GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA

GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 10 | Issue: 01

On 31st March 2024

http://www.research.lk

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GARI Publisher | Biomedical Science | Volume: 10 | Issue: 01

Article ID: IN/GARI/JOU/2024/172 | Pages: 55-77 (23)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 13.02.2024 | Publish: 31.03.2024

LILY MEDIATED GREEN SYNTHESIS OF SILVER NANOPARTICLES AND THE EVALUATION OF THEIR ANTIOXIDANT, PHOTOCATALYTIC, AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Plant-mediated synthesis of silver nanoparticles (AgNPs) has gained recognition around the globe because it is safer, rapid, and environmentally friendly. The current research seeks to evaluate the antioxidant, photocatalytic, and antibacterial activities of AgNPs synthesized using 5 varieties of Lilium species. Three Asiatic varieties (orange, pink, and yellow), one trumpet variety (white), and one oriental variety (hot pink) of lilies were utilized in this study. Out of the five varieties, only white trumpet lilies synthesized AgNPs (WAgNPs) readily at room temperature. The formation, shape, and size of WAgNPs were characterized using UV-visible spectroscopy and transmission electron microscope (TEM). WAgNPs with a 20 nm diameter of spherical morphology were observed. Antioxidant assays including TFC, TPC, TAC, and DPPH of the Lilium flower extracts and WAgNPs were evaluated. The highest activity was observed in WAgNPs for all antioxidant assays. The photocatalytic degradation of Eriochrome Black-T was achieved using 100 ppm WAgNPs and 4000 ppm WAgNPs. The latter exhibited a slightly higher rate constant under sunlight. Staphylococcus aureus and Escherichia coli were utilized to test for antibacterial activity of WAgNPs and of the flower extracts. Although the antibacterial activity of WAgNPs and the water extracts against Escherichia coli were not greater than Staphylococcus aureus, all samples exhibited approximately 2 cm inhibition zones. Hence, a sustainable approach was discovered through the utilization of antioxidant, photocatalytic, and antibacterial properties of Lilium species. This could be used to cure diseases triggered by free radicals, remediate pollution generated by organic dyes, and resolve antibiotic-resistant catastrophes.

Keywords: nanoparticle, antioxidants, plant-mediated synthesis

INTRODUCTION

Ever since Nobel laureate Richard Feynman introduced the term nanotechnology in his well-known speech “there’s plenty of room at the bottom” (Feynman, 1960), several groundbreaking advancements have occurred in the nanotechnology field. Nanotechnology is a promising field that deals with nanoparticles that range in size between 1 and 100 nm. Upon size reduction to the nanoscale range and its subsequent shape change, nanoparticles acquire beneficial chemical, biological and physical properties that are distinct from those of its components at bulk scale (Gatoo et al., 2014). Due to unique properties of nanoparticles, they are utilized in various applications in industries like textile, food science, pharmaceutical and wastewater treatments (Thiruvengadam and Kumar, 2019). Specifically, metallic nanoparticles

(MNPs) including gold and silver are now of major interest as they are beneficial in catalytic processes and are effective anti-cancer and anti-microbial agents (Kumar et al., 2020).

Silver nanoparticles (AgNPs) are most intensely studied and are distinctive among MNPs in terms of their unique optical, electrical, and thermal properties, as well as strong electrical conductivity (Chen et al., 2009, Naderi et al., 2012 and Coetzee et al., 2020). Hence, owing to these properties, AgNPs function as nanosensors (Prosposito, 2016), conductive nanowires (Markus Diantoro, 2018) or as secondary fillers in polymer composites (Sanmuham et al., 2021). Moreover, studies have shown that AgNPs are effective against antibiotic-resistant strains of bacteria (Barros et al., 2018, Loo et al., 2018 and Khatoon et al., 2019). There are two main approaches to synthesizing MNPs (Figure 01). Bulk material is broken down into nanoparticles via the top-down approach. This includes physical methods such as laser ablation, sputtering and milling (Ahmed et al., 2021). Although these methods can almost flawlessly regulate the size and shape of nanoparticles, they are expensive. The bottom-up approach involves the build-up of nanoparticles from atomic level (Vikas Sarsar, 2014). These include chemical methods like pyrolysis and sol-gel process which contribute to environmental toxicity. Hence, at present, there is a rising demand to design environmental-friendly synthesis techniques that do not involve toxic compounds.

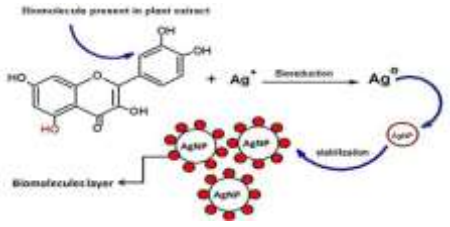

Figure 02. Mechanism of AgNP green synthesis (Zafar and Zafar, 2019)

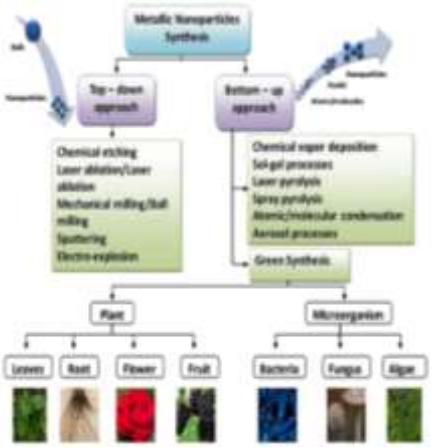

Figure 01. Approaches to synthesize MNPs (Singh et al., 2018)

Green synthesis includes bacteria, algae, plants, fungi and yeast to synthesize MNPs (Gour and Jain, 2019). However, major drawbacks of microbes as a source to synthesize nanoparticles are production of MNPs with imperfect nanostructures (Bahrulolum et al., 2021) and requirement of aseptic conditions making it a relatively expensive process (Rautela, Rani and Das, 2019).

