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# Microwave-assisted ecofriendly silver nanoparticle synthesis by varieties of *Chrysanthemum morifolium* Ramat: Assessing their antioxidant, photocatalytic and antibacterial activities

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**Abstract**

Based on recent developments in the field of Nanobiotechnology, this study is aimed at the assessment of antioxidant, photocatalytic and antibacterial activities of silver nanoparticles (AgNPs) synthesized using *Chrysanthemum morifolium* Ramat varieties (brown, yellow, purple, pink, salmon pink and white), where five varieties produced AgNPs except white. Antioxidant assays such as total flavonoid content, total phenolic content and total antioxidant capacity and free radical scavenging assays such as ferric reducing antioxidant power and DPPH radical scavenging assays were performed. Photocatalytic activity was assessed by the degradation of the model dye methylene blue. Antibacterial activity was determined on *Staphylococcus aureus* and *Escherichia coli*. Performed assays indicated higher antioxidant contents and activities in AgNPs compared to water extracts. The lowest minimum inhibitory concentration was obtained for AgNPs of brown variety indicating a higher antioxidant activity. Photocatalytic activity assessment on brown AgNPs of 3175 ppm and 212 ppm showed no difference in their rate constants indicating a similar photocatalytic activity for both concentrations. Antibacterial activity was higher in water extracts compared to AgNPs for both types of bacteria used. Biosynthesized AgNPs show a wide array of properties which could be beneficial in the treatment of free radical-related diseases, environmental pollution, and antibiotic resistance.

## 1. Introduction

Nanotechnology is an emerging field of science where matter is manipulated and controlled on a nanoscale using scientific knowledge and technology in order to be utilized for various biomedical and industrial applications. The nanoscale is considered to be having one or more dimensions in the 1 nm to 100 nm range. Unknowingly, people from the past had been using nanomaterials for various purposes; as dyes imparting colours to fabrics, and nanoscale silver and gold particles that impart deep yellow and ruby red coloured stains on glass by artists [1,2].

The Nobelist Richard Feynman first put forward the concept of nanotechnology and since then, the field of nanotechnology has developed vastly resulting in the advancement of fields of health and biomedicine, biotechnology and agriculture, food and nutrition, and defense and security [3]. The high surface area to volume ratio and the quantum effect give rise to the remarkable optical, electrical and magnetic properties of nanomaterials making them ideal for above applications [4,5]. The synthesis of metallic nanoparticles (NPs) in nanobiotechnology utilizes either the bottom up or the top down approaches [6]. Synthesis of NPs such as silver (Ag), gold, zinc, platinum, and aluminum utilizing conventional physical or chemical methods carry the possibility of giving rise to complications such as eco-unfriendliness, expensiveness, toxicity and high consumption of energy, whereas microbial-mediated biological synthesis holds limitations such as being bio-hazardous due to contamination by microbial cells, poor control and low production. Therefore, plant-mediated biosynthesis of eco-friendly, non-toxic and biocompatible silver NPs (AgNPs) has now gained the attention of the scientific community [7-9].

This technique employs secondary metabolites of various natural plant extracts such as phenolic compounds, flavonoids, alkaloids and terpenoids as the reducing agents of the metal ion to its metal and stabilizing/capping agents of the growing metal NP agglomerates [10]. The presence of hydroxyl groups in terpenoids enables the dissociation of protons, giving resonance structures capable of further oxidation. In addition to hydroxyl groups, flavonoids and phenols show tautomeric transformations into keto and enol forms releasing protons to reduce metal ions. These metabolites are also known to show chelating effects enabling the capture of metal ions for the process of reduction followed by stabilization [11]. Low-heat and high-heat synthesis are the major methods of plant-mediated synthesis where suitability of the technique is dependent on the type of plant material utilized in the study. According to scientific literature, microwave-assisted synthesis consumes less energy and reaction time, arrests agglomeration and yields NPs with narrow size distributions compared to conventional methods [12].

In this study, for the synthesis of AgNPs, varieties of *Chrysanthemum morifolium* Ramat flowers were used. *Chrysanthemum*, a member of the Asteraceae family is native to Asia and northeastern Europe, whereas the diversity is observed in China. Having an extensive

diversity of thousands of varieties and being a popular garden hardy or exhibition plant, it is readily available and easily accessible all over the world. It has been used as a medicine and a health food in China over many years due to its antioxidant, anti-inflammation, anti-tumorigenesis, cardiovascular protection, anti-aging, and antibacterial properties. It is evident that these properties are a result of the presence of a significant amount of secondary metabolites [13-15]. The high antioxidant capacities shown by water extracts of *Chrysanthemum* in previous studies [16] is the basis for the selection of this flower for AgNP synthesis and the assessment of its antioxidant and anti-bacterial properties in this study. As *Chrysanthemums* are mainly used as an ornamental and a garden flower in Sri Lanka, utilizing the dried flowers after usage prevents wastage of natural resources.

An antioxidant is a molecule that is stable enough to donate an electron to a free radical to prevent oxidation, by neutralization. Free radicals at higher concentrations, which tend to take up electrons from cellular components giving rise to chain reactions within the human body are terminated by antioxidants, delaying/inhibiting cellular damage and oxidative stress, which otherwise leads to degenerative ailments, namely, arthritis, autoimmune disorders, aging and neurological disorders [17,18]. Studies on mice models have shown that high doses of synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxy-toluene have led to tumor promoting activity, cytotoxicity and hemolysis [19]. Therefore, the necessity for high potential natural and harmless antioxidant sources is high. Apart from the antioxidant properties, AgNPs are widely known for their photocatalytic activity due to its high absorption in the region of visible light, and its prevention of the recombination of hole pairs and electrons during the photocatalytic process [20,21]. This property could be exploited in order to prevent environmental pollution due to the release of toxic, carcinogenic and mutagenic azo compounds to water bodies via industrial effluents which ultimately get accumulated in food chains effecting the life on earth. Scientists are now focusing on the degradation of highly stable azo bonds of azo compounds by the use of AgNPs, which will be assessed in this study using the model dye, methylene blue (MB). Furthermore, as a potential solution for the growing threat of antibiotic resistance worldwide, antibacterial activity of AgNPs and *Chrysanthemum* extracts will be assessed in this study [22,23].

This study has been aimed at the assessment of the antioxidant, photocatalytic and antibacterial activities of AgNPs synthesized using six varieties of *Chrysanthemum morifolium*. Total antioxidant capacity (TAC), total phenolic content (TPC), total flavonoid content (TFC) and other free radical scavenging assays such as Ferric reducing antioxidant power (FRAP) and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) will be performed on AgNPs as well as water extracts of *Chrysanthemum* for comparison. Photocatalytic activity will be assessed with the aid of methylene blue, and the analysis of antimicrobial activity will be performed by the well diffusion method using *Staphylococcus aureus* and *Escherichia coli*. It is expected that this study would pave way to cure free radical-related diseases, and for the betterment of the environment and the life on earth.

## 2. Experimental

### 2.1 Materials

#### 2.1.1 Sample collection

Six varieties of *Chrysanthemum morifolium*, brown, yellow, purple, pink, salmon pink (S. pink) and white were collected from Malsha Plant Nursery, Kadawatha, Sri Lanka. The flowers of these plants (Figure 1) were utilized for this study.

