Universal Journal of Green Chemistry
http://ojs.wiserpub.com/index.php/UJGC/

Research Article

Evaluation of *Gerbera jamesonii* Derived Silver Nanoparticles for Photocatalysis, Antibacterial Activity and Detection of Melamine Adulteration in Milk

Mathivathani Kandiah*, Bagya Kavirathne

School of Science, Business Management School (BMS), Sri Lanka
E-mail: mathi@bms.ac.lk

Received: 20 August 2024; **Revised:** 9 October 2024; **Accepted:** 11 October 2024

Abstract: Nanotechnology plays a crucial role in today's world in fields of food technology and textile. The present study focuses on green synthesis of silver nanoparticles using 5 petal extracts of *Gerbera jamesonii* and assessing its photocatalytic and antibacterial activity. The petal extracts were subjected to 30-min incubation at 90 °C for AgNP synthesis. Qualitative and quantitative analysis of antioxidants were carried out and results were statistically analysed in order to determine the highest incorporation of antioxidants in AgNP synthesis. The shape and size of synthesised AgNP were analysed using SEM, which showed spherical AgNPs with a diameter of 50–60 nm. Photocatalytic activity with Malachite Green (MG) in the presence of $NaBH_4$ confirmed a higher rate constant with AgNP diluted at 267 ppm. Antibacterial activity tested using well diffusion technique against *Escherichia coli* showed a higher growth inhibition in red (GerR), white (GerW) and yellow (GerY) petal extracts and *Staphylococcus aureus* showed a higher growth inhibition in AgNPs synthesised with pink (GerP) and yellow (GerY) petal extracts. Melamine adulteration in Milk was assessed using raw milk and 2 mM melamine spiked milk samples. A clear colour change was observed, and absorption peak was visible at 500–540 nm confirming the ability to detect melamine using AgNPs synthesised using GerP extracts. The results therefore suggests that green synthesised AgNPs can be utilised for various applications which will play a crucial role in quality assurance purposes in the food technology and textile industries.

***Keywords*:** silver nanoparticles, *gerbera jamesonii*, melamine adultrations, photocatalysis, antibacterial activity

1. Introduction

Nanotechnology involves matter at a range of 1–100 nm and encompasses in the development and application of chemical, physical, and biological systems having structural similarities to single atoms or molecules [1,2]. In comparison to the features of the bulk materials which are used in nanoparticle synthesis, the nanoparticles carry improved properties based on their size, distribution and morphology [3].

Out of the various nanoparticles synthesised using chemical and biological methods, silver nanoparticles (AgNPs) have been utilised extensively in various applications due to its distinctive chemical and physical properties including thermal, biological, electrical conductivity and optical properties [4]. AgNPs are mainly used in the medical and food technology fields as antibacterial agents, as it has proven to be highly toxic to prokaryotic cells including bacteria while its

DOI: https://doi.org/10.37256/ujgc.2220245540

relatively less toxic to eukaryotic cells[5]. Several studies showed that the difference in cell wall composition, difference between interaction and antioxidant mechanisms against Reactive Oxygen species and differences in genetic and metabolic pathways are several reasons for the elevated toxicity of AgNPs towards prokaryotic cells[6,7]. Furthermore, the removal of toxic dye contaminants and purification of contaminated water has been studied extensively with regards to the AgNPs as it has the ability to degrade dyes through its optical sensor properties[8].

As most conventional methods and chemical synthesis of nanoparticles result in toxic by-products, studies are carried out in bottom-up approaches utilising eco-friendly approaches such as bacterial, fungal and plant-based synthesis techniques. Although bacteria and fungi can be used in synthesis of nanoparticles, green plant-based synthesis is prompted due to its quick development, shorter protocols, non-pathogenicity and shorter incubation periods[9]. The nanoparticles are synthesised through a biochemical reaction, in which the phytochemicals present in the plant extracts rapidly reduce the metal ions into its stabilised form while acting as a capping agent, and a stabilizing agent with the biomolecules present within the extract[10].

Gerbera jamesonii which is a very common ornamental flower belonging to Asteraceae family is native to Asian and African region and a very popular commercial flower[11]. Studies have been carried out on the isolation of active compounds in *Gerbera jamesonii* including apigenin, kampferol, quercetin, Taxifolin, quercetin-4'-O-β-d-glucopyranoside, Rutin, Gerberinside, Gerberin, Protocatechuic acid, stigmasterol and Daucosterol (Figure 1: compounds 1 to 11 respectively), which has proven to act as excellent free radical scavengers, with potent anti-inflammatory, and anti-tumor activity[12]. Although research has been carried out previously on synthesis and characterization of *Gerbera jamesonii* derived AgNPs, no studies were carried out on testing for important applications pertaining to *Gerbera jamesonii* unlike other flowers of the same family[13]. Thus, the present study will focus on nanoparticle synthesis using petal extracts of five colours: Pink (GerP), White (GerW), Yellow (GerY), Maroon (GerM) and Red (GerR) of *Gerbera jamesonii* (Figure 2).

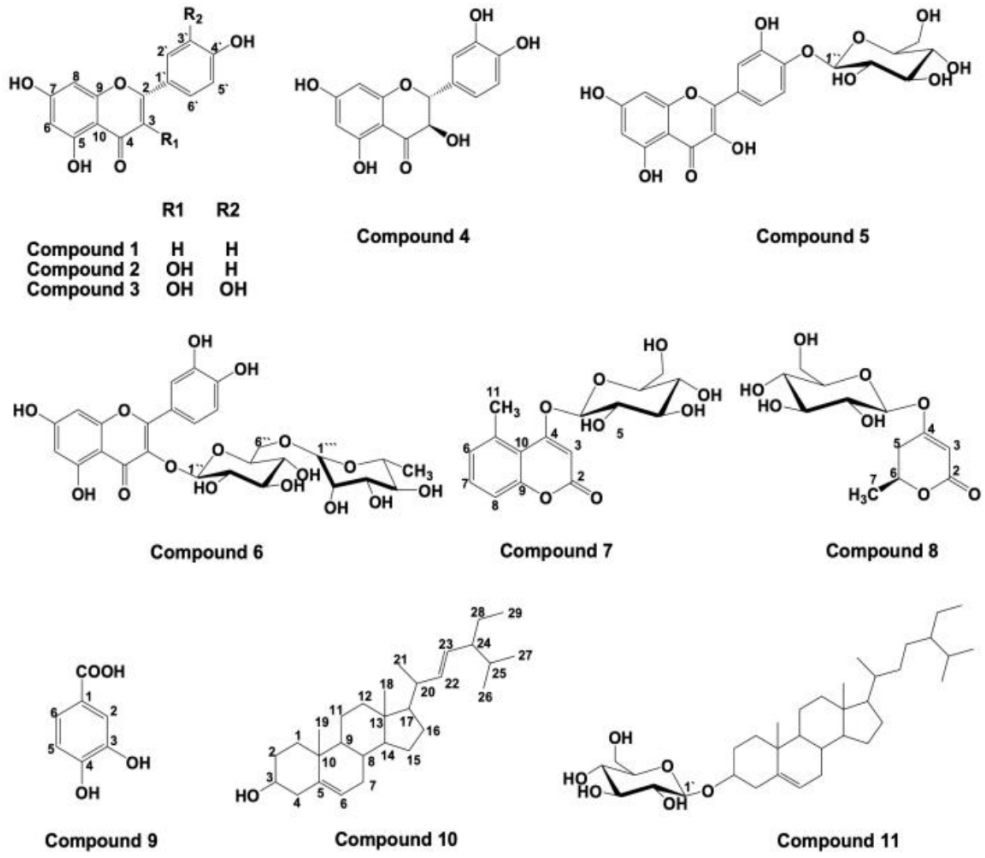

Figure 1. Active compounds isolated from *Gerbera jamesonii*[10]

Figure 2. Five colours of *Gerbera jamesonii* **A**: Pink (GerP) **B**: White (GerW) **C**: Yellow (GerY) **D**: Maroon (GerM) **E**: Red (GerR)

Exposure to toxic substances induce the production of free radicals in the body which can attack and damage cells and tissues leading to chronic health issues. Antioxidants function to deactivate and neutralize these free radicals. Studies show that phenolics in plants have antioxidant properties that enables them to function as reducing agents, hydrogen donors and oxygen quenchers[14]. In addition to plant extracts, AgNP synthesised by biological methods have proven to act as antioxidant agents, as it has the ability to destroy reactive oxygen species through capping agents such as phenolics and flavonoids from the plant extracts[15]. Thus, the green synthesised AgNPs will also be tested for their antioxidant activity in the present study via the Phosphomolybdenum assay and the DPPH free radical scavenging assay.

Non-biodegradable synthetic dyes that are widely used in the textile industry, which are highly mutagenic and carcinogenic has become an ecological issue in the recent past[16]. Conventional methods including chlorination, coagulation etc, has not been successful in removing these, due to their high-water solubility[17]. Studies are being carried out on the plasmon-derived photocatalysis of these azo dyes, using AgNPs by dissociation of azo bonds, resulting in non-toxic biproducts[18]. AgNPs are specifically chosen for the photocatalytic degradation, since they accommodate both UV and visible spectrum for their functions unlike other nanoparticles due to their optical property and surface plasmon resonance[19]. The surface plasmon resonance phenomena play a major role in the degradation, by exciting the conduction band electrons into the valence band causing a resonance with the light wave, leading to a shift in wavelength[20].