A faster reduction of metallic ions and stabilization is achieved by plant extracts in comparison to microbes (Ali et al., 2020). Parts of the plant including stem, flower, bark and roots can be utilized in plant-mediated green synthesis of nanoparticles (Jain and Mehata, 2017 and Alabdallah and Hasan, 2021). Intracellular, extracellular and phytochemical mediated synthesis are the three approaches to synthesize nanoparticles using plants. Cost-effectiveness, non-toxicity, eco-friendliness, use of aqueous solvents, readily availability of plants, and the biocompatibility of extracts derived from plants are all benefits of the above three approaches (Dogiparthi et al., 2021). In this study, phytochemical mediated synthesis is approached to synthesize

AgNPs. Natural reducing agents or biomolecules in the plant extract reduce Ag+ ions into metallic silver (Figure 02). This is also then stabilized and capped by the bioactive compounds present in the plant extract (Ponarulselvam et al., 2012).

Free radicals are molecular entities that possess unpaired electrons in their atomic orbitals. This causes them to be relatively unstable and highly reactive. Hence, they serve as either oxidants or reductants by donation or acceptance of electrons from other substances respectively (Sharifi-Rad et al., 2020). Free radicals that incorporate oxygen as a part of their molecular entity are known as reactive oxygen species (ROS) and commonly found ROS in many diseases include hydroxyl, superoxide, nitric oxide, nitrogen dioxide, peroxyl and lipid peroxyl (Niedzielska et al., 2016). Free radicals are useful in low amounts, partake in defense mechanisms of the immune system, and regulate redox reactions. However, at higher quantities, oxidative stress can cause tissue damage in the human body (Sailaja Rao et al., 2011). Antioxidants protect tissue from free radical damage by hindering radical production, scavenging radicals, or facilitating their breakdown (Poljsak, Šuput and Milisav, 2013). However, studies have shown that synthetic antioxidants like butylated hydroxyanisole and butylated hydroxytoluene exhibit carcinogenesis and induce unfavorable effects on animal liver (Lourenço, Moldão-Martins and Alves, 2019). Hence, the need for natural antioxidants like AgNPs arise which transfer electrons to free radicals whilst discouraging tumorigenesis (Figure 03) (Flieger et al., 2021).

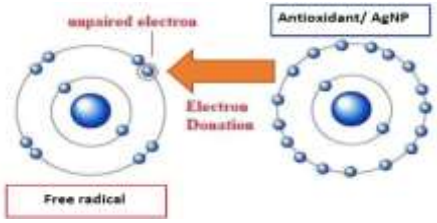

Figure 03. Interaction of free radicals with antioxidants (Verma et al., 2019)

Organic dyes used in textile industries are major organic pollutants and are known to be potential carcinogens. They are non-compostable and restrict sunlight from reaching water bodies resulting in higher biochemical oxygen demand (BOD) and lower photosynthetic rates by aquatic plants. These deleterious effects can result in death of aquatic organisms (Lellis et al., 2019 and Ardila-Leal et al., 2021). Photocatalytic breakdown of toxic pollutants via AgNPs is one conceivable way for accomplishing significant cleanup. Studies indicate that such biological removal technique of dyes is economically feasible and produces no harmful byproducts relative to physicochemical methods like ozonation and adsorption (Saratale et al., 2011).

Furthermore, the antibacterial activity of AgNPs and water extracts of Lilium will be investigated in this study as a viable solution for the increasing danger of antibiotic resistance around the world (Ayukekbong, Ntemgwa and Atabe, 2017). Lilium or lilies are ornamental crops belonging to the Liliaceae family. These herbaceous perennials are widely used as cut flowers in horticulture due to their flamboyant display of petals and availability of large number of hybrids. Lilies possess bioactive compounds like saponins, flavonoids, alkaloids and polysaccharides, which contribute to anti-tumor, antibacterial and antioxidant activities. Asiatic, oriental and trumpet are widely hybridized lily varieties and differ in blooming period, growth rate, fragrance and origin (Herlina, Samijan and Winarto, 2020). To date, there has been no research into synthesizing AgNPs from Lilium species. Therefore, much research needs to be focused on the subject because they have more potential than just providing shade and beauty to the surroundings. Thus, the objective of this study is to

synthesize AgNPs using aqueous flower extracts of five varieties of Lilium species and to evaluate their antioxidant, photocatalytic and antibacterial activity. The antioxidant activity will be assessed using antioxidant assays like TFC, TPC, TAC, and DPPH. The photocatalytic activity will be determined upon the ability to degrade Eriochrome Black-T via synthesized AgNPs and the antibacterial activity will be determined utilizing gram-negative Escherichia coli and gram-positive Staphylococcus aureus. This research is predicted to aid in a high-quality of life whilst providing a safer and greener environment.

Five varieties of Lilium flowers were obtained from Shiro Hana Atelier, Alfred House gardens, Colombo 03, Sri Lanka. This includes oriental hot pink, asiatic orange, white trumpet, asiatic yellow and asiatic pink lilies (Figure 04).

Figure 04. Lilies procured for this study are (A) oriental hot pink (B) asiatic orange (C) trumpet white (D) asiatic yellow and (E) asiatic pink.

Preparation of aqueous flower extract

The samples were completely dried in a hot air oven and were powdered. A mass of 2 g from each crushed sample was suspended in 50 mL distilled water which was then heated to 100°C for 15 minutes in the hot air oven. Water extracts were obtained through filtration using Whatman filter paper no.1 into 50 mL falcon tubes and were stored at 4°C for future use.

Evaluation of phytochemical properties

Water extracts were used to test for the presence of phytochemicals according to the procedure shown in Table 01.