#### 2.1.2 Chemicals and Reagents

Acetic acid ($CH_3COOH$) (CAS no. 7758-99-8), Aluminium chloride ($AlCl_3$) (CAS no. 7446-70-0), Ammonium hydroxide ($NH_4OH$), Ammonium molybdate ($[NH_4]_6Mo_7O_{24}.4H_2O$), Chloroform ($CH_3Cl$) (CAS no. 67-66-3), Copper sulphate ($CuSO_4$) (CAS no. 7758-98-7), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (CAS no. 1898-66-4), Ethanol ($C_2H_5OH$) (CAS no. 64-17-5), Ferric chloride ($FeCl_3$) (CAS no. 7705-08-0), Folin-Ciocalteu phenol reagent, Methanol ($CH_3OH$) (CAS no. 67-56-1), Hydrochloric acid (HCl) (CAS no. 7647-01-01-0), McFarland solution, Methylene Blue (MB) ($C_{16}H_{18}ClN_3S$), Molisch's reagent, Mueller-Hinton agar powder, Nutrient agar powder, Saline, Silver nitrate ($AgNO_3$) (CAS no. 7761-88-8), Sodium acetate ($CH_3OONa$) (CAS no. 127-09-3), Sodium borohydride ($NaBH_4$), Sodium carbonate ($Na_2CO_3$) (CAS no. 497-19-8), Sodium hydroxide (NaOH) (CAS no. 1310-73-2), Sodium nitrate ($NaNO_3$) (CAS no. 7631-99-4), Sodium sulphate ($Na_2SO_4$) (CAS no. 7757-82-6), Sulphuric acid ($H_2SO_4$) (CAS no. 7664-93-9) and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (CAS no. 7757-82-6)

### 2.2 Methodology

The experiments were performed following safety regulations and good lab practice, along with the required protective gear.


**Figure 1.** Six *Chrysanthemum morifolium* varieties used in this study (a) brown variety, (b) yellow variety, (c) purple variety, (d) pink variety, (e) S. pink variety, and (f) white variety.

#### 2.2.1 Preparation of Chrysanthemum morifolium samples

Shade dried, finely cut samples of 2 g were measured and placed in separate beakers. 30 mL of distilled water was added, covered with foil, and heated for 10 min at 90°C. The extracts were thoroughly filtered first using muslin cloth and then with filter paper and transferred to 50 mL falcon tubes. The samples were stored at 4°C until further use.

1 mL of each extract was mixed with 14 mL distilled water in order to prepare the diluted water extract samples and stored in 15 mL falcon tubes at 4°C until further use.

#### 2.2.2 Synthesis and optimization of silver nanoparticles (AgNPs)

Mixtures of 1 mL of each extract and 20 mL of 1 mM silver nitrate solution were mixed and microwaved at 511 W for 5.5 min in a turntable microwave. Absorbance was measured from 300 nm to 500 nm using water as the blank [24]. Optimization was performed in microwave at 511 W for 2.5 min, 4 min and 5.5 min. Heat method was also performed at 90°C for 60 min, 45 min, 30 min, and 15 min using 1 mL of sample and 9 mL of 1 mM $AgNO_3$. 1 mL of each synthesized AgNP sample was mixed with 14 mL distilled water in order to prepare the diluted AgNP samples and stored in 15 mL falcon tubes at 4°C until further use.

#### 2.2.3 Phytochemical screening

Phytochemical screening, in order to identify the composition of each *Chrysanthemum morifolium* sample was performed on 0.5 mL of each flower extract as indicated in Table 1 [25].

#### 2.2.4 Determination of total flavonoid content (TFC)

Triplicates of 1.5 mL of samples and 1.5 mL of 2% $AlCl_3$ were mixed and incubated for 10 minutes. The absorbance was measured at 415 nm. The concentration was expressed in equivalents of Quercetin (QE) in µgQE/100 g [24].

#### 2.2.5 Determination of total phenolic content (TPC)

A mixture of 50 µL of sample, 0.5 mL of 10% Folin-Ciocalteu reagent and 1 mL of distilled water were mixed, and it was left for 3 min at RT and 1.5 mL of 20% sodium carbonate was added and incubated for 1 h in dark. Absorbance was measured in triplicates at 765 nm and concentration was expressed in equivalents of gallic acid (GAE) in mgGAE/100 g [24].

#### 2.2.6 Determination of total antioxidant content (TAC)

Mixtures of 3 mL of sample, 1 mL of solution containing 0.6 M sulphuric acid, 28 mM sodium sulphate and 4 mM ammonium molybdate (1:1:1 ratio) were incubated at 90°C for 90 min. Absorbance was measured at 695 nm in triplicates and the concentration was expressed in equivalents of ascorbic acid (AAE) in mgAAE/100 g [24].

#### 2.2.7 Ferric reducing antioxidant power (FRAP) assay

A mixture of 2.9 mL of FRAP reagent (10:1:1 ratio of acetate buffer at pH = 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$) and 100 µL of each sample were added and the absorbance was measured at 593 nm at 1 min intervals until a drop in the values was observed [26].

#### 2.2.8 DPPH assay

0.004% DPPH in methanol was prepared and initial absorbance measured at 517 nm. 2 mL of DPPH was mixed with 1 mL of each sample and absorbance was recorded after an incubation of 30 min in dark [24].

#### 2.2.9 Median Inhibitory Concentration ($IC_{50}$)

1 mL samples of concentrations 100%, 80%, 60%, 40% and 20% were prepared and 2 mL of DPPH in methanol was mixed and absorbance of each concentration of each sample was measured at 517 nm [24].

#### 2.2.10 Photocatalytic activity of concentrated and diluted AgNPs

1 mL of 2 mM methylene blue and 1 mL of 0.2 M sodium boro-hydride was mixed and topped up to 200 mL with distilled water. 1 mL of 3175 ppm AgNPs of the chosen Brown sample was added and the absorbance was measured from 300 nm to 780 nm for 90 min. The procedure was repeated for Brown 212 ppm AgNPs for 200 min [27].

#### 2.2.11 Antimicrobial activity of samples

*Escherichia coli* and *Staphylococcus aureus* were streaked on to nutrient agar petriplates and incubated overnight at 37°C. A colony from this was diluted with saline and streaked onto 15 mL Mueller Hinton agar plates. 3 wells were made for the negative control (-) of saline, duplicates of each sample (S1 and S2), and Gentamycin was used as the positive control (+). Plates were incubated overnight at 37°C. Zone of inhibition (ZOI) was measured using a ruler.

#### 2.2.12 Statistical analysis

One–way ANOVA was performed using Microsoft Excel 2016 package and $p < 0.05$ was considered to have a significant statistical difference. Statistical correlation was determined using IBM SPSS Statistics 21 software.

**Table 1.** Phytochemical screening procedure [25].

| Phytochemical Test | Procedure |
|---|---|
| Test for carbohydrates | To 0.5 mL of extract, 0.25 mL Molisch's reagent and few drops of c.$H_2SO_4$ were added. |
| Test for tannins | To 0.5 mL extract,1 mL 5% $FeCl_3$ was added. |
| Test for Saponins | To 0.5 mL extract, 0.5 mL distilled water was added and was shaken for 15 mins. |
| Test for terpenoids | To 0.5 mL extract, 2 mL chloroform and 2 mL c.$H_2SO_4$ were added. |
| Test for anthraquinone | To 0.5 mL extract, 2 mL 10% $NH_4OH$ was added. |
| Test for steroids | To 0.5 mL extract, 0.5 mL chloroform and 1 drop of c.$H_2SO_4$ were added. |
| Test for proteins | To 0.5 mL extract, 2 drops of $CuSO_4$ and 1 drop of c.$H_2SO_4$ were added. |

#### 2.2.13 Scanning electron microscopy

SEM analysis was performed using a Hitachi SU6600 SEM at Sri Lanka Institute of Nanotechnology (SLINTEC), Homagama.