Colorimetric detection of toxic and harmful substances using green synthesised AgNPs is a common application studied using various nanoparticles. Melamine adulteration, began in China, with the addition of melamine into dairy products, in order to mask a dilution in protein went undetected at the time, due to ineffective tests, leading to the death of 6 infants and 52,000 hospitalizations[21]. The conventional methods of detecting melamine, including the HPLC and gas-chromatographic techniques were initially developed, during this scandal. Although they were sensitive and reliable, it incurred a very high cost and sophisticated techniques[22]. Since green synthesised AgNPs are eco-friendly and cost-effective, researchers developed colorimetric methods and assays to determine melamine adulterations using AgNPs. The underlying principle for the detection of melamine using AgNP is by the aggregation of melamine in the sample due to the electrostatic attraction between the amino group of melamine and nanoparticle surface, which in turn leads to a significant colour change that can be detected by a shift in the absorbance, when measured through UV-Vis spectrophotometry[23] (Figure 3).

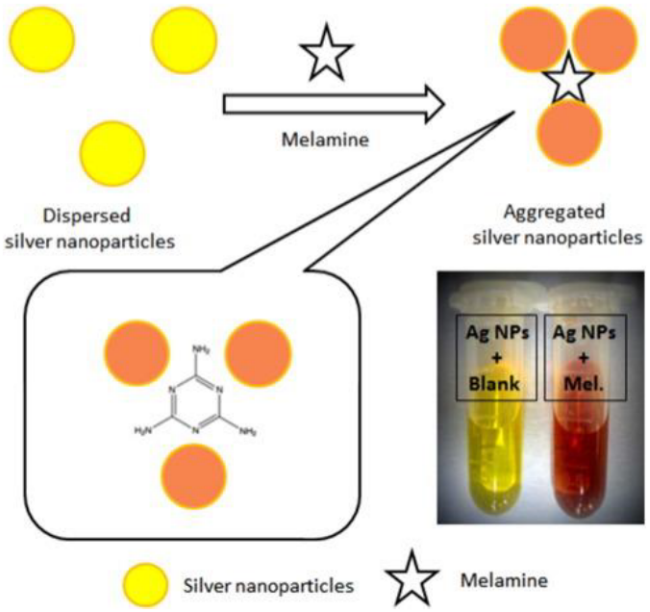

Figure 3. Mechanism of melamine detection in milk using AgNPs[22]

Among the applications of AgNPs, the antibacterial activity exerted by the nanoparticles have been studied extensively due to the properties of these nanoparticles. AgNPs being smaller in size and having a larger surface area, allows these molecules to easily penetrate the bacterial cell walls, damage membranes and cause cell death through production of reactive oxygen species and interfere with the DNA replication process eventually leading to cell death. Thus, the antibacterial activity of these nanoparticles has been extensively studied, using *Staphylococcus aureus* and *E. coli* as microorganisms. The present study will therefore focus on the antibacterial activity of the synthesised AgNPs against *E. coli* and *S. aureus*[24]. Antibacterial activity of AgNPs have been studied prior on many occasions due to the unique properties posed by AgNPs in exerting its activity of bacterial strains. The biocompatibility of AgNPs and their ability to attack multidrug resistant strains of bacteria has made it a promising solution to act as a successful antibacterial agent[25]. AgNPs exert the antibacterial activity through several ways such as interfering with the permeability and respiration of bacteria, penetrating bacterial cell walls and releasing highly reactive silver ions which contributes to the bactericidal affect[25]. Furthermore, AgNPs play a role in improving food packaging and increasing the shelf-life of packaged goods. Traditional food packaging techniques involve petroleum-based plastics which pose significant threat to the environment when discarded improperly. However, in comparison to other materials used in packaging, plastics are easier to be used in active packaging as it can be easily incorporated with bio-active compounds such as nanoparticles[26]. This has led to the development of active packaging, which has incorporated nanoparticles into packages, which has the ability to release antioxidants and antimicrobials into the food, leading to the increase in quality of food packaging and shelf-life[27]. Additionally incorporating these nanoparticles into food packages will lead to absorption of water vapour, regulation of moisture and act as thermal insulators, which will further improve shelf life of food[28].

This novel approach aims in the synthesis of AgNPs using 5 petal extracts of *Gerbera jamesonii*, and to detect their antioxidant activity, antibacterial and photocatalytic activities. Furthermore, the petal extracts will be analysed qualitatively and quantitatively for the presence of phytochemicals. The AgNPs will then be used in the colorimetric detection of melamine in melamine adulterated milk at various concentrations. The antibacterial activity will be tested against *Escherichia coli* and *Staphylococcus aureus* using both petal extracts and synthesised AgNPs. All objectives will be achieved, and test results will be analysed using statistical methods accordingly.

2. Methodology

2.1 *Sample collection and preparation*

Flowers of *Gerbera jamesonii* were collected from Haputhale, Sri Lanka (Figure 1). The petals were dried at 40 °C in hot air oven drying oven. Extraction method used by Arokiyaraj[29] was slightly modified using lower temperature. The petals were slightly grounded and the aqueous extracts were prepared by incubating 2 g of the powder in 50 mL distilled water at 60 °C for 15 min. The samples were filtered, into 50 mL centrifuge tubes using Whattman No. 1 filter paper and stored at 4 °C for further use[30].

2.2 *Phytochemical analysis of petal extracts*

The prepared aqueous extracts were qualitatively analysed for the presence of phytochemicals including tannins and flavonoids by modifying the procedures adapted by Waghmare and Sontakke[31,32] (Table 1).

Table 1. Qualitative analysis of phytochemicals

Phytochemical Tested	Methodology	Inferences
Proteins	To 0.5 mL of extract, 2 drops of Millons reagent was added	Positive results indicate a precipitate formation
Carbohydrates	Molisch reagent was added 3 drops each to 0.5 mL of extract, followed by few drops of conc. H_2SO_4 along the wall.	Positive results indicated a purple colour ring formation at the junction of the two layers.
Tannins	Few drops of freshly prepared 1% $FeCl_3$, was added to 0.5 mL of extract.	Formation of Brownish green or blue-black colour solution indicates presence of tannins.
Flavonoids	Freshly prepared aqueous 1% NH_3, was added to 0.5 mL of extract.	Yellow coloured solution formation indicates positive results
Phenols	To 0.5 mL of extract, 1 mL of distilled water was added followed by addition of 10% $FeCl_3$.	Formation of a green-blue solution indicates positive result
Steroids	1 mL of chloroform followed by 1 mL of conc. H_2SO_4, was added to 0.5 mL of extract.	If steroids are present lower chloroform layer will turn red coloured.
Glycosides	0.2 mL of Glacial acetic acid, with 1 drop of $FeCl_3$, was added to 0.5 mL of extract, followed by few drops of conc. H_2SO_4, along the wall.	Brown ring formation within the solution indicates positive results
Saponins	5 mL of distilled water was added to 0.5 mL extracts, and vortexed for 2 min.	Formation of a stable foam indicate presence of saponin

2.3 *Synthesis and visualisation of AgNPs*

Silver nitrate purchased from HiMedia (CAS: 7761-88-8) was used to prepare 9 mL of 1 mM $AgNO_3$ and added into 1 mL of each of the prepared aqueous extracts[33]. Aliquots of the prepared extracts were incubated at 60 °C and 90 °C for 15 min, 30 min, 45 min, 60 min and at Room temperature for 24 h to optimize and determine the best temperature and time for the synthesis of the AgNPs. The colour change was visualised, and absorbance was measured from 320 nm to 520 nm using the Jenway-6305 UV-Visible spectrophotometer in plastic cuvettes, to confirm the formation of the AgNPs. Samples incubated at 90 °C for 30 min showed the best absorption peak at 400 nm confirming the formation of AgNPs.

2.4 *Particle size analysis of AgNPs*

5 mL of the optimised AgNP synthesised from pink petal extract was aliquoted and centrifuged for 5 min at 12,400 × *g* at room temperature using the Grant Instruments Microspin-12 High speed centrifuge. The supernatant was discarded, and the procedure was repeated to obtain a prominent pellet of AgNPs. The pellet was oven dried to remove any remaining liquid and was gold coated to be analysed using the HITACHI SU6600 SEM, using the secondary electron detector. The images were obtained and analysed using AZtec software in different magnifications at a nanoscale.

2.5 *Determining the photocatalytic activity of AgNPs with Malachite green*

Malachite green Oxalate 64741, purchased from SRL India (CAS: 2437-29-8) was used to prepare 100 mL of 1 mM Malachite Green and added to 1 mL of two different concentration (267 ppm and 4000 ppm) of AgNPs in a beaker and exposed to direct sunlight. The absorbance was measured from 320 nm to 800 nm in 30 min intervals up to 90 min using distilled water as a blank. The procedure was repeated followed by the addition of 1 mL of 0.2 mM $NaBH_4$ (SRL India, CAS: 16940-66-2) solution and absorbance was checked, every 5 min up to 15 min.

2.6 *Determining the antibacterial activity of water extracts and AgNPs*

Antibacterial potential was evaluated against *Escherichia coli* (ATC25922) and *Staphylococcus aureus* (ATC25923) using the well diffusion technique on Mueller-Hinton agar[34]. Three wells were prepared on each plate for the negative control (saline), duplicates of sample and Gentamycin discs (Gen 10, HiMedia) was used as the positive control. 1 mL of 2 g/dm^3 solutions were prepared from water extracts and AgNPs, and 0.4 mL each was added onto the two wells in each plate. The agar plates were left for incubation for 24 h incubation at 37 °C in an incubator. Following that, the zone of inhibitions (ZOI) were measured using a ruler.