Table 01. Procedure performed to test for each phytochemical's presence (Panchal and Parvez, 2019).

Phytochemical	Procedure
Carbohydrate	0.25 mL of water extract was mixed with 0.125 mL of Molisch's reagent. A few drops of concentrated sulfuric acid were added.
Tannins	0.25 mL of water extract was mixed with 0.5 mL of 5% Ferric chloride gently
Saponins	0.25 mL of water extract was mixed with equal amounts of distilled water. The mixture was shaken in a graduated cylinder for 15 minutes lengthwise
Terpenoids	0.25 mL of water extract was mixed with 0.5 mL chloroform. Few drops of concentrated sulfuric acid were added to the mixture.
Anthraquinones	2-3 drops of ammonia solution were mixed with 0.25 mL of water extract
Steroids	0.25 mL of water extract was gently mixed with 0.25 mL of chloroform. 1-2 drops of concentrated sulfuric acid were added to the mixture.

Proteins	0.25 mL of water extract was mixed with 1-2 drops of 0.2% ninhydrin reagent. This was then heated for a few minutes

Green-mediated synthesis and optimization of AgNPs

1 mL of each water extract was mixed with 9 mL of 1 mM AgNO3 solution. Optimization was performed in a hot air oven at 90°C and 60°C for 15 minutes, 30 minutes, 45 minutes and 60 minutes. The prepared solution was also kept at RT for 72 hours. Absorbance was measured from 300 nm to 520 nm against a water blank.

Dilution: 1mL of water extracts and 1mL of synthesized AgNP sample were diluted 15 times and stored at 4°C for future use. These diluted samples were used to analyze further assays.

Evaluation of Total Flavonoid Content (TFC)

TFC was evaluated using an AlCl3 colorimetric method adopted from Yadav and Agarwala with slight modifications. A volume of 1 mL of the sample was mixed with 1 mL of 2% AlCl3.6H20 and incubated at RT for 60 minutes. The absorbance was measured in triplicates at 415 nm against a distilled water blank. Results were demonstrated in µg Quercetin per 100g (µg QE/100g) (Yadav and Agarwala, 2011).

Evaluation of Total Phenolic Content (TPC)

TPC was evaluated using Folin-Ciocalteu method from Fafal et al with minor modifications. A volume of 0.4 mL of the sample was dissolved in 0.6 mL distilled water and 0.25 mL of 5% Folin-Ciocalteau reagent in triplicates. The solution was then mixed and incubated at RT for 2 minutes followed by the addition of 750 µL of 15% Na2CO3 and incubated at RT in the dark for 2 hours. Absorbance was measured in triplicates at 765 nm against a distilled water blank. Results were demonstrated in mg of gallic acid per 100g (mg GAE/100g) (Fafal et al., 2017).

Evaluation of Total Antioxidant Activity (TAC)

TAC was evaluated using phosphomolybdenum assay from Shabbir, Khan and Saeed with minor modifications. A reagent solution was prepared with 0.6 M H2SO4, 28 mM Na3PO4 and 4 mM of (NH4)6Mo7O24 in a 1:1:1 ratio. To 2 mL of this reagent solution, 0.2 mL of the sample was added. The prepared mixture was incubated at 95°C for 90 minutes. After samples cooled down to 25°C, the absorbance was measured in triplicates at 695 nm against a distilled water blank. Results were demonstrated in mg of ascorbic acid per 100g (mg AAE/100g) (Shabbir, Khan and Saeed, 2013).

Evaluation of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

DPPH scavenging activity was evaluated according to the procedure provided by Mahdi-Pour et al with minor modifications. A volume of 1 mL of the sample was dissolved in 100 mL of 0.004% DPPH solution. The mixture was incubated at RT for 30 minutes. The absorbance was measured at 517 nm against a methanol blank. % DPPH scavenging activity was formulated according to the equation below (Mahdi-Pour et al., 2012).

$$\text{Inhibition}\,(\%) = A_{\text{control}} - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100.$$

Evaluation of the photocatalytic activity of AgNPs

1 mL of 100 ppm WAgNPs was added to 100 mL of 2 mM EBT in a glass beaker and enclosed by a watch glass. This was then kept under direct sunlight. Absorbance was recorded at equal intervals every 30 minutes from 320 nm to 720 nm against a distilled water blank for 120 minutes. The procedure was repeated

for 4000 ppm WAgNPs for 150 minutes (Sharma et al., 2015).

Evaluation of the antibacterial activity

15 mL Mueller Hinton agar plates were prepared and Staphylococcus aureus and Escherichia coli were used to determine the antibacterial activity via well diffusion method. The two strains were streaked onto the agar plates separately and wells were made for a negative control of saline and for the two copies of the same sample (S1 and S2). Gentamycin was placed as the positive control. Plates were incubated overnight at 37°C and the zone of inhibition was measured using a ruler.

Petri plates were labelled as below (Figure 05)

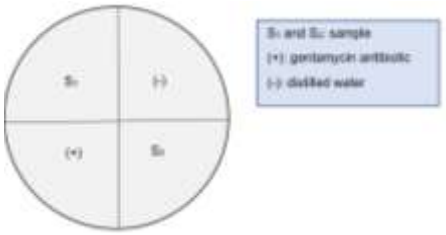

Figure 05. Labelling of petri plates for antibacterial assay.

TEM analysis

10 mL of WAgNP sample was transferred to a 15 mL Eppendorf tube and was centrifuged at 5000 rpm for 5 minutes. The cycle was repeated 6 times and was then completely dried in hot air oven. Using JEOL JEM-2100 transmission electron microscope at Sri Lanka Institute of Nanotechnology (SLINTEC), Homagama, TEM analysis was performed.

