text{ Scavenging} = A_c - A_s / A_c * 100$$

Where, A_c = absorbance of the control and A_s = absorbance of the samples/standard.

Catalytic Activity Analysis of AgNPs: The synthesized AgNPs were evaluated for their catalytic activity against a toxic dye methylene blue (MB) using sodium borohydride ($NaBH_4$) as a reducing agent. 10 ml of 0.00005 M solution of MB was added to 5 ml of 0.005 M $NaBH_4$ solution. A total of 4 samples were prepared. In three samples, 1, 2 & 4 ml of the synthesized nanoparticles were added to a mixture containing MB & $NaBH_4$. One sample was prepared without sample/standard and taken as blank. The final volume was made upto 25 ml with Milli-Q water in all the reaction mixtures. The color of the reaction mixtures containing AgNPs slowly changed in color from deep blue to light blue and finally to colorless. The reduction reaction was monitored regularly using a UV-Vis spectrophotometer (Shimadzu UV 1800) at intervals of 2 min. The absorption maxima values were recorded and compared.

RESULTS & DISCUSSION:

UV-vis Spectral Analysis: In this study, the extract of *M. zapota* plant was added to 1 mM $AgNO_3$ solution. The reaction mixture instantly changed color to yellowish-brown, which deepened to brown after 30 minutes. The formation of AgNPs was indicated by the appearance of brownish color in the reaction mixture. It is well established that AgNPs in aqueous solution show yellowish-brown color due to the characteristic surface plasmon vibrations [13]. The phytochemicals present in the extract might have carried out the reduction of silver ions. *M. zapota* is rich in polyphenols (quercitin, dihydromyricetin, methyl chlorogenate, myricitrin, (+)-gallocatechin, (-)-epicatechin, (+)-catechin, and gallic acid) [11]. Previous studies reported that an increase in the number of peaks indicated decreased symmetry of nanoparticles, and the appearance of a single peak around 400-420 nm was attributed to spherical nanoparticles [14].

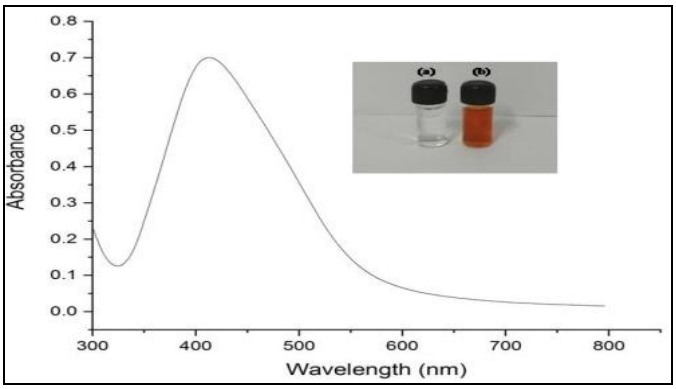

FIG. 1: UV-VIS SPECTRA SHOWING THE FORMATION OF AGNPS USING FRUIT EXTRACT OF *M. ZAPOTA* IN OPTIMAL CONDITIONS (A) $AgNO_3$ SOLUTION (B) AGNPS SOLUTION

A very clear SPR band centered at around 420 nm can be clearly seen in **Fig. 1**, which is assumed to be an indication of spherical nanoparticles.

Further, with an increase in the reaction time, the peak remained at the same position (even after 6 months), indicating that the nanoparticles are extremely stable, with no evidence of flocculation.

Transmission Electron Microscopy (TEM) Analysis: TEM analysis was done to evaluate the shape and size of the nanoparticles. The study revealed that the nanoparticles were almost spherical and monodispersed.

The size of the nanoparticles was found to be below 10 nm. This data has been found to be in good agreement with the available reports. For instance, Guan et al. (2019) reported an average diameter of 15 nm of the biologically synthesized AgNPs [15].