## 3. Results and discussion

### 3.1 Results

#### 3.1.1 Phytochemical screening

Overall, the phytochemical screening tests performed on the samples showed carbohydrates, tannins, saponins, terpenoids and steroids except anthraquinones and proteins [Table 2].

#### 3.1.2 Synthesis of Silver Nanoparticles

Microwave assisted synthesis of AgNPs indicated a colour change from colourless to brown [Figure 2(a)], and the UV/Vis spectrophotometric analysis indicated clear maximum absorption peaks corresponding to the surface plasmon behavior of AgNPs, for 5 sample out of 6 samples from 420 nm to 480 nm [Figure 2(b)]. Optimization of microwave assisted synthesis showed satisfactory results for all time durations except for the white sample [Table 3]. Further characterization was performed on the brown AgNPs using SEM analysis [Figure 3].

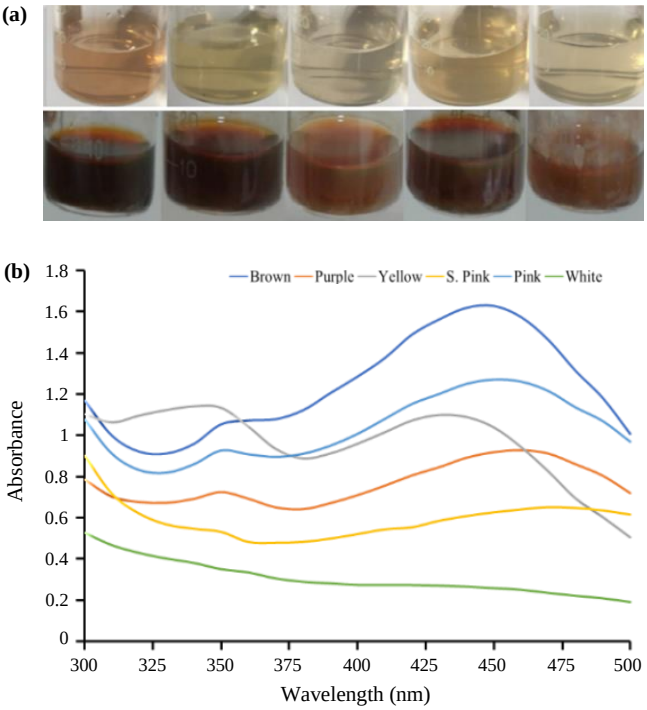


**Figure 2.** Microwave assisted synthesis and characterization of AgNPs. (a) Row 1: Before synthesis, Row 2: After synthesis. (b) UV/Vis characterization of synthesized AgNPs.

**Table 2**. Phytochemical screening tests for *Chrysanthemum* water extracts.

| Phytochemical Test | Brown | Yellow | Purple | Pink | S. Pink | White |
|---|---|---|---|---|---|---|
| Carbohydrates | + | + | + | + | + | + |
| Tannins | + | + | + | + | + | - |
| Saponins | - | - | - | + | + | + |
| Terpenoids | + | + | + | + | + | + |
| Anthraquinone | - | - | - | - | - | - |
| Steroids | + | + | + | + | - | - |
| Proteins | - | - | - | - | - | - |

**Table 3.** Optimization of the microwave-assisted synthesis of AgNPs.

| Sample | Time (min) | | |
|---|---|---|---|
| | 2.5 (511 W) | 4.0 (511 W) | 5.5 (511 W) |
| Brown | √ | √ | √ |
| Yellow | √ | √ | √ |
| Purple | √ | √ | √ |
| Pink | √ | √ | √ |
| S. Pink | √ | √ | √ |
| White | x | x | x |

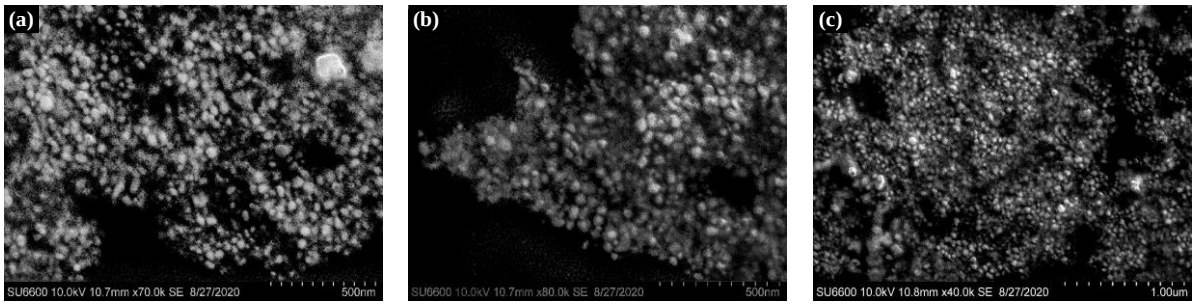


**Figure 3.** SEM images of Brown AgNPs at different magnifications. (a) 70 kX, (b) 80 kX, and (c) 40 kX.

### 3.1.3 Antioxidant and free radical scavenging assays

Antioxidant assays TFC, TPC, TAC, FRAP, and DPPH radical scavenging assays were performed on water extracts and AgNPs. The antioxidant contents [Table 4] and activities [Figure 4 and Figure 5] for AgNPs of all samples were higher than that of their respective water extracts. This was further verified by performing statistical analysis using ONE – way ANOVA on the results obtained. $IC_{50}$ determination performed on the results of DPPH assay indicated lower $IC_{50}$ values for AgNPs [Table 5].

### 3.1.4 Photocatalytic degradation of methylene blue

The reaction showed a gradual and continuous decrease in the colour of the MB dye from blue to colourless. Photocatalytic degradation of MB by 212 ppm and 3175 ppm brown AgNPs showed a gradual decrease of the maximum absorption peak of MB at 660 nm, from $0^{th}$ min to $200^{th}$ min and $0^{th}$ min to $90^{th}$ min respectively (Figure 6(a) and 6(b)).

### 3.1.5 Antibacterial activity

The ZOI obtained for all the AgNPs were lower than that of water extracts for both *S. aureus* and *E. coli* [Figure 7 and Table 6], which was verified using ONE – way ANOVA [Table 7].