Statistical analysis

Microsoft® Excel 2019 software was used to perform single-factor ANOVA and Two-sample T-test. Statistical difference was defined as $p < 0.05$ and F crit < F value respectively. IBM SPSS Statistics 25 was used to determine statistical correlation among TFC, TPC and TAC.

Results

Table 02. Phytochemical analysis using water extracts

Phytochemical	Yellow (Y)	Orange (O)	Hot pink (HP)	Light pink (LP)	White trumpet (W)
Carbohydrates	✓	✓	✓	✓	✓
Saponins	✓	✓	✓	✓	✓
Steroids	✓	✓	✓	✓	✓
Proteins	x	x	x	x	x
Terpenoids	x	x	x	x	x
Anthraquinones	x	x	x	x	x
Tannins	x	x	x	x	x

Results of the phytochemical analysis indicated the presence of carbohydrates, saponins and steroids in all water extracts.

Biosynthesis of silver nanoparticles (AgNPs)

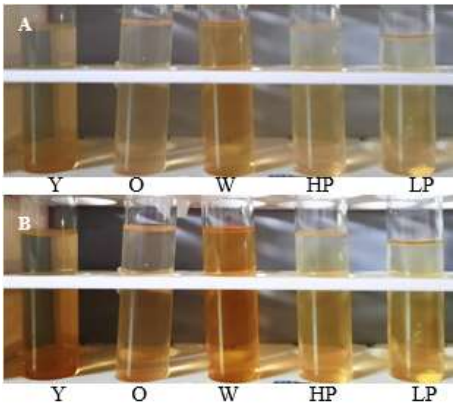

Figure 06. AgNP bio-synthesis using aqueous extracts of Lilium spp. (A) before and (B) after optimization at RT for approximately 72 hours.

Change in color from light brown to reddish-brown was observed in W water extract

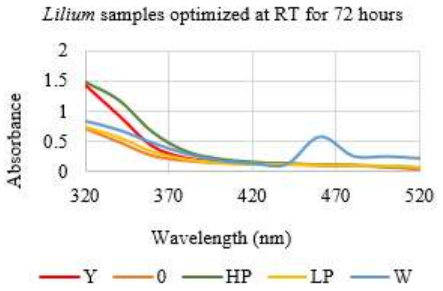

Figure 07. UV-Visible spectrum for AgNP synthesis at RT for 72 hours

UV-Visible spectrum indicated an absorbance peak at 460 nm for W sample. Other 4 samples did not produce an absorbance peak indicating the dissolution of AgNPs.

Table 03. Optimization of the biosynthesis of AgNPs.

	60°C				90°C				RT
Sample code	15 min.	30 min.	45 min.	60 min.	15 min.	30 min.	45 min.	60 min.	72 hrs.
W	✓	✓	x	x	x	x	x	x	✓
HP	x	x	x	x	x	x	x	x	x
Y	x	x	x	x	x	x	x	x	x
LP	x	x	x	x	x	x	x	x	x
O	x	x	x	x	x	x	x	x	x

W produced an absorbance at 420 nm at RT, 60°C (15 min and 30 min). Hence, W sample optimized at RT was used for further analysis

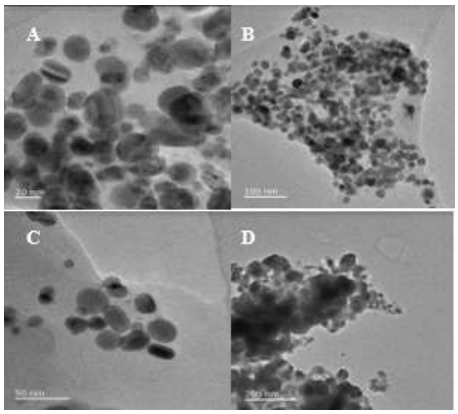

Figure 08. TEM images of WAgNPs at magnifications of (A) 20 nm (B) 50 nm (C) 100 nm (D) 200 nm

TEM analysis revealed the presence of WAgNPs of spherical morphology with an average diameter of 20 nm.

Evaluation of Total Flavonoid Content (TFC)

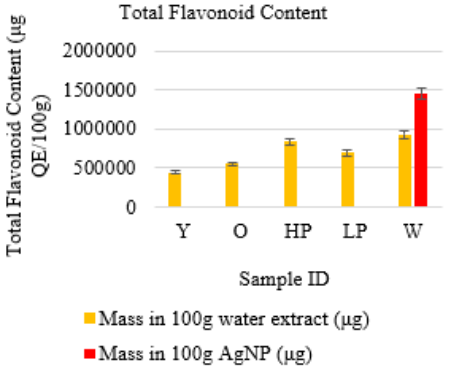

Figure 09. TFC of water extracts and WAgNPs expressed in quercetin equivalents

Highest TFC is observed in WAgNPs relative to all water extracts. W and HP have the highest TFC among all water extracts followed by LP>O>Y.

Table 04. Single-factor ANOVA between water extracts of the TFC assay

SUMMARY						
Groups	Count	Sum	Average	Variance		
Y	3	35.333 33333	11.777 77778	0.01484358		
O	3	43.875	14.625	0.13269949		
HP	3	67	22.333 33333	2.42344661		
LP	3	55.457 5	18.479 16667	0.09877258		
W	3	74.187 5	24.729 16667	0.02479719		
ANOVA						
Variation source	SS	df	MS	F	P-value	F crit
Between Groups	340.91 86921	4	85.229 67305	158.26 8879	5.3 82E-09	3.47 805
Within Groups	5.3851 18879	10	0.5385 11888			
Total	346.30 3811	14				

Table 05. Two-Sample T-test between W water extract and WAgNPs of the TFC assay

t Stat	11.29870875
P(T<=t) two-tail	2.01808E-08
t Critical two-tail	2.144786688

Statistical analysis revealed a significant difference between the water extracts and between WAgNPs and W water extract.