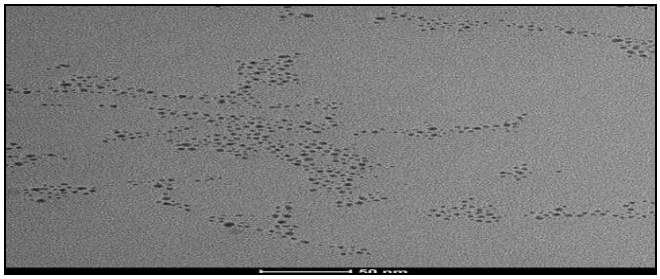

FIG. 2: TEM IMAGE OF THE SYNTHESIZED AGNPS

FT-IR Spectroscopy Analysis: FT-IR spectral studies are important for understanding the possible involvement of biomolecules present in *M. zapota* fruit extract in the synthesis of AgNPs. The FT-IR spectra of *M. zapota* extract and the synthesized silver nanoparticles were recorded, and the major peaks are shown in **Fig. 3**.

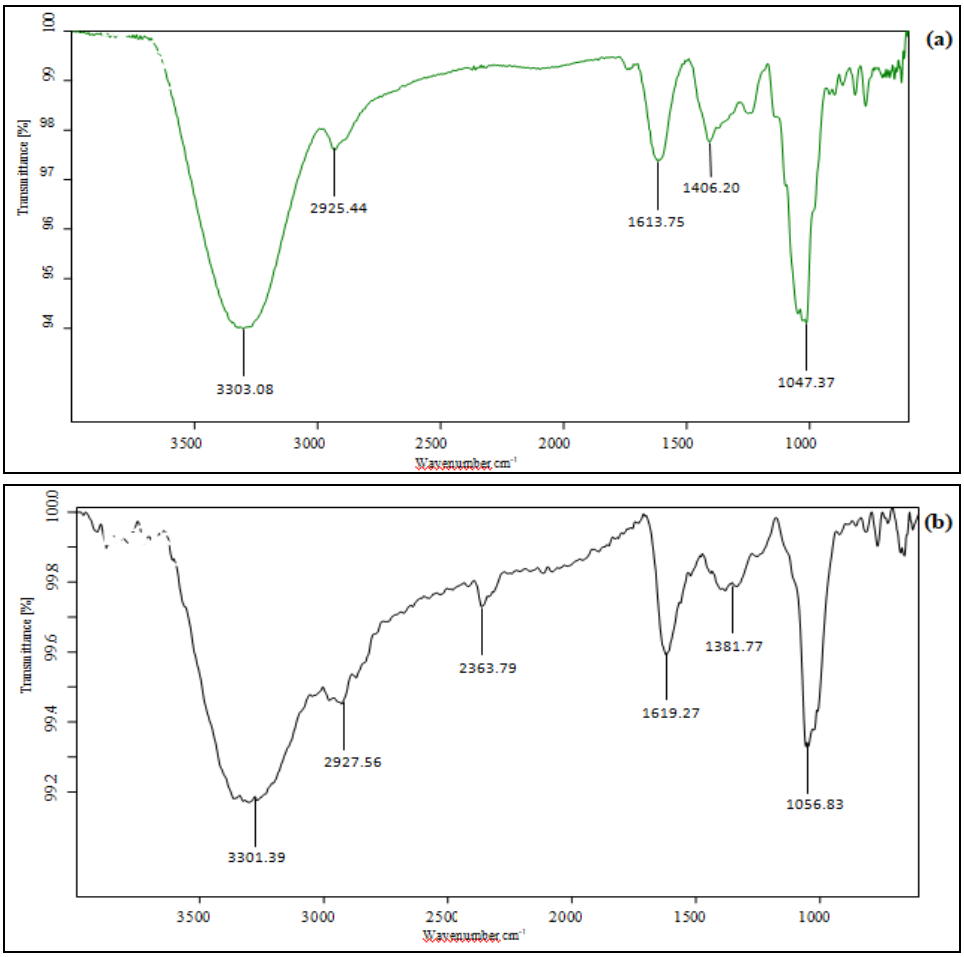

FIG. 3: FT-IR SPECTRA OF (A) *M. ZAPOTA* EXTRACT (B) *M. ZAPOTA* AGNPS

The FT-IR spectrum of *M. zapota* extract displayed a broad peak at 3303.08 cm^{-1} due to the intermolecular H-bonded –OH group of phenolic compounds present in the extract. While the FT-IR spectra of AgNPs showed a distorted peak at 3301.39 $cm^{-1,}$ which might be due to new interactions between the extract biomolecules and silver metal. The peaks at 2925.44 (in extract) and 2927.56 (in AgNPs) were assigned to the stretching frequency of alkanes. The peak at 1406.20 seen in the extract was due to C-O-H bending. The appearance of strong peaks at 1047.37 (in extract)

and 1056.83 (in AgNPs) were assigned to C-O group of phenols/alcohols. Other strong peaks at 1613.75 (extract) and 1619 (AgNPs) demonstrated NH bending, which might be due to the presence of amino acids. The peaks at 1406.20 (extract) and 1381.77 (AgNPs) were assigned to the carboxylate group. An interesting feature seen in the FT-IR spectrum of AgNPs was the appearance of a new peak at 2363.79 absent in the extract, suggesting the possible interaction between the extract and the silver metal. The presence of these peaks confirmed that the nanoparticles were capped by biomolecules such as phenols, amino acids, aldehyde, carboxylic acid and others present in the fruit extract. The presence of these functional groups on the surface of as-synthesized AgNPs confers stability to them by preventing their agglomeration. Our findings are well supported by the previous studies, which demonstrated the involvement of phenolics, flavonoids, aldehydes, carboxylic acid, and amino acids in the synthesis of AgNPs [16].