2.7 *Melamine detection in milk and melamine spiked water using AgNPs*

2.7.1 *Melamine detection in water using AgNPs*

The standard lab test proposed by Ramalingam[23] was carried out for the detection of melamine in water, with modifications. Series of concentrations of melamine (HiMedia, India, CAS108-78-1) was prepared from 2 mM to 10 mM. 0.6 mL of the prepared solutions were added with 0.3 mL of GerP AgNP. The absorbance was measured from 320 nm to 700 nm to observe the peak shift at ~540 nm during melamine aggregation[22]. Distilled water was used as the blank.

2.7.2 *Colorimetric detection of melamine in milk using AgNPs*

The method for detection of melamine in milk was adapted from Ping et al.[35], with modifications. 4 mL non-spiked pasteurized milk was taken into a 15 mL centrifuge tube and diluted up to 10 mL with distilled water. 2 mL from 10% TCA and chloroform solution, was added to the centrifuge tube and vortexed for 16 min. The solution was centrifuged for 30 min at 4000 rpm, and the supernatant was transferred to a fresh centrifuge tube. The pH of the supernatant was adjusted to 8, with 10% $NaHCO_3$, and centrifuged at 4000 rpm for 30 min. 0.6 mL of the resultant supernatant was added with 0.8 mL of the GerP AgNP. The absorbance was taken from 320 nm to 700 nm to observe for the peak shift at ~540 nm[22], using water as the blank. The same procedure was repeated to pasteurized milk spiked with 2 mM melamine.

2.8 *Quantitative analysis of antioxidant activity*

Triplicates of each optimised sample was analysed quantitatively for the presence of antioxidants including phenols and flavonoids.

Total Flavonoid content was quantified by modifying the method from Ayub et al[36]. 1 mL of sample was added, followed by addition of 0.3 mL of 5% (*w*/*v*) $NaNO_2$ and an incubation of 5-min. 0.6 mL of 10% $AlCl_3$ was then added to the incubated solution, followed by a 5-min incubation at room temperature. 2 mL of 1M NaOH was finally added into the resulting solution, and absorbance was measured at 510 nm, using distilled water as the blank and results were expressed µg Quercetin equivalents per 100 g (µg/QE/100 g).

The Total Phenolic content was analysed using the Folin-Ciocalteu method adapted from Ingkasupart et al[37]. 0.4 mL of the petal extracts were added with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with distilled water). The solution was incubated for 2 min at RT, followed by the addition of 1.5 mL of 7.5% (*w*/*v*) Na_2CO_3 solution. The resulting solution was incubated in the dark at room temperature for 2 h and the absorbance was measured at 760 nm, using distilled water as the blank. The results were expressed in g gallic acid equivalents per 100 g (g/GAE/100 g).

The Total Antioxidant capacity was determined quantitatively by using a Phosphomolybdenum assay employed by Anvari and Rashid Jamei [38]. A reagent solution was prepared by mixing 0.6M H_2SO_4 with 2 mM NaH_2PO_4 solution and 4 mM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ solution in the 1:1:1 ratio. 1 mL of the prepared reagent was added into 2 mL samples each, and the absorbance was measured at 695 nm after incubating for 90 min at 95 °C, using distilled water as the blank. The results were expressed as g ascorbic acid equivalents per 100 g (g/AAE/ 100 g).

The 2,2-Diphenyl-1-picrylhydrazyl Radical Scavenging Capacity assay was carried out using the methodology proposed by Negm El-Dein with modifications [12]. 2 mL of 0.4 mM methanolic DPPH (extrapure, 95%, SRLIndia, CAS: 1898-66-4) solution was added to 1 mL of sample, and incubated at 37 °C, for 20 min to obtain the absorbance at 517 nm, using methanol as the blank. Percentage activity was measured using Equation (1) DPPH percentage activity:

$$Percentage\ Activity\ = \frac{(Absorabnce\ of\ DPPH - Absorbance\ of\ sample)}{Absrobance\ of\ DPPH} \times 100\% \quad (1)$$

2.9 *Statistical analysis*

All the results were statistically analysed, using one-way ANOVA, and IBM SPSS v.28 was used to calculate the correlation coefficients for each phytochemical test that was carried out. Quantitative analysis of antioxidants and the antibacterial activity against *E. coli* and *S. aureus* was carried out using independent *t*-test by IBM SPSS v.28 where *p*-value ≤ 0.05 was considered significant.

3. Results and discussion

Synthesis of nanoparticles using various methods have been extensively studied in the past. However, due to the use of toxic chemicals in traditional methods, researchers now focus on the green synthesis of these nanoparticles, using plant extracts. Out of these nanoparticles, AgNPs have proven to be effective in exerting efficient antibacterial activity and has shown promising effects in many other fields, which led the present study to be focused on synthesising AgNPs using eco-friendly approaches [39]. The results of the present study might have been influenced by the method employed, as drying of the petals was a major challenge faced due to possible fungal growth. Therefore, the samples were dried within a shorter period using the hot-air oven at 40 °C.

Synthesising of AgNPs using the petal extracts of *Gerbera* has been carried out previously in order to analyse the role of the phytochemicals in stabilising AgNPs [13]. A study was also carried out to isolate various phytochemicals from methanolic extracts of *Gerbera*, revealing excellent free radical scavenging phytochemicals [10]. Since this study was an eco-friendly approach to synthesis AgNP, distilled water was used to extract the antioxidants and the phytochemicals from *Gerbera jamesonii.* Using water as an extraction solvent is beneficial over, using chemical reagents, due to its low cost and non-toxic effects and absence of toxic biproducts which require treatment prior to release [40].

3.1 *Phytochemical analysis*

Since phytochemicals are important in reducing the Ag^+ ions into stabilised forms, phytochemicals present in the plant extracts were tested qualitatively, as studies with the water extracts of these flowers have not been studied prior. The results proved the presence of tanins, carbohydrates and soluble proteins in all samples (Table 2). The presence of these phytochemicals is beneficial in green synthesis of these nanoparticles, as it doesn't require additional stabilising agents [41].

Table 2. Phytochemical analysis (+refers as presence and refers as not present)

Test	GerP	GerM	GerW	GerR	GerY
Millons test	+	+	+	+	+
Molisch test	+	+	+	+	+
Tannin test	+	+	+	+	+
Saponin test	–	–	–	–	–
Flavonoid test	+	–	+	–	+
Phenol test	–	–	–	–	–
Steroids test	+	+	–	+	+
Glycosides test	+	+	–	+	+

3.2 *Synthesis and visualisation of nanoparticles*

AgNPs synthesised at 90 °C for 30 mins showed a distinct colour change from light colour (Figure 4A) to a darker yellowish and brown colour (Figure 4B) confirming the synthesis of nanoparticles (Figure 2A). The colour changes due to the excitation of electrons by the surface plasmon resonance effect and the reduction of Ag^{+} to Ag^{0} is a definite indication of the formation of the nanoparticles as shown by previous studies synthesising AgNPs[42]. The intensity of the colour in each of the solutions act as a direct indication of the nanoparticles present in the solution[41]. Further confirmation of the synthesised nanoparticles can be confirmed through visualisation by UV-Visible spectrophotometry. The UV/Visible spectrophotometry results (Figure 2C) indicate clear absorption peaks at 400 nm in all synthesised samples confirming presence of AgNPs.

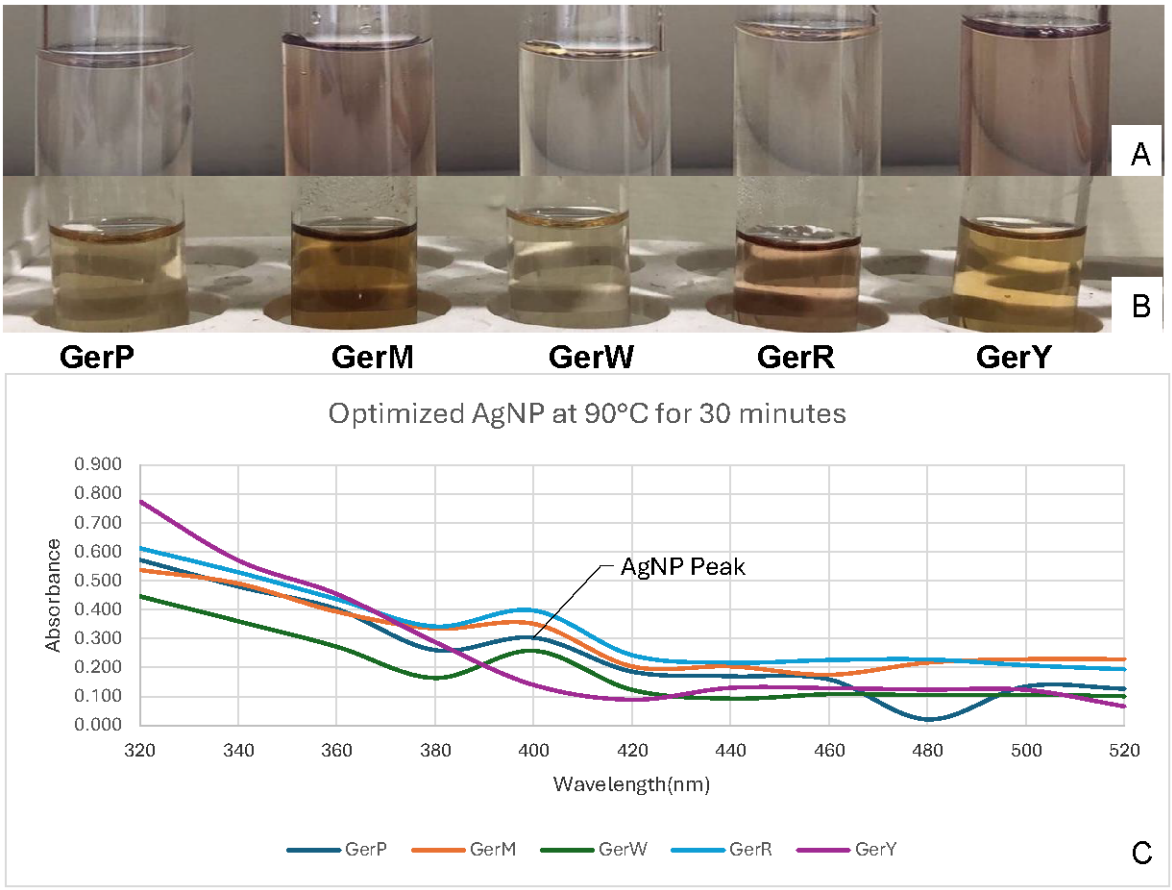

Figure 4. (**A**) Plant extracts before incubation (**B**) Synthesised AgNP (**C**) UV/Vis spectrophotometric results