Table 06. Single-factor ANOVA between water extracts of the TPC assay

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Y	3	40.98285714	13.66095238	0.476547765		
O	3	40.41142857	13.47047619	0.086645535		
HP	3	40.72571429	13.5752381	0.070658659		
LP	3	39.06857143	13.02285714	0.005328646		
W	3	42.01142857	14.00380952	0.53624798		
ANOVA						
Variation source	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.509115646	4	0.377278912	1.604856802	0.247595	3.47805
Within Groups	2.350857168	10	0.235085717			
Total	3.859972814	14				

Table 07. Two-Sample T-test between W water extract and WAgNPs of the TPC assay.

t Stat	67.38231494
P(T<=t) two-tail	4.88704E-20
t Critical two-tail	2.131449546

Statistical analysis revealed a significant difference between WAgNPs and W water extract but no statistical difference is evident between water extracts.

Evaluation of Total Antioxidant Capacity (TAC)

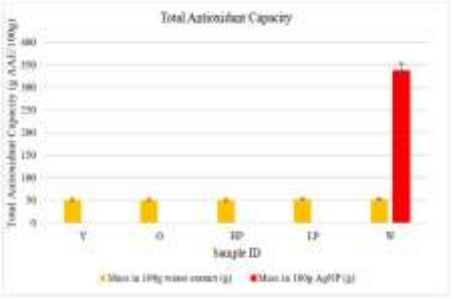

Figure 11. TAC of water extracts and WAgNPs expressed in ascorbic acid equivalents.

WAgNPs extract has the highest TAC. All water extracts constitute approximately equal amounts of antioxidants.

Table 08. Single-factor ANOVA between water extracts of the TAC assay.

SUMMARY						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Y	3	4.048181818	1.349393939	0.003405264		
O	3	4.020909091	1.340303030	0.008128394		
HP	3	4.066363636	1.355454545	0.005225976		
LP	3	4.157272727	1.385757576	0.001356137		
W	3	4.157454545	1.391818182	0.000931177		
ANOVA						
Variation source	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	0.006280992	4	0.001570248	0.412204464	0.79617	3.47804
Within Groups	0.038093893	10	0.003809389			
Total	0.044374884	14				

Table 09. Two-Sample T-test between W water extract and WAgNPs of the TAC assay

t Stat	6.753233
P(T<=t) two-tail	0.021231
t Critical two-tail	4.302653

Statistical analysis revealed a significant difference between WAgNPs and W water extract but no statistical difference is evident between water extracts.

DPPH Radical Scavenging Activity

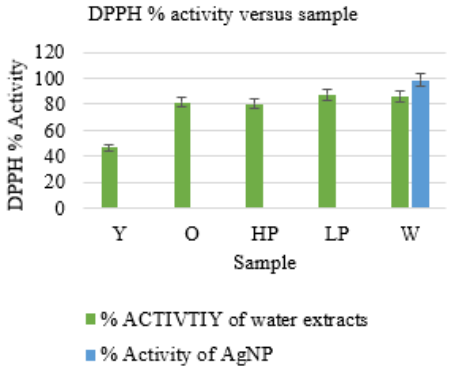

Figure 12. DPPH % activity of Lilium aqueous water extracts and WAgNPs.

A significant difference is evident between DPPH% activity of W water extract and WAgNPs. Among water extracts, the DPPH% activity of water extracts increases in the order of Y<HP=O<LP=W.

Photocatalytic activity of WAgNPs

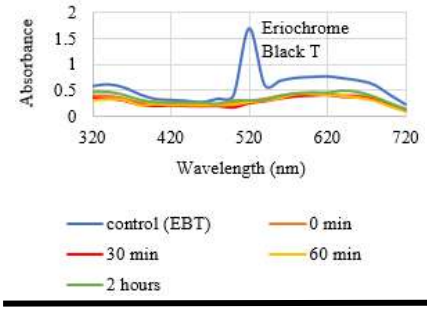

Figure 13. Photocatalytic activity of 100 ppm WAgNPs under direct sunlight

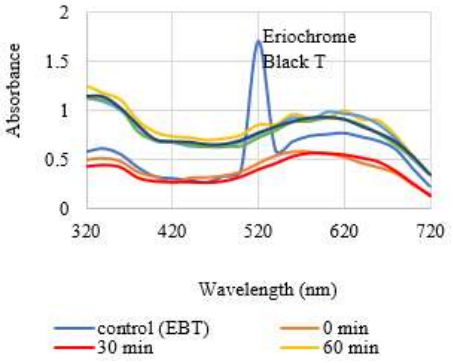

Figure 14. Photocatalytic activity of 4000 ppm WAgNPs under direct sunlight.

Eriochrome Black T dye degraded within 30 minutes in the presence of 100 ppm WAgNPs and 4000 ppm WAgNPs.

Evaluation of the antibacterial activity Escherichia coli

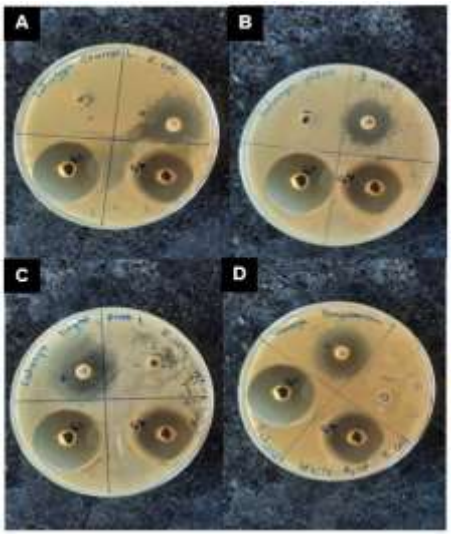

Figure 15. Antibacterial activity of (A) orange (B) yellow (C) white and (D) WAgNPs against Escherichia coli

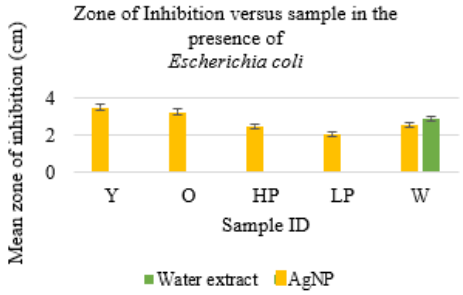

Figure 16. Zone of Inhibitions produced by water extracts and WAgNPs against Escherichia coli.