Dynamic Light Scattering (DLS) Analysis of AgNPs: The average size of the nanoparticles was found to be 90.28 nm. As observed from the results, the measured size of AgNPs using DLS was found to be larger as compared to the TEM measurements. This difference reveals that DLS measures the particle diameter alongwith molecules or ions attached to the surface of AgNPs whereas TEM purely is based on a number base size distribution and is devoid of any capping agent [17]. Zeta potential is an important parameter ascertaining the stability of aqueous nano suspensions. Higher the zeta potential values of nanoparticles (either positive or negative), higher will their stability towards agglomeration [18].

The zeta potential value of the as synthesized nanoparticles was found to be -24.8 mV, indicating that the particles have reasonably good stability. PDI of the synthesized nanoparticles (0.147) was below 0.4 suggesting that the nanoparticles were almost monodisperse in nature.

XRD Analysis of AgNPs: XRD pattern of the AgNPs is shown in Figure 4. The appearance of characteristic diffraction peaks at 38.14º (111), 44.41 (200), 64.54 (220) and 77.53 (311) can be indexed to face center cubic (FCC) structure of silver (JCPDS file no. 89- 3722).

The XRD pattern clearly revealed that the nanoparticles formed are crystalline in nature. Similar results were reported by Sudha *et al.*, (2017) [12].

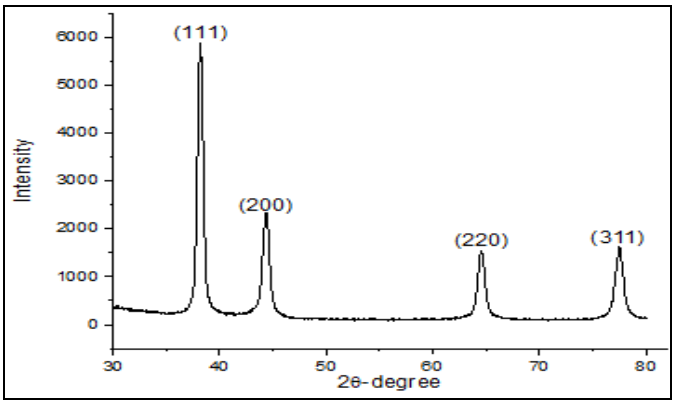

FIG. 4: XRD OF THE AGNPS EXHIBITING THE FACETS OF CRYSTALLINE SILVER

***In-vitro* Antioxidant Activity of AgNPs:** The synthesized AgNPs and plant extract were evaluated for *in-vitro* antioxidant using two substrates, DPPH and hydrogen peroxide, as shown in **Fig. 5 (A)** & **(B)**.