3.3 *SEM visualization of synthesised AgNP*

The SEM images obtained in 500 nm scale (Figure 5), indicated the synthesised nanoparticles were spherical in shape and had a diameter of 50–60 nm. The band gap energy was calculated using the equation (Equation (2): Bandgap energy) which confirmed all synthesised AgNPs act as semiconductors, as they had a band gap energy of <3 eV. Although all silver nanoparticles are metallic conductors, the synthesised nanoparticles may show semi-conductor like properties due to the SPR affect, although previous studies have not shown semiconductor behavious of AgNPs. The band gap energy will aid in determining the conductivity of the nanoparticle which can be used in fabrication of electrodes [43].

$$\begin{aligned} &\text{h-Planck's constant } (6.626 \times 10^{-34}\text{J/s}) \\ &\text{C-speed of light } (3 \times 10^{8}\text{m/s}) \\ &\lambda\text{—the cut off wavelength value} \end{aligned} \tag{2}$$

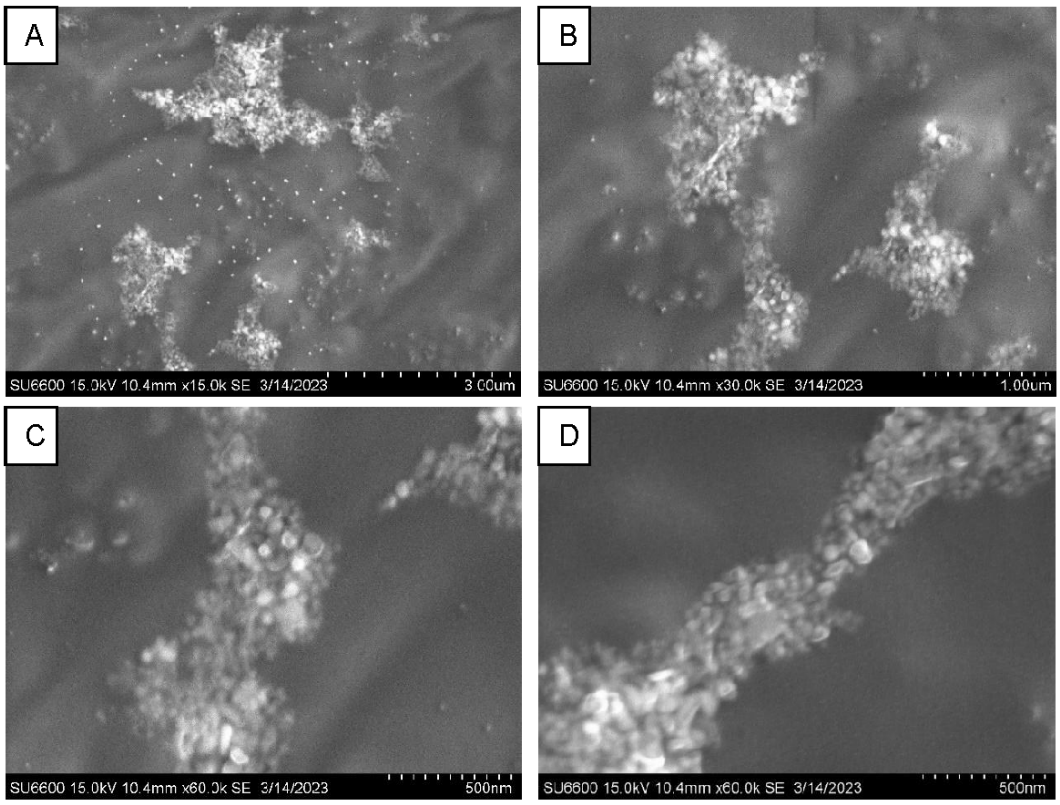

Figure 5. SEM analysis at 15.0 kV (**A**) 10.4 mm × 15.0 k 3 µm (**B**) 10.4 mm × 30.0 k 1 µm (**C**) 10.4 mm × 60.0 k 500 nm (**D**) 10.4 mm × 60.0 k 500 nm

3.4 *Quantitative analysis of antioxidants*

To quantitatively analyse the presence of the phytochemicals and antioxidants which involve in stabilising and capping the AgNPs, the TFC, TPC and the TPC and the DPPH assay was carried out. The results were analysed using One-way ANOVA, and flavonoids, phenolics showed higher concentrations in synthesised AgNPs than the water extracts (Figure 6A,B). The statistical difference observed between the TFC and TPC in water extracts and AgNPs suggests a possibility of significant contribution of antioxidants in stabilising the AgNPs. Previous studies done on other species of family Asteraceae has shown that flavonoids and phenols acting as free radical scavengers, and capping agents and increase the

surface area, which can be beneficial in incorporating these AgNPs as potential drug carriers in personalised medicine and targeted drug therapy[44,45]. A significant difference was observed when the absorbance was measured at 695 nm and analysed through a one-way ANOVA (Figure 6C/D). The scavenging activity was higher in the synthesised AgNPs than the water extracts suggesting that AgNPs show excellent radical scavenging activity when compared to water extracts, which will play a role in exerting antioxidant effects against reactive oxygen species. Calculation of correlation of phenols and flavonoids with the total antioxidant capacity showed that phenols were moderately correlated while flavonoids did not show any correlation suggesting that the total flavonoids in the AgNPs have not incorporated in exerting the antioxidant activity shown by the AgNPs, while the phenols have. Studies have proposed that phenols infer the antioxidant activity by scavenging free radicals such as reactive oxygen species (ROS), suppressing ROS through enzyme inhibition or chelating metals involved in free radicals production and by the up regulation of antioxidant defense[46].

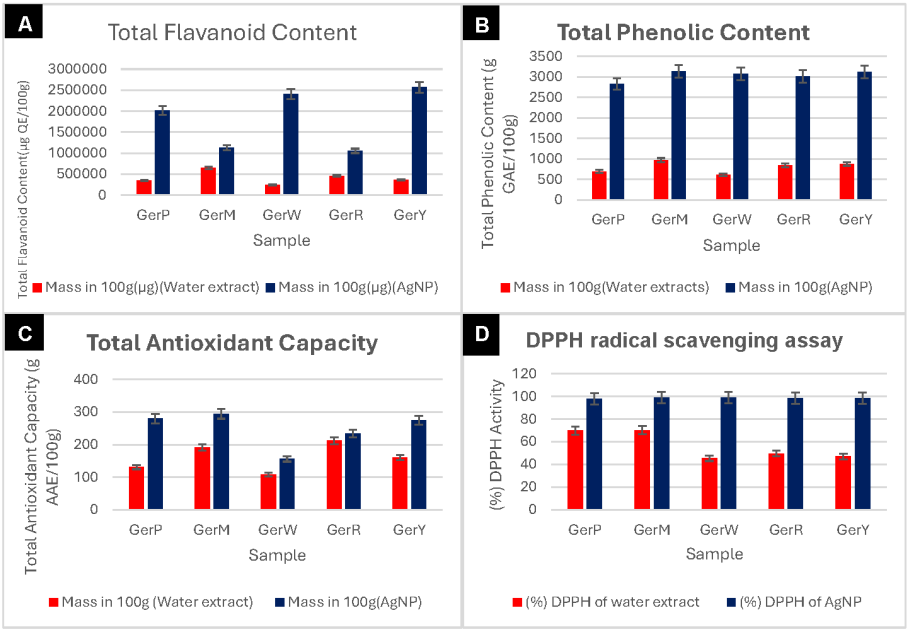

Figure 6. (**A**) TFC (**B**) TPC (**C**) TAC (**D**) Radical scavenging activity of AgNPs