The ZOI is significantly larger in WAgNPs than in W water extract. Y and O water extracts exhibited a larger ZOI than other water extracts.

Table 10. Two-sample T-test for WAgNPs vs W water extract against Escherichia coli

t Stat	-0.295819792
P(T<=t) two-tail	0.816897113
t Critical two-tail	12.70620474

No significant difference in ZOI between W water extract and WAgNPs against Escherichia coli.

Staphylococcus aureus

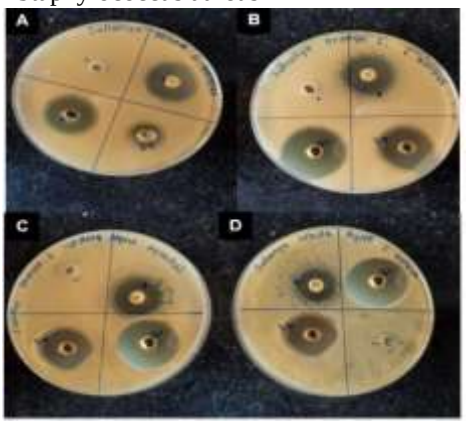

Figure 17. Antibacterial activity of (A) orange (B) yellow (C) white and (D) WAgNPs against Staphylococcus aureus

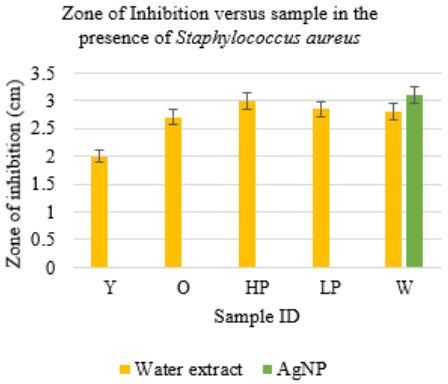

Figure 18. ZOI produced by water extracts and WAgNPs against Staphylococcus aureus.

No significant difference in ZOI between WAgNPs and W water extract. Y water extract exhibited the smallest ZOI among all samples.

Table 11. Two-sample T-test for WAgNPs vs water extracts against Staphylococcus aureus.

t Stat	-2.347006799
P(T<=t) two-tail	0.051313022
t Critical two-tail	2.364624252

Table 12. Two-sample T-test for antibacterial activity between all Escherichia coli and Staphylococcus aureus samples.

t Stat	0.238976763
P(T<=t) two-tail	0.813335767
t Critical two-tail	2.073873068

Statistical analysis revealed no significant difference in ZOI between WAgNPs and W water extract in the presence of Staphylococcus aureus. Insignificant difference between all samples of Escherichia coli and Staphylococcus aureus is observed.

DISCUSSION

Advancements in numerous scientific sectors have led to the world's globalization. One major way that this advancement has been made possible is through the green synthesis of AgNPs whilst decreasing energy use and by means of renewable materials (Rauwel et al., 2015). AgNPs are in high demand due to their antioxidant, photocatalytic, and antibacterial activities (Sriramulu and Sumathi, 2017). The purpose of using Lilium spp. in this research is to learn more about their importance in the synthesis of AgNPs than being merely used as cut flowers in floral displays. The characteristics of synthesized AgNPs are investigated in this study utilizing aqueous extracts of 5 varieties of Lilium flowers. As an alternative to organic solvent extraction, water extraction has been used in this study to minimize environmental hazards.

Phytochemicals and other biologically active molecules play a crucial role in the biosynthesis of AgNPs. Hence, the presence of phytochemicals in each water extract was studied (Table 02). Additionally, other secondary metabolites including flavonoids and phenols have also been known to contribute to the reduction of Ag+ into Ag0 (Shaikh and Patil, 2020). After optimization at RT, W sample produced a peak absorbance at 460 nm (Figure 07), signifying the synthesis of AgNPs (WAgNPs). W sample was found to be heat-sensitive since the bio-reduction of AgNPs by secondary metabolites was favored at low temperatures and AgNPs formation was absent when heated to higher temperatures (Table 03). Although no available literature on synthesis of AgNPs using species of Lilium are available, studies on species of flowers closely related to lilies like fritillaria revealed the successful synthesis of AgNPs at RT. Hence, the correct temperature is critical for efficacious AgNP synthesis (Akintelu, Bo and Folorunso, 2020). However, the other four varieties did not synthesize AgNPs even at RT. This could be due to relatively lesser concentrations of secondary metabolites in the sample resulting in lower reducing capability (Singh et al., 2020). After optimization at RT, intensification of sample color was also observed (Figure 06) owing to a phenomenon termed surface plasmon resonance (SPR). SPR is the resonant oscillation of excited electrons on the AgNPs' outer surface under the influence of specific wavelengths of light (Smiechowicz, Niekraszewicz and Kulpinski, 2021). Furthermore, TEM analysis on WAgNPs revealed spherical AgNPs with an average diameter of 20 nm (Figure 08).