As evident from the results, with increasing concentration, the antioxidant activity of AgNPs and the plant extract increased.

The DPPH scavenging activity of AgNPs and plant extract was 51.32 % and 38.03 %, respectively at a concentration of 100 µg/ml. At the same time, ascorbic acid showed higher scavenging activity of 91.58 % at the same concentration. DPPH is a stable free radical and accepts electrons or hydrogen from AgNPs.

The hydrogen peroxide scavenging activity of AgNPs, plant extract, and ascorbic acid was 59.06%, 54.91%, and 56.22 %, respectively, at a concentration of 100 µg/ml. AgNPs demonstrated higher H_2O_2 scavenging activity as compared to the standard ascorbic acid.

Notably, the H_2O_2 scavenging effect of AgNPs was higher than the standard ascorbic acid. At a low dose H_2O_2 can accelerate the dissolution of AgNPs inside a cell and results in strong oxidative stress [19]. The antioxidants like flavonoids and phenols capped on the surface of the nanoparticles might have contributed to the DPPH and H_2O_2 scavenging activity [20].

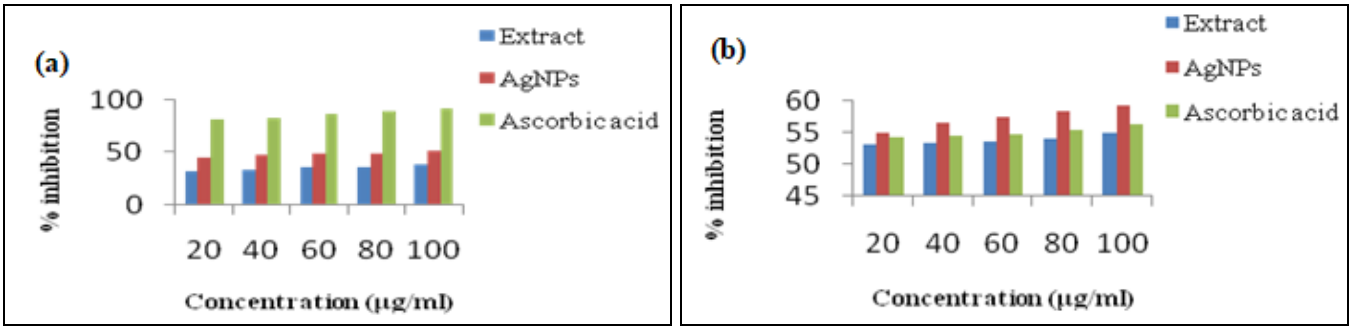

FIG. 5: DPPH RADICAL (A) AND H_2O_2 (B) SCAVENGING ACTIVITIES OF PLANT EXTRACT, AGNPS & ASCORBIC ACID

Catalytic Activity of AgNPs: The synthesized AgNPs were evaluated for their catalytic activity through the reduction of MB dye in the presence of $NaBH_4$ as a reductant. In its oxidized state, MB has blue color and upon reduction, it loses its color due to the formation of colorless leucomethylene blue. The UV-Vis spectrum of methylene blue shows characteristic peaks at 612 nm and 664 nm [21]. **Fig. 6 (A)** represents the UV-Vis spectrum of the degradation of MB by $NaBH_4$ alone without AgNPs and **Fig. 6 (B)**, **(C)** & **(D)** shows the UV-Vis spectrum of the degradation of MB by $NaBH_4$ with increasing amount of the synthesized AgNPs i.e., 1, 2 & 4 ml respectively. With the progress of reaction, the absorption maxima at 664 nm decreases accompanied by the appearance of a new band at 432 nm which intensifies with time.