3.5 *Photocatalytic activity of AgNPs*

Due to the complex chemical content, synthetic organic dyes highly contribute to water pollution. Due to the industrial effluents water pollution have led to sever health hazards and environmental concerns. The removal of these dyes is currently approached with various complex physical and chemical treatment which are time consuming, cost-intensive and shows lesser effectivity[47]. Photocatalytic dye degradation using AgNPs is thus approached to degrade these dyes, without production of any toxic by-products, due to their excellent surface properties and chemical reactivity. Photocatalytic dye degradation is carried out by the formation of oxygen radicals and hydroxyl radicals from the photogenerated electrons due to the SPR effect, attacking the dye and adsorbing onto the AgNPs and degrading it[48] (Figure 7). Although studies have shown malachite green (MG) to have an λ_{max} of 618 nm, and degradation in the absence of the $NaBH_4$, the AgNPs in the present study were unable to degrade the dye due to the larger redox potential between the electron donor and the acceptor which usually limits the reaction rate, in the absence of a catalyst[49]. However, with the addition of $NaBH_4$ catalyst which donates electrons for the formation of free radicals, the electron donor easily crosses the activation energy barrier in the catalytic degradation reaction causing complete degradation of the malachite green dye within 0–5 min which may have resulted in the color change of the dye from blue green to colorless (Figure 8), which was detected through the absorbance values of the UV-Vis spectroscopic analysis (Figure 9). The absorbance levels shown by the UV-Vis spectroscopic analysis

further proves the complete degradation of the dye within 5 min with a reduction in λ_{ma} absorbance level. The calculated rate constants (Figure 10) shows that degradation of malachite green has happened faster at 4000 ppm concentration of AgNPs (K = 1.3919), suggesting that a higher concentration of AgNPs degrades the dye faster in the presence of $NaBH_4$ than at 267 ppm (K = 1.2721). Several studies have shown that the rate of degradation of Malachite green was higher when relatively higher concentrations of AgNPs were present [50]. Therefore, it is evident that the AgNPs demonstrate higher chances of an effective light sensitive photocatalyst and can be used effectively in the degradation of organic pollutants in wastewater treatments in an eco-friendly and cost-effective way.

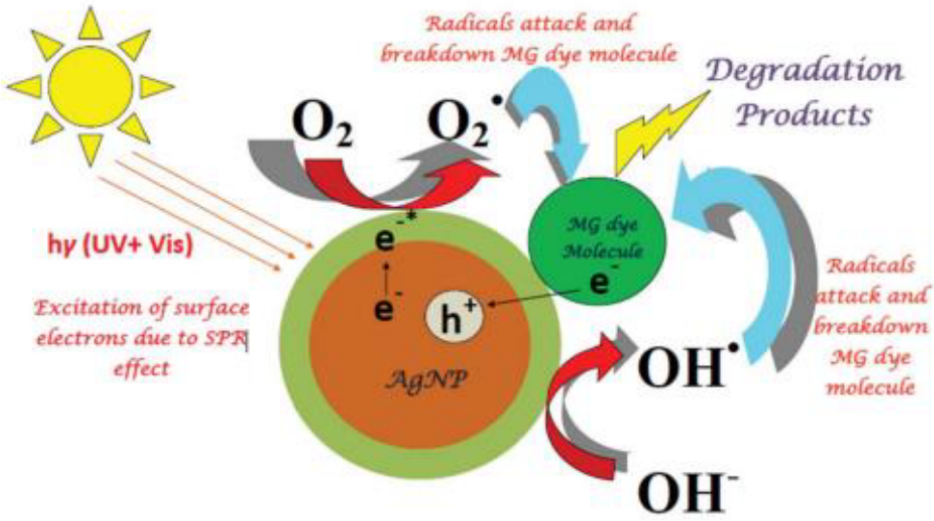

Figure 7. Mechanism for photocatalytic MG dye degradation from AgNPs [41]

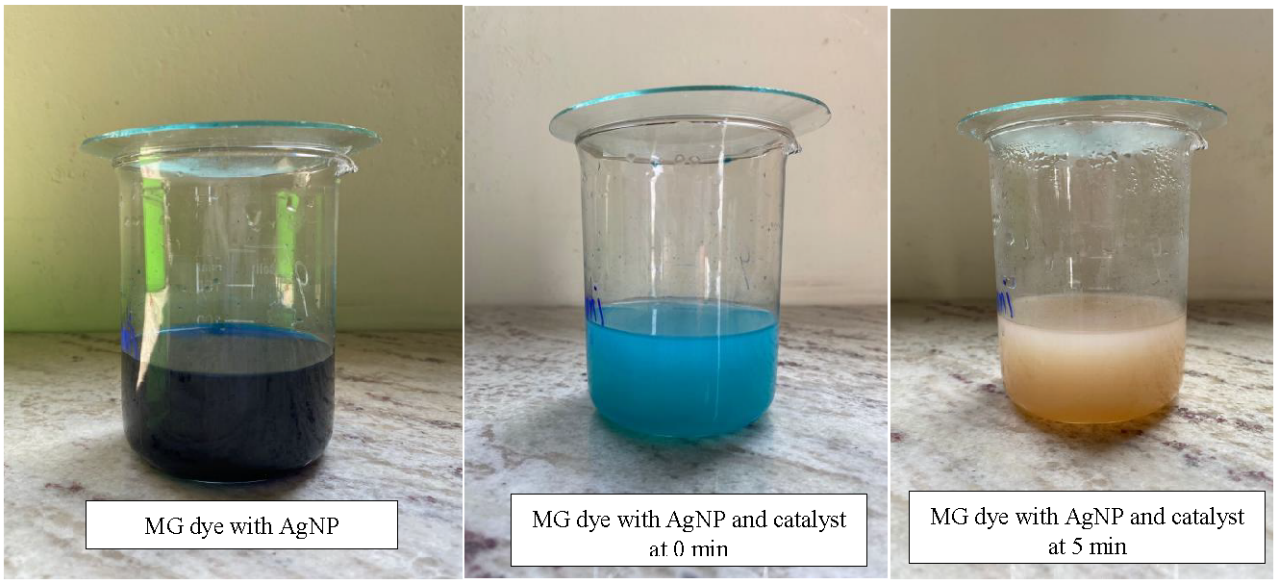

Figure 8. Degradation of MG dye with $NaBH_4$ 0–5 min at 267 ppm AgNP concentration

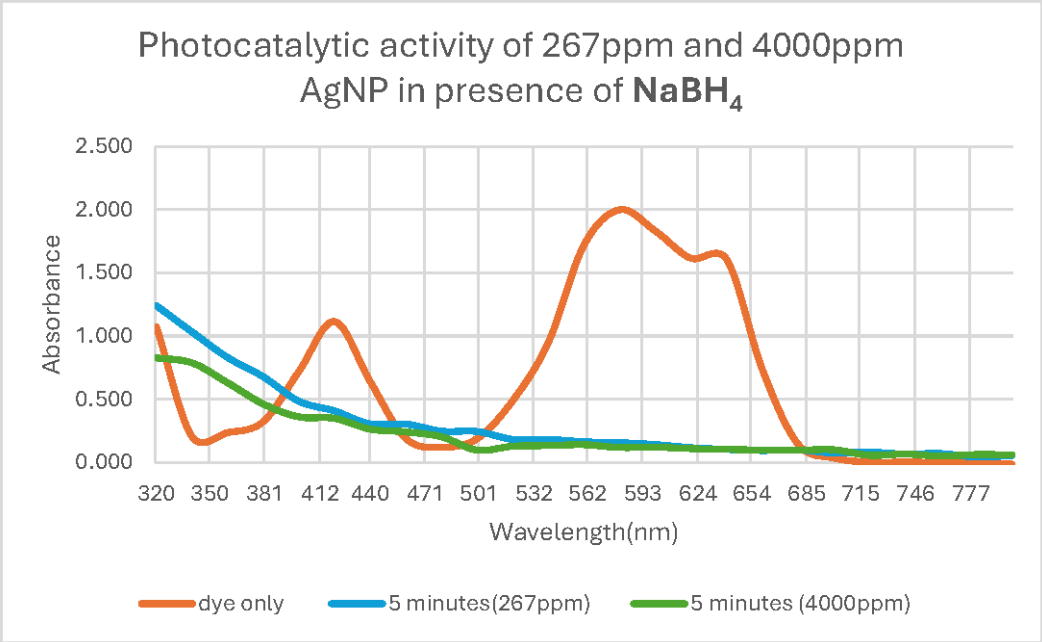

Figure 9. Photocatalytic activity of GerP AgNP with $NaBH_4$ 0–5 min at 4000 ppm and 267 ppm AgNP concentration

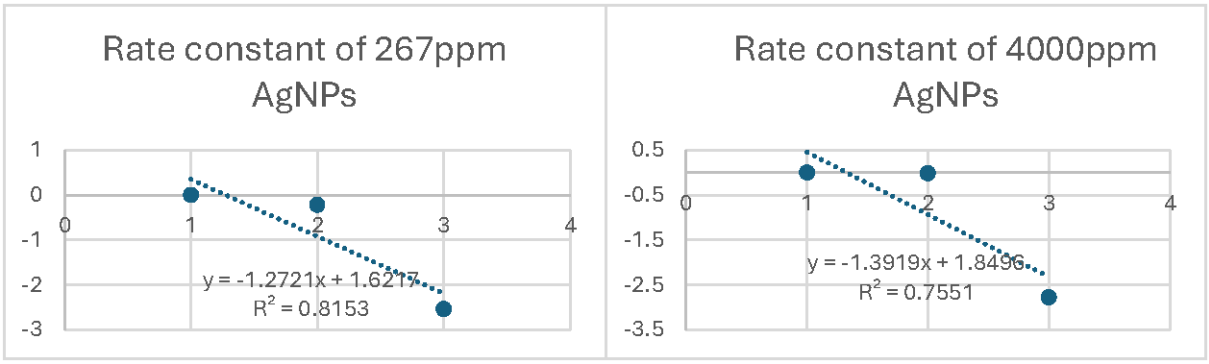

Figure 10. Rate constant graphs at 267 ppm and 4000 ppm

3.6 *Melamine adulteration in milk*

Melamine is a highly nitrogenous compound considered as protein in several protein measuring tests and is added to dairy products to boost the protein content[51]. Since it is added in very small quantities it is usually difficult to detect using the conventional methods which are costly and time-consuming. Thus, colorimetric detection of melamine can be approached as green synthesised AgNPs are relatively less costly and higher in accuracy due to its ability to aggregate melamine present in minute quantities. As melamine binds with AgNPs, it causes a decrease in the inter-particle distance, leading to a strong overlap of the Plasmon fields in the nearby nanoparticles. This in turn leads to the occurrence of colour change from pale yellow to red and the aggregation of melamine can be quantified using UV-Vis spectrophotometry which will show a distinct peak between 500–540 nm[35]. In the present study out of the various concentrations of melamine prepared, all concentrations of melamine showed a distinct colour change (Figure 11). Aggregation of melamine by AgNPs were quantified in the 2 mM sample which showed the presence of a peak at 520–540 nm confirming the aggregation of melamine and change in absorbance when added with the GerP AgNPs prepared.