**Table 4.** Antioxidant contents of *Chrysanthemum* extracts and AgNPs based on antioxidant assays.

| Sample | TFC (µgQE/100 g) | TPC (mgGAE/100 g) | TAC (mgAAE/100 g) |
|---|---|---|---|
| Brown extract | 1669477.761 | 15.27094 | 88.25133 |
| Brown AgNPs | 12790480.00 | 2419.790 | 3312.406 |
| Yellow extract | 2229354.073 | 10.15207 | 91.86316 |
| Yellow AgNPs | 17398332.00 | 1083.958 | 4739.037 |
| Purple extract | 1590610.945 | 6.939387 | 93.29426 |
| Purple AgNPs | 8592579.000 | 1047.976 | 2689.280 |
| Pink extract | 1928723.138 | 7.774684 | 3.679978 |
| Pink AgNPs | 13233227.10 | 1115.442 | 5312.969 |
| S. Pink extract | 500530.9845 | 5.461555 | 63.10481 |
| S. Pink AgNPs | 4165104.950 | 1299.850 | 1787.388 |
| White extract | 398238.3808 | 6.875134 | 79.93730 |

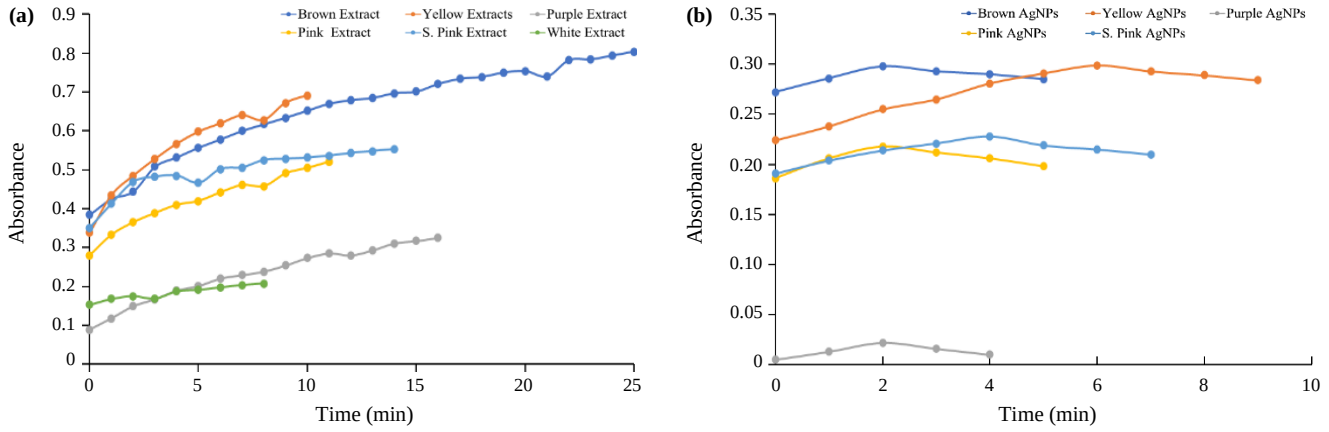


**Figure 4.** FRAP assay for *Chrysanthemum* samples. (a) FRAP for water extracts, and (b) FRAP for AgNPs.

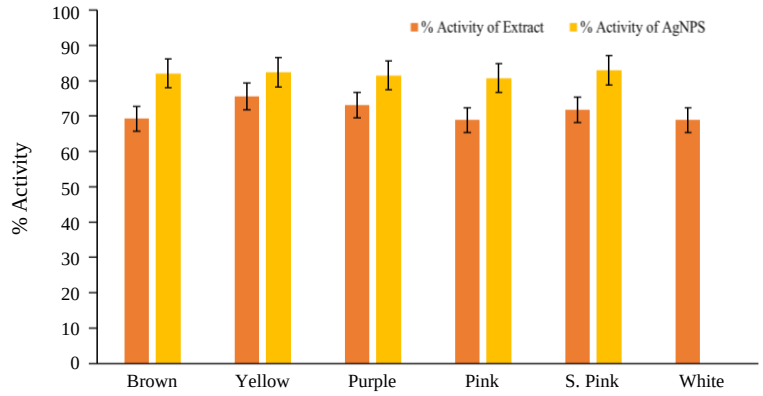


**Figure 5.** DPPH activity of *Chrysanthemum* water extracts and AgNPs (%activity). Error bars of 95% confidence interval are indicated.

**Table 5.** Minimum Inhibitory Concentration (MIC) of each sample.

| Sample | $IC_{50}$ of water extract (%) | $IC_{50}$ of AgNPs (%) |
|---|---|---|
| Brown | 33.54 | 2.63 |
| Yellow | 31.76 | 3.87 |
| Purple | 31.07 | 18.46 |
| Pink | 34.78 | 3.59 |
| S. Pink | 35.16 | 25.31 |
| White | 50.00 | - |

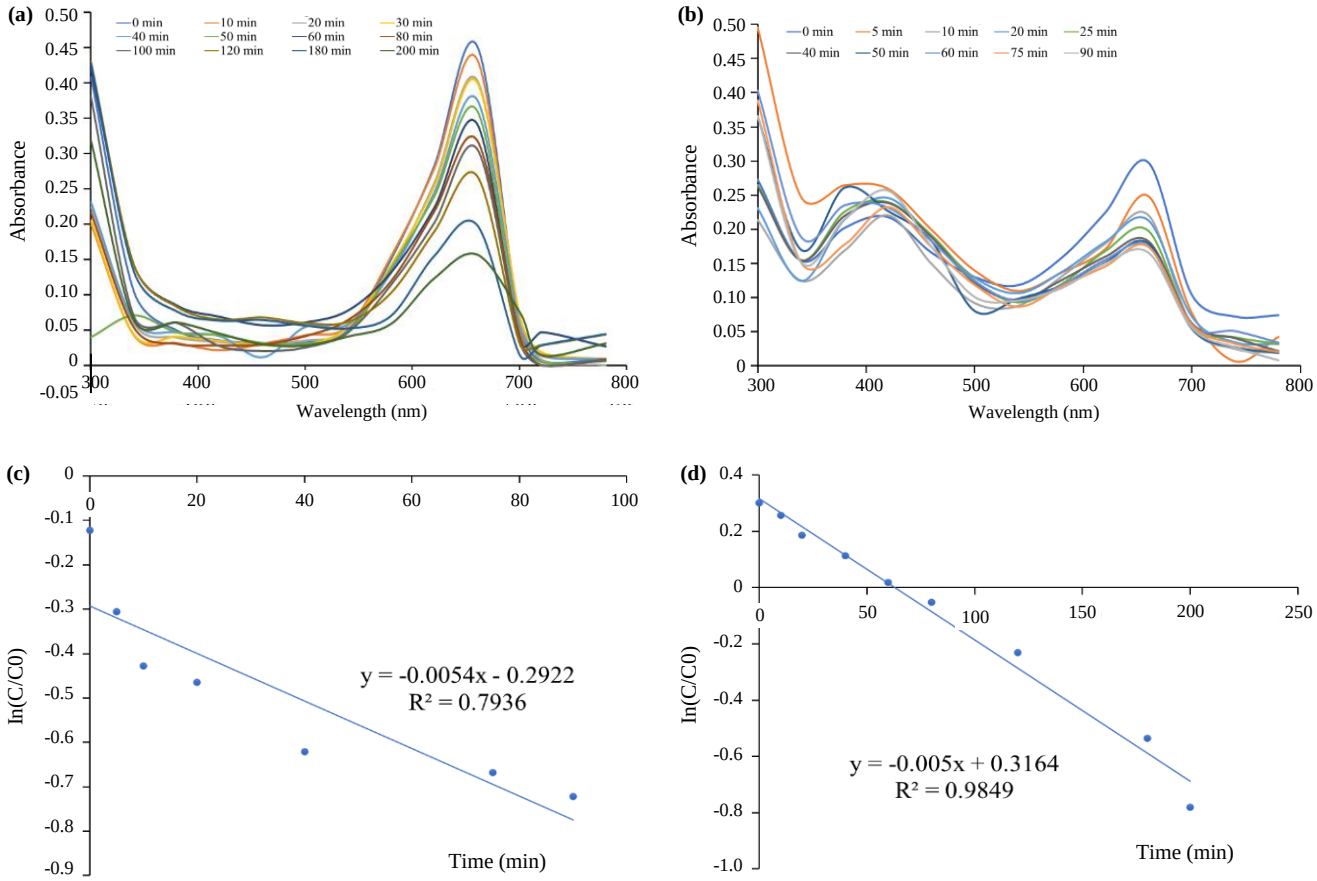


**Figure 6.** Photocatalytic degradation of MB by brown AgNPs. (a) MB degradation by brown AgNPs of 212 ppm, (b) MB degradation by brown AgNPs of 3175 ppm, (c) Plot for rate determination of AgNPs of 3175 ppm, and (d) Plot for rate determination of AgNPs of 212 ppm.