Optical studies were performed on WAgNPs to determine its bandgap. Bandgap defines the variance in energy between the lowest conduction band and the highest valence band (Dittmer et al., 2019). Upon reaching bandgap energy, excited electrons engage in conductivity. Based on bandgap energy value, nanoparticles can either be categorized as insulators (>4eV) or semiconductors (<3eV). Synthesized WAgNPs were found to be semiconductors (Table 13).

The equation below was used to calculate the bandgap energy of WAgNPs (Figure 19):

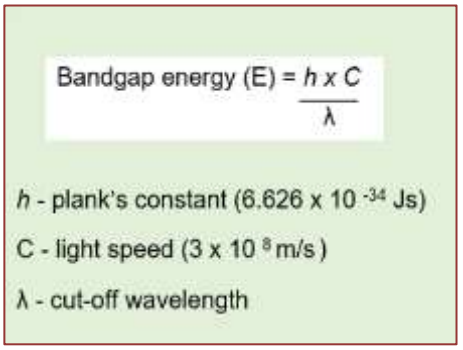

Figure 19. Equation for calculating bandgap energy (Shao, Lin and Shao, 2020).

Table 13. Bandgap energy for WAgNPs.

AgNP sample	Bandgap energy (E)	Classification
White lily	2.954 eV	Semiconductor

Antioxidant assays were performed to evaluate the antioxidant activities of water extracts and WAgNPs. The TFC was measured using the aluminum chloride colorimetric method, which works on the principle that AlCl3 binds to hydroxyl groups in C3 and C5 and C4 keto groups of flavones and flavanols to yield acid-stable complexes (Figure 20) (Ahmed, 2018). Single-factor ANOVA revealed that all water extracts had significantly different flavonoid concentrations as evident by F value > F crit (Table 04 and 14). WAgNPs have the highest concentration of flavonoids among all the samples assessed (Figure 09) including W water extract (Table 05 and 15). Highest flavonoid content in AgNPs compared to water extract was also observed in Aloe barbadensis; a species closely related to Lilium (Sohal et al., 2019).

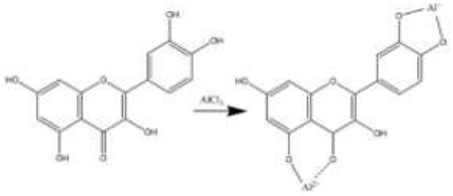

Figure 20. Interaction of AlCl3 with flavonoids (Ilmi, Elya and Handayani, 2020).

For TPC evaluation, Folin-Ciocalteu assay was performed. A redox reaction occurs between phosphotungstates and phosphomolybdates of Folin-Ciocalteu reagent and polyphenols in the sample resulting in a blue chromophore formed by phosphotungstic-phosphomolybdenum complexes under alkaline conditions. Higher phenolic content in the sample produces an increased intensity of blue due to the progressive formation of chromophores (Blainski, Lopes and De Mello, 2013).

The highest phenolic content was observed in WAgNPs with approximately 3-fold greater phenol concentration compared to all water extracts (Figure 10). No significant difference in phenolic content was observed between water extracts as F value < F crit (Table 06 and 14) despite a significant difference in the concentration of phenols between W water extract and WAgNPs (Table 07 and 15). A study on phylogenetically related Allium sativum also revealed higher concentration of phenols in AgNPs relative to their corresponding water extracts (Abbas et al, 2022).

For TAC evaluation, phosphomolybdenum assay was performed. At acidic pH, antioxidants in the sample reduce Phosphate-Mo (VI) to Phosphate-Mo (V) resulting in the development of a blue-green complex (Figure 21) (Khan et al., 2012). There was no significant difference in the amounts of antioxidants between water extracts as F value < F crit (Table 08 and 14). However, significant difference was observed between WAgNPs and its water extract (Table 09 and 15). As expected, WAgNPs had the highest amounts of antioxidants with 6-fold more antioxidants compared to all water extracts (Figure 11). This supports a study in which AgNP synthesis using Hyacinthus orientalis yielded AgNPs with higher antioxidants than the flower extracts (Bunghez et al., 2011). Hence, the above antioxidant assays confirm that WAgNPs have more antioxidants than plant extracts.

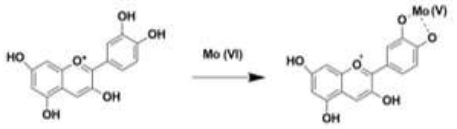

Figure 21. Reduction of Phosphate-Mo (VI) by antioxidants in the sample (Fowsiya and Madhumitha, 2017)

Table 14. Single-factor ANOVA for TFC, TPC, and TAC

Antioxidant assay	Result	Interpretation
TFC	158.268 > 3.478 F value > F crit	significant difference
TPC	1.604 < 3.478 F value < F crit	non-significant difference
TAC	0.412 < 3.478 F value < F crit	non-significant difference

Table 15. Two-sample T-test for TFC, TPC and TAC

Antioxidant assay	Result	Interpretation
TFC	2.01808E-08 < 0.05 P value < 0.05	significant difference
TPC	4.88704E-20 < 0.05 P value < 0.05	significant difference
TAC	0.021231 < 0.05 P value < 0.05	significant difference

The Pearson correlation coefficient graph demonstrates the correlation between TFC, TPC and TAC (Figure 22). A strong positive correlation between TPC and TAC (R2 =0.999) suggests that phenolic compounds contribute to a majority of antioxidant properties of the flower extracts. A moderate positive correlation between TFC-TAC (R2=0.754) and TFC-TPC (R2=0.757) was also observed. Similar findings were observed by Jin et al in which phenolic compounds were the primary contributor to antioxidant property (Jin et al., 2012).