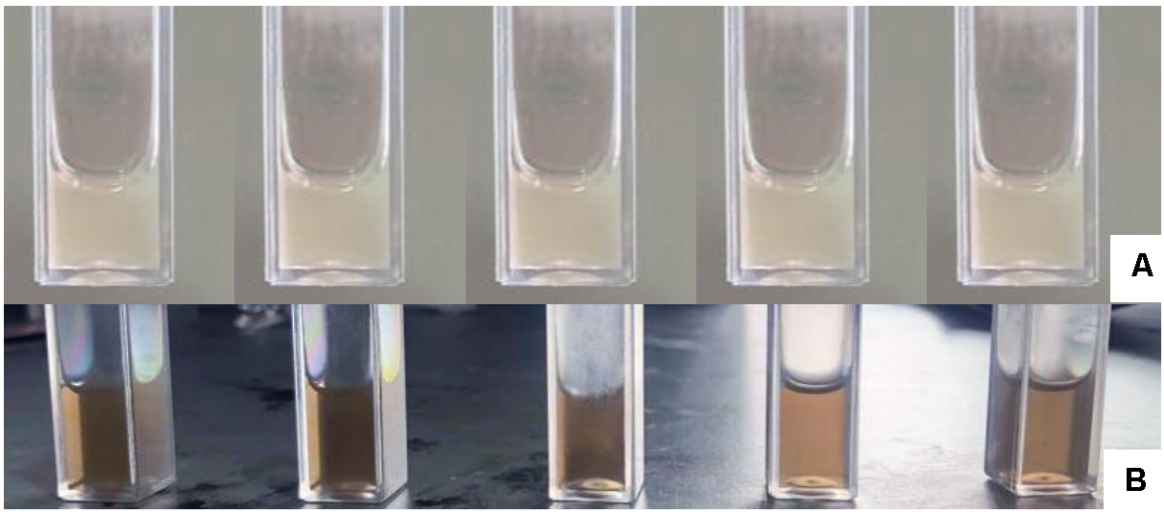

Figure 11. Aggregation of Melamine in concentration series (**A**) Before incubation (**B**) After incubation

2 mM melamine spiked milk and raw milk also confirmed the presence of melamine in the samples, when quantitatively analysed through UV-Vis spectroscopy as a clear absorbance peak was observed between 500–520 nm (Figure 12) which was also confirmed through a colorimetric change in the sample. Although colorimetric detection using AgNPs is relatively less costly and have high sensitivity, they may have several limitations when compared with techniques like HPLC or gas chromatography. One of the main limitations lie in the sample matrix and the pH affect. As several milk products may differ in pH values, the ability to detect melamine concentrations accurately may differ from sample to sample. However, enhancing the AgNP activity and developing light sensitive probes, due to the catalytic effects, higher extinction coefficient and magnetic effects, minute amounts of additives present in milk can be detected using AgNPs through cost-effective ways in future[52].

3.7 *Antibacterial activity of AgNPs*

The present study focused on the antibacterial activity on *S. aureus* and *E. coli* using both the aqueous plant extract and synthesised AgNPs (Figure 13). The zone of inhibition was measured and statistically analysed using one-way ANOVA. According to the results, the mean ZOI in all water extracts accounted for 2.71 cm ± 0.2 cm, while the AgNPs showed a mean ZOI of 2.74 cm ± 0.2 cm, suggesting that the activity of AgNPs were relatively higher than the water extracts. The one-way ANOVA, however, did not show a significant difference between the zone of inhibition of the aqueous extracts and AgNPs. As the surface area-to-volume ratio of silver nanoparticles heavily impacts its antibacterial activity, the results suggest that the antibacterial activity exerted by the antioxidants and compounds of the synthesised AgNPs might not be as strong as the antioxidant activity and the photocatalytic activity shown by these nanoparticles.

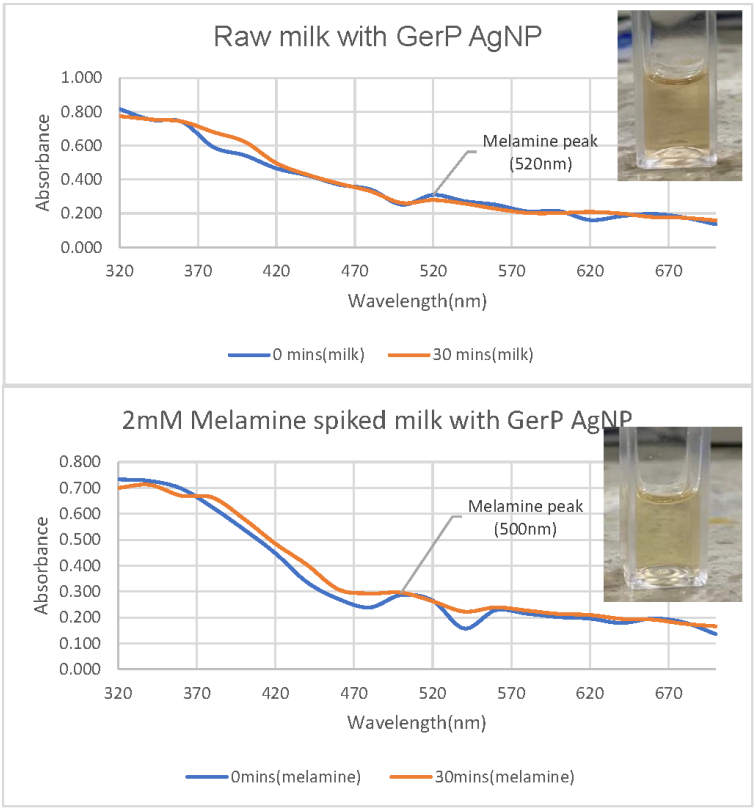

Figure 12. UV-Vis spectroscopy analysis of melamine detection in milk

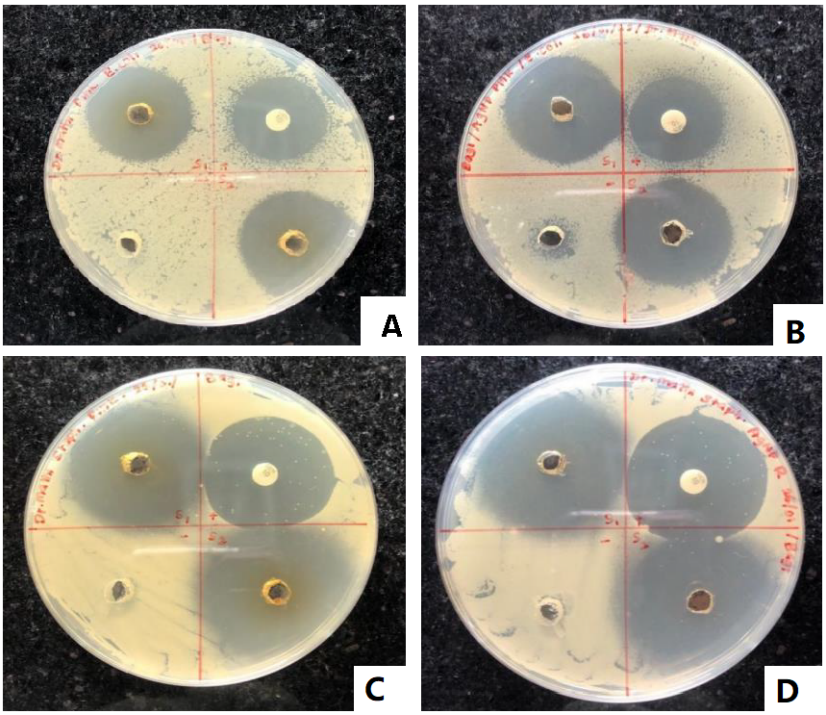

Figure 13. ZOI in *E. coli* (**A**) GerP aqueous extract (**B**) GerP AgNP ZOI in *S. aureus* (**C**) GerP Aqueous extract (**D**) GerP AgNP