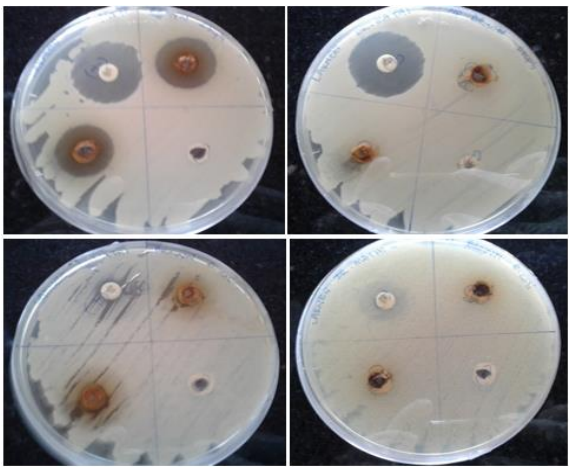

**Figure 7.** Antibacterial activity determination of *Chrysanthemum* water extracts and AgNPs. Top left: Brown water extract against *S. aureus*, Top right: Brown AgNPs against *S. aureus*, Bottom left: Brown water extract against *E. coli*, Bottom right: Brown AgNPs against *E. coli*.

**Table 6.** Antibacterial activity of Chrysanthemum water extracts and AgNPs.

| Sample | ZOI for *S. aureus* (mm) | ZOI for *E. coli* (mm) |
|---|---|---|
| Brown extract | 2.10 | 1.95 |
| Brown AgNPs | 1.15 | 1.00 |
| Yellow extract | 1.85 | 1.40 |
| Yellow AgNPs | 1.75 | 0.75 |
| Purple extract | 1.65 | 2.00 |
| Purple AgNPs | 0.85 | 1.05 |
| Pink extract | 1.50 | 1.65 |
| Pink AgNPs | 1.10 | 1.25 |
| S. Pink extract | 0.00 | 1.35 |
| S. Pink AgNPs | 0.00 | 0.85 |
| White extract | 0.00 | 0.00 |

**Table 7.** Statistical analysis for antibacterial activity determination.

| Single factor ANOVA test | P value | F value and Fcrit |
|---|---|---|
| Between water extracts and AgNPs on *Staphylococcus aureus* | 0.449177 | F value < Fcrit |
| | P value > 0.05 | 0.620366 < 4.964603 |
| Between water extracts and AgNPs on *Escherichia coli* | 0.129589 | F value < Fcrit |
| | P value > 0.05 | 2.728367 < 4.964603 |
| Between all samples of *Staphylococcus aureus* and *Escherichia coli* | 0.72112 | F value < Fcrit |
| | P value > 0.05 | 0.130736 < 4.30095 |

## 3.2 Discussion

Scientific research on microwave-assisted AgNP synthesis has gained attraction of the scientific community due to its less energy consumption and reaction time, ability to arrest agglomeration and narrower size distribution of AgNPs. Microwave-assisted (511 W), plant-mediated synthesis of AgNPs was performed in this study by utilizing the secondary metabolites such as flavonoids, phenolic compounds, terpenoids and alkaloids found in flowers of varieties of *Chrysanthemum morifolium* Ramat. This was followed by the assessment of antioxidant, photocatalytic and antibacterial properties of the synthesized AgNPs.

### 3.2.1 Phytochemical screening

Phytochemical screening indicated the presence and absence of carbohydrates, tannins, saponins, terpenoids, anthraquinones, steroids and proteins in each sample (Table 2).

### 3.2.2 Synthesis of silver nanoparticles

Water was used as the choice of solvent for AgNP synthesis as other organic solvents have been identified to show comparatively lower yields and possible toxicities. The colour change observed during the synthesis from colourless/pale yellow to brown (Figure 2(a)) indicated the formation of AgNPs, which is a result of the surface plasmon behaviour of the AgNPs. This was confirmed by spectrophotometric analysis [Figure 2(b)], which indicated clear maximum plasmon peaks from 420 nm to 480 nm for Brown, Yellow, Purple, Pink and S. Pink samples. As the conduction and valence bands (CB and VB) of AgNPs lie close, the free movement of electrons gives rise to a collective oscillation corresponding to the incident light, showing the maximum plasmon resonance peak [28,29].

Although optimization performed on microwave-assisted synthesis at 511 W showed NPs for all time durations (Table 3), the best UV/Vis spectra were obtained for the 5.5-min time duration. Thereby, the products of 5.5 min were chosen for further analysis. Spectrophotometric analysis following 6 months of synthesis showed the plasmon resonance peak between 420 nm to 480 nm indicating the stability of the synthesized AgNPs over time. Conventional low heat method of AgNP synthesis did not show satisfactory spectrophotometric results although a clear colour change was observed. SEM analysis performed on Brown AgNPs (Figure 3) indicated AgNPs of spherical shape with a size of 40 ± 1.2 nm, which lies in the range of the nanoscale.

The assessment of the band gap energy could be utilized for the determination of the conductivity of AgNPs. For this purpose, the energy gap between the CB and VB bands is measured in electron volts (eV), which is the minimum energy required for an electron to excite from the VB to the CB. This enables classification of AgNPs into insulators (> 4 eV) or semiconductors based on the equation, $E = {}^{hC}/_{\lambda}$ where E = band gap energy, h = Plank's constant ($6.629 \times 10^{-34}$ Js), C = speed of light and $\lambda$ = corresponding maximum absorption wavelength of each sample. Accordingly, the AgNPs of all the samples were classified to be semiconductors (Table 8).

### 3.2.3 Antioxidant and free radical scavenging assays

This research focuses on determination of antioxidant properties of Chrysanthemum water extracts and AgNPs. However, so far studies have not been reported for antioxidant assays on *Chrysanthemum* AgNPs. Colorimetric methods were utilized for all the assays performed.

The TFC assay was performed using the $AlCl_3$ method, where the complexation of $AlCl_3$ with C-4 keto groups, C-3/C-5 hydroxyl groups and A/B rings of ortho dihydroxide groups in flavones, flavonols and flavonoids was measured at 415 nm [30]. TFC of AgNPs

was higher than that of the water extracts (Table 4), where yellow, pink, and brown showed higher TFC in both AgNPs and water extracts compared to other samples. Statistical analysis using ONE-way ANOVA indicated a significant difference between the TFC values of water extracts and AgNPs ($P < 0.05$ / $P = 0.001$). F-value > Fcrit ($F = 22.8422$, Fcrit = 5.117355) also indicated a significant difference between the two groups.