It is evident from **Fig. 6 (A)** that the rate of degradation reaction is much slower without AgNPs than in the presence of AgNPs. Moreover, only 20-30% of MB is degraded after 24 minutes. As the concentration of AgNPs increases, the rate of reduction reaction also increases, which can be seen in **Fig. 6 (B)**, **(C)** & **(D)**.

The time required for 100% degradation of MB was found to be 24, 14 & 10 minutes with 1, 2 & 4 ml AgNPs solution, respectively. From the results, it is evident that the degradation process of MB becomes much more efficient after the addition of AgNPs. Our results are well supported by the previous studies, which demonstrated the catalytic potential of AgNPs [22].

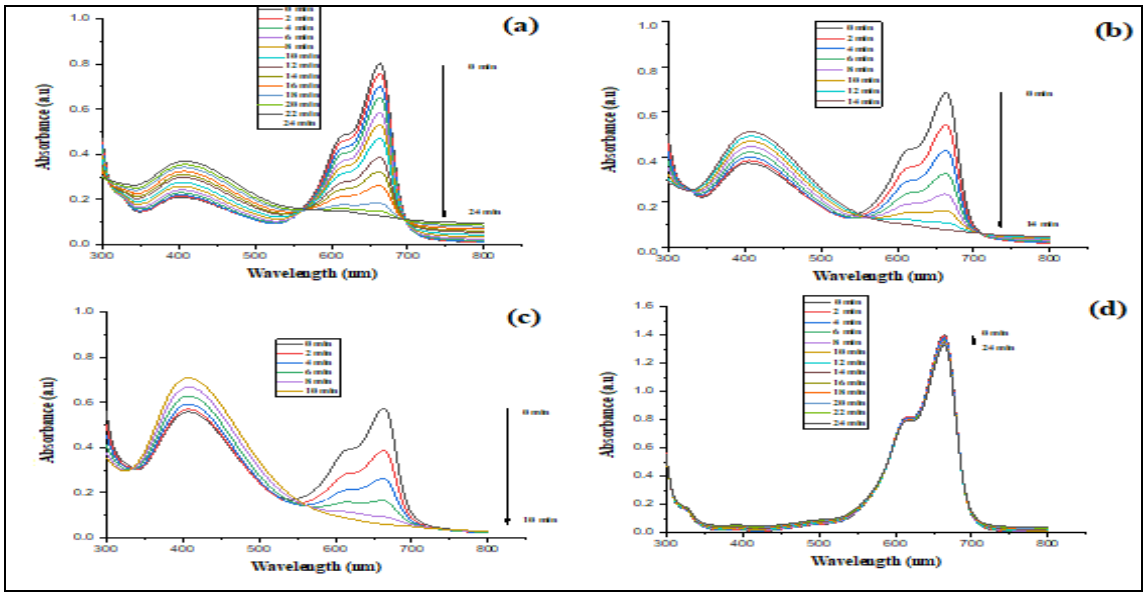

FIG. 6: UV-VIS ABSORBANCE SPECTRA SHOWING REDUCTION OF MB BY $NaBH_4$ AT 2 MIN INTERVALS: (A) WITH 1 ML AGNPS (B) WITH 2 ML AGNPS (C) WITH 4 ML AGNPS (D) WITHOUT AGNPS

CONCLUSION: Present work demonstrated the successful production of highly stable AgNPs by a green approach using the fruit extract of *M. zapota*. The method was easy, rapid, economical, and eco-friendly, proving advantageous over conventional physical and chemical methods. The AgNPs

demonstrated excellent activity as a catalyst in degrading MB dye and good antioxidant activity in scavenging DPPH and H_2O_2 free radicals. The biosynthesized AgNPs can further be exploited to develop useful agents for biomedical and pharmaceutical applications.

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CONFLICTS OF INTEREST: The authors declare no competing personal or financial interests.

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