4. Conclusions

In conclusion, 5 samples of *Gerbera jamesonii* were used to synthesise AgNPs, out of which the samples optimised at 90 °C for 30 min, was taken as the optimised samples, to carry out the photocatalytic activity, and melamine detection of synthesised GerP AgNP. The SEM analysis shows, AgNPs synthesised with GerP, had a spherical shape and was 40 nm in diameter. The antioxidant assays carried out proved the highest antioxidant capacity was observed in the synthesised AgNPs, with GerP AgNP having similar phenolic and antioxidant capacities to other AgNPs. The photocatalytic activity of the AgNPs were proved by the degradation of Malachite green dye within 2 min of adding $NaBH_4$ as the catalyst. Low concentrations of melamine (2 mM) were detected in both the raw milk and the melamine spiked milk which was confirmed through the absorbance peaks observed. Furthermore, the antibacterial activity was highest in AgNPs synthesised with Pink (GerP), against *S. aureus* and Red (GerR) against *E. coli* which proves that these AgNPs can be used in treating antibiotic resistance diseases. These non-toxic eco-friendly AgNPs can be used as a solution to address most ecological and health issues faced today including organic dye degradation and melamine detection. Furthermore, future studies can be carried out using these nanoparticles in improving the food packaging quality and antibacterial purposes. Future studies can particularly focus on incorporating these nanoparticles into food and textile industries to improve quality standards following a toxicity analysis. As studies have shown potential toxicity of AgNPs in biological systems, prolonged exposure of food items to AgNPs may have a toxic effect on living cells, which needs further analysis. However, incorporation of these eco-friendly AgNPs may lead to a significant change in quality of life, and living conditions

Acknowledgements

The authors would like to acknowledge the Business Management School (BMS) for funding and Sri Lanka Institute of Nanotechnology (SLINTEC) for facilitating the analysis of nanoparticles using the Hitachi SU6600 SEM.

Conflict of interest

The authors declare no competing financial interest

References

[1] Mahmoud, N.; Mohaddeseh, S.; Mohammad, S. S.; Zahra, I. Green Nanotechnology. *Interface Sci. Technol.* **2019**, *28*, 145-198. https://doi.org/10.1016/B978-0-12-813586-0.00005-5.

[2] Rodrigues, S. M.; Demokritou, P.; Dokoozlian, N.; Hendren, C. O.; Karn, B.; Mauter, M. S.; Sadik, O. A.; Safarpour, M.; Unrine, J. M.; Viers, J.; et al. Nanotechnology for Sustainable Food Production: Promising Opportunities and Scientific Challenges. *Environ. Sci. Nano* **2017**, *4*, 767–781. https://doi.org/10.1039/C6EN00573J.

[3] Song, J. Y.; Kim, B. S. Rapid Biological Synthesis of Silver Nanoparticles Using Plant Leaf Extracts. *Bioprocess Biosyst. Eng.* **2008**, *32*, 79–84. https://doi.org/10.1007/s00449-008-0224-6.

[4] Zhang, X. F.; Liu, Z. G.; Shen, W.; Sangiliyandi, G. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. *Int. J. Mol. Sci.* **2016**, *17*, 1534. https://doi.org/10.3390/ijms17091534.

[5] Anand, K. K.; Srivastava, R.; Singh, P.; Virendra, B. Y.; Nath, G. Antioxidant and Antibacterial Activity of Silver Nanoparticles Synthesized by Cestrum Nocturnum. *J. Ayurveda Integr. Med.* **2020**, *11*, 37–44. https://doi.org/10.1016/j.jaim.2017.11.003.

[6] Liao, C.; Li, Y.; Tjong, S. C. Bactericidal and Cytotoxic Properties of Silver Nanoparticles. *Int. J. Mol. Sci.* **2019**, *20*, 449. https://doi.org/10.3390/ijms20020449.

[7] Yin, I. X.; Zhang, J.; Zhao, I. S.; Mei, M. L.; Li, Q.; Chu, C. H. The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. *Int. J. Nanomed.* **2020**, *15*, 2555–2562. https://doi.org/10.2147/IJN.S246764.

[8] Amir Reza, S.; Ghasemi, E.; Azam, P.; Seyedeh, M. H.; Reza, T. G. Highly Sensitive Surface Plasmon Resonance Sensor for Detection of Methylene Blue and Methylene Orange Dyes Using NiCo-Layered Double Hydroxide. *Opt. Commun.* **2023**, *529*, 129057. https://doi.org/10.1016/j.optcom.2022.129057.

[9] Jha, A. K.; Prasad, K.; Kulkarni, A. R. Synthesis of TiO_2 Nanoparticles Using Microorganisms. *Colloids Surf. B Biointerfaces* **2009**, *71*, 226-229. https://doi.org/10.1016/j.colsurfb.2009.02.007.

[10] Singh, M.; Goyal, M.; Devlal, K. Size and Shape Effects on the Band Gap of Semiconductor Compound Nanomaterials. *J. Taibah Univ. Sci.* **2018**, *12*, 470–475. https://doi.org/10.1080/16583655.2018.1473946.

[11] Cioć, M.; Dziurka, M.; Pawłowska, B. Changes in Endogenous Phytohormones of Gerbera Jamesonii Axillary Shoots Multiplied under Different Light Emitting Diodes Light Quality. *Molecules* **2022**, *27*, 1804. https://doi.org/10.3390/molecules27061804.

[12] Negm El-Dein, S.; Hussein, A.; Abu-Bakr, M. S.; Negm El-Dein, A.; Awad, H. M.; Ragab, E. A. Phytochemical Analysis and Determination of Antioxidant, Anti-Cholesterol, Anti-Inflammatory and Anti-Proliferative Activities of Gerbera Jamesonii Flowers. *Adv. Tradit. Med.* **2022**, *23*, 863-875. https://doi.org/10.1007/s13596-022-00659-x.

[13] Udaya Prakash, N. K.; Sripriya, N.; Aishwarya, V.; Preethy, S.; Benetta, S.; Bhuvaneswari, S. Synthesis of AgNPs Using Flowers Exhibiting Different Photoresponse. *IOP Conf. Ser.: Mater. Sci. Eng.* **2019**, *574*, 012005. https://doi.org/10.1088/1757-899x/574/1/012005.

[14] Abdel-Aziz, M. S.; Shaheen, M. S.; El-Nekeety, A. A.; Abdel-Wahhab, M. A. Antioxidant and Antibacterial Activity of Silver Nanoparticles Biosynthesized Using Chenopodium Murale Leaf Extract. *J. Saudi Chem. Soc.* **2014**, *18*, 356–363. https://doi.org/10.1016/j.jscs.2013.09.011.

[15] Mikhailova, E. O. Silver Nanoparticles: Mechanism of Action and Probable Bio-Application. *J. Funct. Biomater.* **2020**, *11*, 84. https://doi.org/10.3390/jfb11040084.

[16] Latha, D.; Arulvasu, C.; Prabu, P.; Narayanan, V. Photocatalytic Activity of Biosynthesized Silver Nanoparticle from Leaf Extract of Justicia Adhatoda. *Mater. Sci. Eng. J.* **2017**, *9*, 10. https://hal.science/hal-01497956.

[17] Lellis, B.; Fávaro-Polonio, C. Z.; Pamphile, J. A.; Polonio, J. C. Effects of Textile Dyes on Health and the Environment and Bioremediation Potential of Living Organisms. *Biotechnol. Res. Innov.* **2019**, *3*, 275–290. https://doi.org/10.1016/j.biori.2019.09.001.

[18] Marques, N. R. S.; Lima, M. T. A.; Pereira, G. A. L.; Pereira, G. Catalytic Degradation of Azo Dyes by Silver Nanoparticles. *Eng. Proc.* **2023**, *31*, 54. https://doi.org/10.3390/ASEC2022-13952.

[19] Rani, P.; Kumar, V.; Singh, P. P.; Matharu, A. S.; Zhang, W.; Kim, K.-H.; Singh, J.; Rawat, M. Highly Stable AgNPs Prepared via a Novel Green Approach for Catalytic and Photocatalytic Removal of Biological and Non-Biological Pollutants. *Environ. Int.* **2020**, *143*, 105924. https://doi.org/10.1016/j.envint.2020.105924.

[20] Sarina, S.; Waclawik, E. R.; Zhu, H. Photocatalysis on Supported Gold and Silver Nanoparticles under Ultraviolet and Visible Light Irradiation. *Green Chem.* **2013**, *15*, 1814. https://doi.org/10.1039/C3GC40450A.

[21] Xiaofang, P. The China Melamine Milk Scandal and Its Implications for Food Safety Regulation. *Food Policy* **2011**, *36*, 412-420. https://doi.org/10.1016/j.foodpol.2011.03.008.

[22] Kumar, N.; Kumar, H.; Mann, B.; Seth, R. Colorimetric Determination of Melamine in Milk Using Unmodified Silver Nanoparticles. *Spectrochim. Acta, Part A* **2016**, *156*, 89–97. https://doi.org/10.1016/j.saa.2015.11.028.

[23] Ramalingam, K.; Devasena, T.; Senthil, B.; Kalpana, R.; Jayavel, R. Silver Nanoparticles for Melamine Detection in Milk Based on Transmitted Light Intensity. *IET Sci. Meas. Technol.* **2017**, *11*, 171–178. https://doi.org/10.1049/iet-smt.2016.0215.

[24] Huq, M. A.; Ashrafudoulla, M.; Rahman, M. M.; Balusamy, S. R.; Akter, S. Green Synthesis and Potential Antibacterial Applications of Bioactive Silver Nanoparticles: A Review. *Polymers* **2022**, *14*, 742. https://doi.org/10.3390/polym14040742.

[25] Bruna, T.; Maldonado-Bravo, F.; Jara, P.; Caro, N. Silver Nanoparticles and Their Antibacterial Applications. *Int. J. Mol. Sci.* **2021**, *22*, 7202. https://doi.org/10.3390/ijms22137202.