TPC assay is performed by means of the Folin-Ciocalteu reagent, where heteropolyphosphotungstates – molybdates which give a yellow colour are reduced to a pale blue colour by the phenolic compounds in the samples, which was quantified at 765 nm [31]. TPC of AgNPs were higher than that of the water extracts (Table 4), in the order, brown > S. pink > pink = yellow = purple, whereas for water extracts, the order was brown > yellow > pink > purple = white > S. pink. ONE-way ANOVA analysis showed a significant difference between the TPC of water extracts and AgNPs ($P < 0.05$ / $P = 0.000358$). F-value > Fcrit ($F = 30.76146$, Fcrit = 5.117355) also showed a significant difference in the values obtained for the two groups.

The TAC assay was determined using the phosphomolybdenum method, where the reduction of molybdenum (VI) to Mo (V) phosphate complex by the antioxidants in the sample was measured at 695 nm [32,33]. TAC of AgNPs was higher than that of water extracts (Table 4). Higher TAC shown by pink, yellow and brown AgNPs correlates with results of TFC and TPC. The order for the TAC of water extracts was purple = yellow = brown > white > S. pink > pink. ONE-way ANOVA analysis indicated a significant difference of TAC between water extracts and AgNPs ($P < 0.05$ / $P = 0.00021$). Also, F – value > Fcrit ($F = 35.63044$, Fcrit = 5.117355) for the two groups indicating a significant difference.

FRAP assay measures the time required for the $Fe^{3+}$ ions in TPTZ to reduce to $Fe^{2+}$ forming a blue complex by the antioxidants in the samples at 593 nm [34,35]. Highest reducing power was observed in AgNPs (2 min to 6 min) whereas water extracts showed comparatively low reducing power (2 min to 20 min) (Figure 4). Confirming the results, previous studies too have shown higher reducing power for *Chrysanthemum* varieties [36,37], which could be further exploited for the prevention of autoxidation of ferrous ions due to the action of free radicals in the body [38].

DPPH radical scavenging assay was performed to determine the ability of the antioxidants in samples to quench the stable DPPH free radical by donating an electron, which changes its colour from violet to colourless/yellow, measured at 517 nm [39-41]. Overall, both AgNPs and water extracts showed higher quenching activity, although the activity of AgNPs was slightly higher (Figure 5). Similarly, previous studies have shown higher radical scavenging activities of *Chrysanthemum* water extracts [36,37].

Based on scientific literature, lower the IC50, a sample would show a higher the antioxidant activity [39,42,43]. IC50 calculated for the samples showed lower IC50 in AgNPs (brown > pink > yellow > purple > S. pink) compared to water extracts (purple > yellow > brown > pink > S. pink > white) (Table 5) indicating a higher antioxidant level in AgNPs. The higher antioxidant activities of brown, yellow, and pink AgNPs correlate with the higher antioxidant contents shown in all antioxidant assays. Pearson correlation factor (PCF) (Figure 8) was performed on all antioxidant assays, and the statistical correlations of all assays were higher than 0.700 indicating a strong positive linear statistical correlation. This implies that antioxidant content and antioxidant activities are directly proportional between each assay.

### 3.2.4 Photocatalytic degradation of methylene blue

Another compelling property of AgNPs, the photodegradation of azo dyes under UV/sunlight, sometimes, with the aid of a catalyst like sodium borohydride ($NaBH_4$), was assessed during this study. Once irradiated with sunlight, both the dye and the AgNPs absorb photons to generate electron and hole pairs due to the surface plasmon resonance effect. The dye, acting as a photosensitizer adsorbs on to AgNPs donating electrons to the CB of AgNPs, which then reacts with molecular $O_2$ to form superoxide and hydrogen peroxide radicals. Photogenerated holes react either with the dye directly or with $H_2O$ to form hydroxyl radicals and the generated radicals collectively degrade the azo dye by breaking the azo bonds [20,44-46]. For this purpose, MB was used as the model dye and $NaBH_4$ as the catalyst. From $0^{th}$ min to the $200^{th}$ min and from $0^{th}$ min to $90^{th}$ min, a gradual decrease of the maximum absorption peaks of MB are observed at 660 nm, in the degradation caused by the 212 ppm (Figure 6(a)) and 3175 ppm (Figure 6(b)) brown AgNPs respectively. The absence of the AgNP absorbance peak in Figure 10(a) is due to the deposition of 212 ppm AgNPs with time, whereas Figure 6(b) shows clear AgNP peaks indicating the stability of 3175 ppm AgNPs throughout the photocatalytic reaction. The rate constant obtained for both 3175 ppm and 212 ppm Brown AgNPs was 0.005 based on the gradients obtained for the graphs (Figure 6(c)-(d)) by using the equation $ln\left(C/C_0\right) = -kT$ where, C = concentration of MB at each time interval, $C_0$ = initial MB concentration, T = time interval and k = rate constant. Therefore, the change in concentration of AgNPs has not affected the rate of the photocatalytic degradation of MB with AgNPs. Further studies need to be performed using AgNPs of higher concentrations in order to study the effect on the rate of the reaction by concentration of AgNPs.

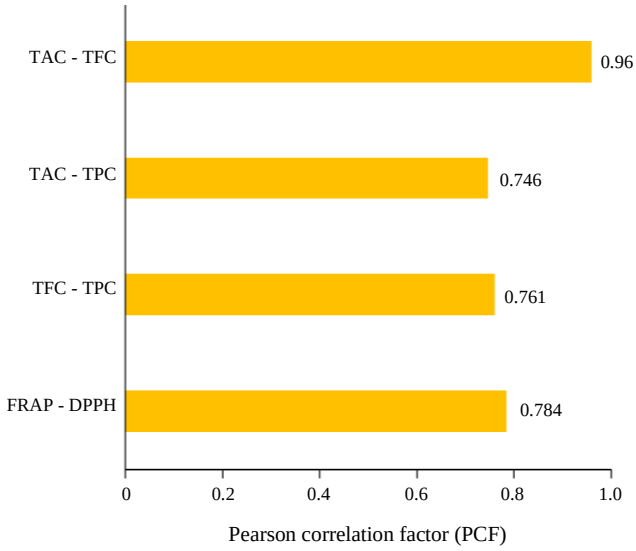


**Figure 8.** Statistical correlation between antioxidant assays.

**Table 8.** Conductivity classification of synthesized AgNPs.

| Sample | Band Gap Energy (eV) | Classification |
|---|---|---|
| Brown | 2.76 | Semiconductor |
| Yellow | 2.89 | Semiconductor |
| Purple | 2.70 | Semiconductor |
| Pink | 2.76 | Semiconductor |
| S. Pink | 2.59 | Semiconductor |

### 3.2.5 Antibacterial activity

AgNPs as well as *Chrysanthemum morifolium* flowers are well-known for their antibacterial properties. Early studies postulated that the toxicity of AgNPs on microorganisms is similar to that of silver ions, where they condense the nuclear DNA inhibiting replication, ultimately preventing the cell survival, and also bind to sulfhydryl groups of proteins decreasing the activity of multiple enzymes. Studies carried out later has showed a higher toxicity of AgNPs causing structural damage of cell membrane, loss of proper cell function and ultimately, death [47-49]. Previous studies have shown satisfactory results for antibacterial activity of *Chrysanthemum* AgNPs [15,16]. However, the expected outcome was not shown by any of the AgNPs against *S. aureus* nor *E. coli* (Table 7), since water extracts indicated higher ZOI. The mechanism behind this phenomenon needs to be further explored. None of the single factor ANOVA performed on antibacterial tests showed a significant difference for P-value nor F-value and Fcrit (Table 8).