[26] Simbine, E. O.; Rodrigues, L. D. C.; Lapa-Guimaraes, J.; Kamimura, E. S.; Corassin, C. H.; Oliveira, C. A. F. D. Application of Silver Nanoparticles in Food Packages: A Review. *Food Sci. Technol.* **2019**, *39*, 793–802. https://doi.org/10.1590/fst.36318.

[27] Sadrolhosseini, A. R.; Ghasemi, E.; Pirkarimi, A.; Hamidi, S. M.; Ghahrizjani, R. T. Highly Sensitive Surface Plasmon Resonance Sensor for Detection of Methylene Blue and Methylene Orange Dyes Using NiCo-Layered Double Hydroxide. *Opt. Commun.* **2023**, *529*, 129057. https://doi.org/10.1016/j.optcom.2022.129057.

[28] Drago, E.; Campardelli, R.; Pettinato, M.; Perego, P. Innovations in Smart Packaging Concepts for Food: An Extensive Review. *Foods* **2020**, *9*, 1628. https://doi.org/10.3390/foods9111628.
[29] Arokiyaraj, S.; Dinesh Kumar, V.; Elakya, V.; Kamala, T.; Park, S. K.; Ragam, M.; Saravanan, M.; Bououdina, M.; Arasu, M. V.; Kovendan, K.; et al. Biosynthesized Silver Nanoparticles Using Floral Extract of *Chrysanthemum Indicum* L.—Potential for Malaria Vector Control. *Environ. Sci. Pollut. Res.* **2015**, *22*, 9759–9765. https://doi.org/10.1007/s11356-015-4148-9.
[30] Kandiah, M.; Chandrasekaran, K. N. Green Synthesis of Silver Nanoparticles Using Catharanthus Roseus Flower Extracts and the Determination of Their Antioxidant, Antimicrobial, and Photocatalytic Activity. *J. Nanotechnol.* **2021**, *2021*, 5512786. https://doi.org/10.1155/2021/5512786.
[31] Siddharth, W.; Santosh, W. S. Preliminary Phytochemical Analysis of Some Asteraceae Members Found in Local Area of Barshitakli. *Vidyabharati Int. Interdiscip. Res. J.* **2020**, 380-383.
[32] Sontakke, K. S.; Shinde, S. L. Evaluation of the Phytochemical Potential of Brassica Juncea Seeds. *Vidyabharati Int. Interdiscip. Res. J.* **2020**, *2*, 25-29.
[33] Song, J. Y.; Kim, B. S. Rapid Biological Synthesis of Silver Nanoparticles Using Plant Leaf Extracts. *Bioprocess Biosyst. Eng.* **2008**, *32*, 79–84. https://doi.org/10.1007/s00449-008-0224-6.
[34] Mohd Yusof, H.; Abdul Rahman, N.; Mohamad, R.; Zaidan, U. H. Microbial Mediated Synthesis of Silver Nanoparticles by Lactobacillus Plantarum TA4 and Its Antibacterial and Antioxidant Activity. *Appl. Sci.* **2020**, *10*, 6973. https://doi.org/10.3390/app10196973.
[35] Ping, H.; Zhang, M.; Li, H.; Li, S.; Chen, Q.; Sun, C.; Zhang, T. Visual Detection of Melamine in Raw Milk by Label-Free Silver Nanoparticles. *Food Control* **2012**, *23*, 191–197. https://doi.org/10.1016/j.foodcont.2011.07.009.
[36] Ayub, M. A.; Hussain, A. I.; Hanif, M. A.; Chatha, S. A. S.; Kamal, G. M.; Shahid, M.; Janneh, O. Variation in Phenolic Profile, β-Carotene and Flavonoid Contents, Biological Activities of Two Tagetes Species from Pakistani Flora. *Chemistry Biodivers.* **2017**, *14*, e1600463. https://doi.org/10.1002/cbdv.201600463.
[37] Ingkasupart, P.; Manochai, B.; Song, W. T.; Hong, J. H. Antioxidant Activities and Lutein Content of 11 Marigold Cultivars (*Tagetes* spp.) Grown in Thailand. *Food Sci. Technol.* **2015**, *35*, 380–385. https://doi.org/10.1590/1678-457X.6663.
[38] Anvari, D.; Jamei, R. Evaluation of Antioxidant Capacity and Phenolic Content in Ethanolic Extracts of Leaves and Flowers of Some Asteraceae Species. *Recent Pat. Food, Nutr. Agric.* **2022**, *9*, 42–49.
[39] Bordoloi, M.; Sahoo, R. K.; Tamuli, K. J.; Saikia, S.; Dutta, P. P. Plant Extracts Promoted Preparation of Silver and Gold Nanoparticles: A Systematic Review. *Nano* **2020**, *15*, 2030001. https://doi.org/10.1142/s1793292020300017.
[40] Lajoie, L.; Fabiano-Tixier, A.-S.; Chemat, F. Water as Green Solvent: Methods of Solubilisation and Extraction of Natural Products—Past, Present and Future Solutions. *Pharmaceuticals* **2022**, *15*, 1507. https://doi.org/10.3390/ph15121507.
[41] Velgosova, O.; Dolinská, S.; Podolská, H.; Mačák, L.; Čižmárová, E. Impact of Plant Extract Phytochemicals on the Synthesis of Silver Nanoparticles. *Materials* **2024**, *17*, 2252. https://doi.org/10.3390/ma17102252.
[42] Krishnaraj, C.; Jagan, E. G.; Rajasekar, S.; Selvakumar, P.; Kalaichelvan, P. T.; Mohan, N. Synthesis of Silver Nanoparticles Using Acalypha Indica Leaf Extracts and Its Antibacterial Activity against Water Borne Pathogens. *Colloids Surf., B* **2010**, *76*, 50–56. https://doi.org/10.1016/j.colsurfb.2009.10.008.
[43] Elisa, A.; Pereira, A. C.; Asevedo, M.; Ferreira, L. F. Synthesis of a Silver Nanoparticle Ink for Fabrication of Reference Electrodes. *Talanta Open* **2022**, *5*, 100085. https://doi.org/10.1016/j.talo.2022.100085.
[44] Mierziak, J.; Kostyn, K.; Kulma, A. Flavonoids as Important Molecules of Plant Interactions with the Environment. *Molecules* **2014**, *19*, 16240–16265. https://doi.org/10.3390/molecules191016240.
[45] Balciunaitiene, A.; Viskelis, P.; Viskelis, J.; Streimikyte, P.; Liaudanskas, M.; Bartkiene, E.; Zavistanaviciute, P.; Zokaityte, E.; Starkute, V.; Ruzauskas, M.; et al. Green Synthesis of Silver Nanoparticles Using Extract of *Artemisia Absinthium* L., *Humulus Lupulus* L. and *Thymus Vulgaris* L., Physico-Chemical Characterization, Antimicrobial and Antioxidant Activity. *Processes* **2021**, *9*, 1304. https://doi.org/10.3390/pr9081304.
[46] Dai, J.; Mumper, R. J. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules* **2010**, *15*, 7313–7352. https://doi.org/10.3390/molecules15107313.
[47] Marimuthu, S.; Antonisamy, A. J.; Malayandi, S.; Rajendran, K.; Tsai, P.-C.; Pugazhendhi, A.; Ponnusamy, V. Silver Nanoparticles in Dye Effluent Treatment: A Review on Synthesis, Treatment Methods, Mechanisms, Photocatalytic Degradation, Toxic Effects and Mitigation of Toxicity. *J. Photochem. Photobiol., B* **2020**, *205*, 111823. https://doi.org/10.1016/j.jphotobiol.2020.111823.

[48] Sumi, M. B.; Devadiga, A.; Shetty, K. V.; MB, S. Solar Photocatalytically Active, Engineered Silver Nanoparticle Synthesis Using Aqueous Extract of Mesocarp of Cocos Nucifera (Red Spicata Dwarf). *J. Exp. Nanosci.* **2016**, *12*, 14–32. https://doi.org/10.1080/17458080.2016.1251622.

[49] Shaikh, W. A.; Chakraborty, S.; Owens, G.; Islam, R. U. A Review of the Phytochemical Mediated Synthesis of AgNP (Silver Nanoparticle): The Wonder Particle of the Past Decade. *Appl. Nanosci.* **2021**, *11*, 2625–2660. https://doi.org/10.1007/s13204-021-02135-5.

[50] Xu, Z.; Zada, N.; Habib, F.; Ullah, H.; Hussain, K.; Ullah, N.; Bibi, M.; Ghani, H.; Khan, S.; Cai, X. Enhanced Photocatalytic Degradation of Malachite Green Dye Using Silver—Manganese Oxide Nanoparticles. *Molecules* **2023**, *28*, 6241. https://doi.org/10.3390/molecules28176241.

[51] Rajpoot, M.; Bhattacharya, R.; Sharma, S.; Gupta, S.; Sharma, V.; Sharma, A. K. Melamine Contamination and Associated Health Risks: Gut Microbiota Does Make a Difference. *Biotechnol. Appl. Biochem.* **2020**, *68*, 1271-1280. https://doi.org/10.1002/bab.2050.

[52] Kanchi, S. One-Pot Biosynthesis of Silver Nanoparticle Using Colocasia Esculenta Extract: Colorimetric Detection of Melamine in Biological Samples. *J. Photochem. Photobiol. A* **2020**, *391*, 112310. https://doi.org/10.1016/j.jphotochem.2019.112310.