## 4. Conclusions

In conclusion, five out of six samples produced AgNPs and SEM images indicated the presence of spherical AgNPs of 40 ± 1.2 nm, which were classified under semiconductors. All antioxidant assays performed showed higher antioxidant contents and activities in AgNPs, where a strong correlation higher than PCF = 0.7 was observed in between each assay. Photocatalytic degradation of the azo dye MB by 3175 ppm and 212 ppm AgNPs indicated the same rate constant, 0.005. Therefore, it could be concluded that AgNPs of lower concentration (i.e. 212 ppm) would be sufficient for the degradation of MB. The mechanism behind the higher antibacterial activity of water extracts than that of AgNPs requires further investigation. Therefore, the antioxidant, photocatalytic and antibacterial properties of AgNPs of *Chrysanthemum morifolium* Ramat could be further exploited as remedies for prevailing biomedical and environmental issues.

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## References

[1] J. Jeevanandam, A. Barhoum, Y. S. Chan, A. Dufresne, and M. K. Danquah, "Review on nanoparticles and nanostructured materials: History, sources, toxicity and regulations," *Beilstein Journal of Nanotechnology*, vol. 9, pp. 1050-74, 2018.

[2] P. I. Dolez, "Nanomaterials definitions, classifications, and applications," in *Nanoengineering: Global approaches to health and safety issues*, Oxford: Elsevier, 2015, pp. 3-33.

[3] P. Iqbal, J. A. Preece, and P. M. Mendes, "Nanotechnology: The "top-down" and "bottom-up" approaches", in *Supramolecular Chemistry: From molecules to nanomaterials*, J. W. Steed, and P. A. Gale, Eds., West Sussex: Wiley, 2012, pp. 3589-3602.

[4] N. H. Hong, *Introduction to Nanomaterials: Basic properties, synthesis, and characterization*, Cambridge: Elsevier Inc, 2019, pp. 1-19.

[5] C. K. Ghosh, "Quantum effect on properties of nanomaterials," in *Introduction to Nano Engineering Materials*, A. Sengupta, and C. Sarkar, Eds., Berlin: Springer, 2015, pp. 73-111.

[6] Y. Liu, S. Mai, N. Li, C. K. Y. Yiu, J. Mao, D. H. Pashley, and F. Tay, "Differences between top-down and bottom-up approaches in mineralizing thick, partially-demineralized collagen scaffolds", *Acta Biomaterialia*, vol. 7, pp. 1742-1751, 2011.

[7] X. Fang, Y. Wang, Z. Wang, Z. Jiang, and M. Dong, "Micro-organism assisted synthesized nanoparticles for catalytic applications", *Energies*, vol. 12(190), 2019.

[8] N. Kulkarni, and U. Muddapur, "Biosynthesis of metal nano-particles: A review", *Journal of Nanotechnology*, 2014.

[9] V. Mody, R. Siwale, A. Singh, and H. Mody, "Introduction to metallic nanoparticles," *Journal of Pharmacy and Bioallied Sciences*, vol. 2, pp. 282-289, 2010.

[10] R. Balachandar, P. Gurumoorthy, N. Karmegam, and H. Barabadi, "Plant-mediated synthesis, characterization and bactericidal potential of emerging silver nanoparticles using stem extract of *Phyllanthus pinnatus*: A recent advance in phytonanotechnology", *Journal of Cluster Science*, vol. 30, pp. 1481-1488, 2019.

[11] V. V. Makarov, A. J. Love, O. V. Sinitsyna, S. S. Makarova, I. V. Yaminsky, M. E. Taliansky, and N. O. Kalinina, "Green nanotechnologies: synthesis of metal nanoparticles using plants," *Acta Naturae*, vol. 6, pp. 35-44, 2014.

[12] S. Iravani, H. Korbekandi, S. V. Mirmohammadi, and B. Zolfaghari, "Synthesis of silver nanoparticles: Chemical, physical and biological methods," *Research in Pharmaceutical Sciences*, vol. 9, pp. 385-406, 2014.

[13] S. Arokiyaraj, M. V. Arasu, S. Vincent, N. U. Prakash, S. H. Choi, Y. K. Oh, K. C. Choi, and K. H. Kim, "Rapid green synthesis of silver nanoparticles from *Chrysanthemum indicum* land its antibacterial and cytotoxic effects: An in vitro study," *International Journal of Nanomedicine*, vol. 9, pp. 379-388, 2014.

[14] S. M. Haqq, H. Pandey, M. Gerard, and A. Chattree, "Bio-fabrication of silver nanoparticles using *Chrysanthemum*

*coronarium* flower extract and its' in vitro antibacterial activity," *International Journal of Applied Pharmaceutics*, vol. 10, pp.209-213, 2018.

[15] Y. He, Z. Du, H. Lv, Q. Jia, Z. Tang, X. Zheng, K. Zhang, and F. Zhao, "Green synthesis of silver nanoparticles by *Chrysanthemum morifolium* Ramat. extract and their application in clinical ultrasound gel," *International Journal of Nanomedicine*, vol. 8, pp. 1809-1815, 2013.

[16] S. Jing, X. Zhang, and L. J. Yan, "Antioxidant activity, antitumor effect and antiaging property of proanthocyanidins extracted from *Kunlun Chrysanthemum* flowers," *Oxidative Medicine and Cell Longevity*, 2015.

[17] Y. Anuj, K. Rewa, Y. Ashwani, J. P. M, S. Seweta, and P. Shashi, "Antioxidants and its functions in human body", *Research in Environment and Life Sciences*, vol. 9, pp. 1328-1331, 2016.

[18] V. Lobo, A. Patil, A. Phatak, and N. Chandra, "Free radicals, antioxidants and functional foods: Impact on human health", *Pharmacognosy Reviews*, vol. 4, pp. 118-126, 2010.

[19] E. M. Atta, N. H. Mohamed, and A. A. M. Abdelgawad, "Antioxidants: An Overview on the Natural and Synthetic Types", *European Chemical Bulletin*, vol. 6, pp. 365-406, 2017.

[20] M. Mavaei, A. Chahardoli, Y. Shokoohinia, A. Khoshroo, and A. Fattahi, "One-step synthesized silver nanoparticles using isoimperatorin: Evaluation of photocatalytic, and electrochemical activities", *Scientific Reports*, vol. 10, no. 1762, 2020.

[21] S. Marimuthu, A. Jayanthi, and S. Malayandi, "Silver nanoparticles in dye effluent treatment: A review on synthesis, treatment methods, mechanisms, photocatalytic degradation, toxic effects and mitigation of toxicity", *Journal of Photochemistry and Photobiology B: Biology*, vol. 205, 2020.

[22] Y. N. Slavin, J. Asnis, U. O. Häfeli, and H. Bach, "Metal nanoparticles: Understanding the mechanisms behind antibacterial activity," *Journal of Nanobiotechnology*, vol. 15, no. 65, 2017.

[23] Y. Y. Loo, Y. Rukayadi, and C. H. Kuan, "In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens," *Frontiers in Microbiology*, vol. 9, 2018.

[24] B. R. Perera, and M. Kandiah, "Microwave assisted one-pot green synthesis of silver nanoparticles using leaf extracts from *Vigna unguiculate*: Evaluation of antioxidant and antimicrobial activities," *International Journal of Multidisciplinary Studies*, vol. 5, pp. 62-78, 2018.

[25] R. Roghini, and K. Vijayalakshmi, "Phytochemical screening, quantitative analysis of flavonoids andminerals in ethanolic extract of *Citrus paradisi*," *International Journal of Pharmaceutical Science and Research*, vol. 9, pp. 4859-4864, 2018.

[26] N. Singh, A. Chatterjee, K. Chakraborty, S. Chatterjee, and J. Abraham, "Cytotoxic effect on MG-63 cell line and antimicrobial and antioxidant properties of silver nanoparticles synthesized with seed extracts of Capsicum sp," *Records of Natural Products*, vol. 10, pp. 47-57, 2016.

[27] K. Sharma, G. Singh, G. Singh, M. Kumar, and V. Bhalla, "'Silver nanoparticles': facile synthesis and their catalytic application for the degradation of dyes," *RSC Advances*, vol. 5, pp. 25781-25788, 2015.

[28] H. I. Badiah, F. Seedeh, G. Supriyanto, and A. H. Zaidan, "Synthesis of silver nanoparticles and the development in analysis method," *IOP Conference Series: Earth and Environmental Science*, vol. 217, 2019.

[29] N. Chouhan, "Silver nanoparticles: Synthesis, Characterization and Applications," in *Silver Nanoparticles - Fabrication, Characterization and Applications*, Khan Maaz, Ed., Intech-Open; 2018, pp. 21-58.

[30] T. P. Kumar, P. Kalita, T. K. Barman, T. K.Chatterjee, and S. Maity, "Quantification of Total Flavonoid Content and antioxidant activity in comparison to a reference flavonoid as in vitro quality evaluation parameter for assessing bioactivity of biomarkers in herbal extracts or formulations," *JPR: BioMedRx: An International Journal*, vol. 1, pp. 757-66, 2013.

[31] J. C. Sánchez-Rangel, J. Benavides, J. B. Heredia, L. Cisneros-Zevallos, and D. A. Jacobo-Velázquez, "The Folin-Ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination," *Analytical Methods*, vol. 5, pp. 5990-5999, 2013.

[32] D. Gupta, "Methods for determination of antioxidant capacity: A review," *International Journal of Pharmaceutical Sciences and Research*, vol. 6, pp. 546-566, 2015.

[33] P. Prieto, M. Pineda, and M. Aguilar, "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E", *Analytical Biochemistry*, vol. 269, pp. 337-341, 1999.

[34] M. El Jemli, R. Kamal, I. Marmouzi, A. Zerrouki, Y. Cherrah, and K. Alaoui, "Radical-Scavenging activity and ferric reducing ability of *Juniperus thurifera* (L.), *J. oxycedrus* (L.), *J. phoenicea* (L.) and *Tetraclinis articulata* (L.)", *Advances in Pharmacological Sciences*, 2016.

[35] K. Pérez-Cruz, M. Moncada-Basualto, J. Morales-Valenzuela, G. Barriga-González, P. Navarrete-Encina, L. Núñez-Vergara, J. A. Squella, and C. Olea-Azar, "Synthesis and antioxidant study of new polyphenolic hybrid-coumarins," *Arabian Journal of Chemistry*, vol. 11, pp. 525-537, 2018.

[36] J. Gong, B. Chu, L. Gong, Z. Fang, X. Zhang, S. Qiu, J, Wang, Y. Xiang, G. Xiao, H. Yuan, and F, Zheng, "Comparison of phenolic compounds and the antioxidant activities of fifteen *Chrysanthemum morifolium* Ramat cv. 'Hangbaiju' in China," *Antioxidants*, vol. 8, 2019.

[37] L. Yang, A. Nuerbiye, P. Cheng, J. H. Wang, and H. Li, "Analysis of floral volatile components and antioxidant activity of different varieties of *Chrysanthemum morifolium*," *Molecules*, vol. 22, no. 1790, 2017.

[38] A. Phaniendra, D. B. Jestadi, and L. Periyasamy, "Free radicals: Properties, sources, targets, and their implication in various diseases", *Indian Journal of Clinical Biochemistry*, vol. 30, pp. 11-26, 2015.

[39] N. Jadid, D. Hidayati, S. R. Hartanti, B. A. Arraniry, R. Y. Rachman, and W. Wikanta, "Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay". *AIP Conference Proceedings*, 2017.

[40] K. Pyrzynska, and A. Pękal, "Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant

capacity of food samples," *Analytical Methods*, vol. 5, pp. 4288-4295, 2013.

[41] M. A. Khan, A. A. Rahman, S. Islam, P. Khandokhar, S. Parvin, B. Islam, M. Hossain, M. Rashid, G. Sadik, S. Nasrin, M. N. H. Mollah, and A. H. M. K. Alam, "A comparative study on the antioxidant activity of methanolic extracts from different parts of Morus alba L. (Moraceae)," *BMC Research Notes*, vol. 6, no. 24, 2013.

[42] Y. Hangun-balkir and M. L. Mckenney, "Green Chemistry Letters and Reviews Determination of antioxidant activities of berries and resveratrol", *Green Chemistry Letters and Reviews*, vol. 5, no. 1, pp. 47-53, 2012.

[43] T. S. Himamura, Y. S. Umikura, T. Y. Amazaki, A. T. Ada, T. K. Ashiwagi, H. Ishikawa, T. Matsui, N. Sugimoto, H. Akiyama, and H. Ukeda, "Applicability of the DPPH Assay for Evaluating the Antioxidant Capacity of Food Additives – Inter-laboratory Evaluation Study," *Analytical Sciences*, vol. 30, pp. 717-721, 2014.

[44] K. Roy, C. K. Sarkar, and C. K. Ghosh, "Photocatalytic activity of biogenic silver nanoparticles synthesized using yeast (*Saccharomyces cerevisiae*) extract," *Applied Nanoscience*, vol. 5, pp. 953-959, 2015.

[45] P. C. Nagajyothi, S. V. P. Vattikuti, K. C. Devarayapalli, K. Yoo, and T. V. M. Sreekanth, "Green synthesis: Photocatalytic degradation of textile dyes using metal and metal oxide nanoparticles-latest trends and advancements," *Critical Reviews in Environmental Science and Technology*, vol. 50, pp. 2617-2723, 2019.

[46] M. Phukan, "Characterizing the ion-selective nature of Fe0-based systems using azo dyes: Batch and column experiments," *Freiberg Online Geoscience*, vol. 42, 2015.

[47] Y. Qing, L. Cheng, R. Li, G. Liu, Y. Zhang, X. Tang, J. Wang, H. Liu, and Y. Qin, "Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies," *International Journal of Nanomedicine*, vol. 13, pp. 3311-3327, 2018.

[48] X. Yan, B. He, L. Liu, G. Qu, J. Shi, L. Hu L, and G. Jiang, "Antibacterial mechanism of silver nanoparticles in *Pseudomonas aeruginosa*: Proteomics approach," *Metallomics*, vol. 10, pp. 557-564, 2018.

[49] B. Le Ouay, and F. Stellacci, "Antibacterial activity of silver nanoparticles : A surface science insight," *Nano Today*, vol. 10, pp. 339-354, 2015.