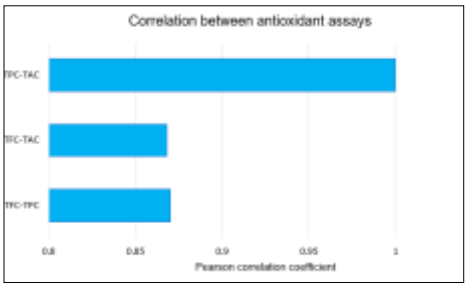

Figure 22. Pearson correlation between antioxidant assays

The ability of Lilium water extracts and WAgNPs to scavenge free radicals was verified by DPPH radical scavenging assay since they can donate hydrogen atoms or electrons to DPPH radicals (Rahman et al., 2015). The color change from violet of the DPPH radical to yellow of the stabilized DPPH permits spectrophotometric evaluation of antioxidant activity. (Gangwar et al., 2014). In the current study, the %DPPH activity of WAgNPs was the highest relative to all water extracts (Figure 12).

Accumulation of azo dyes and the inability to easily break their azo bonds poses serious environmental threats endangering the aquatic ecosystems. Degradation of such dyes by chemical methods generate byproducts that are toxic themselves and can be prevented by the utilization of AgNPs (Pinheirro, 2022). When UV-Visible light reaches the surface of AgNPs with a bandgap equal to or less than the bandgap of AgNPs, electrons in the valence band are excited to the conduction band and oscillates collectively producing a SPR effect (Figure 23). AgNPs can create ‘holes’ in the valence band as a result of electron transfer which tries to recoup the electron shortfall either from the dye or from surrounding water molecules that are adsorbed after losing the electron. This

interaction leads to the formation of hydroxyl and superoxide radicals which in turn break down the azo bonds of azo dyes (Kolya et al., 2015).

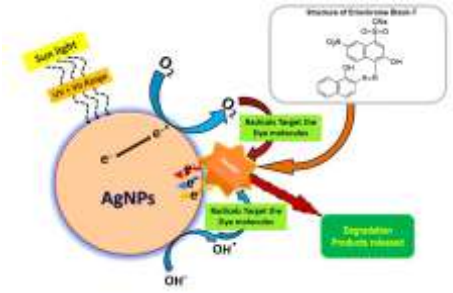

Figure 23. Photocatalytic degradation of azo dyes using AgNPs (Marimuthu et al., 2020)

The photocatalytic activity of WAgNPs was measured by degrading Eriochrome Black-T (EBT) under direct sunlight using 100 ppm WAgNPs and 4000 ppm WAgNPs. The rate constants for 100 ppm WAgNPs and 4000 ppm WAgNPs were calculated using the equation below (Figure 24).

$$\ln \frac{C}{C_0} = kt$$

C=concentration

C_0=initial concentration

k=rate constant

t=time

Figure 24. Equation to calculate the rate constant for photocatalytic degradation of azo dyes (Derbalah et al., 2019)

The highest absorption peak was observed at 520 nm for EBT and decreased within 30 minutes, depicting the dye's degradation by WAgNPs without the use of a catalyst (Figure 13 and 14). The rate constant for 100 ppm and 4000 ppm WAgNPs were 0.0003 and 0.0041 respectively (Figure 25). This indicates that photocatalytic degradation of EBT was more rapid and optimal using 4000 ppm WAgNPs than 100 ppm WAgNPs due to the higher concentration of WAgNPs and large amounts of capping agents required to stabilize WAgNPs (Rani et al., 2020). The semiconducting property (Table 13) of WAgNPs justifies their photocatalytic performance in the degradation of organic dyes (Vella et al., 2021).

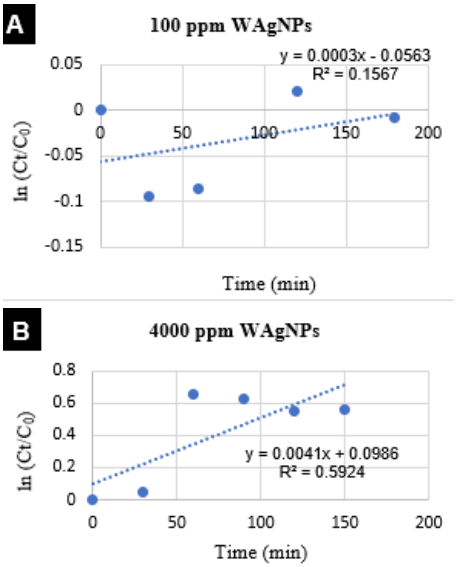

Figure 25. Kinetic graphs for (A) 100 ppm WAgNPs (B) 4000 ppm WAgNPs' photocatalytic activity

Although the exact mechanism of how AgNPs can destroy bacteria remains uncertain, several mechanisms have been proposed over the years. One such mechanism depicts a constant release of silver ions by AgNPs and their binding to bacterial cell walls through electrostatic attractions with sulfur proteins, allowing silver ions to penetrate the cell and cytoplasmic membranes (Wang, Hu and Shao, 2017). In the cytoplasm, the

function of respiratory enzymes can be disrupted by producing reactive oxygen species (ROS) which inhibit ATP production after active uptake of silver ions into bacterial cells (Figure 26). The association of silver ions with sulfur and phosphate groups in DNA can result in DNA replication abnormalities and problems in bacterial binary fission (Qing et al., 2018).

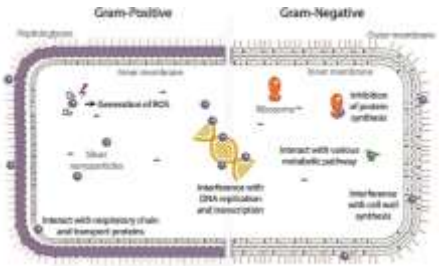

Figure 26. Mechanisms within a bacterium after AgNP interaction (Ahmad et al., 2020)

Studies have indicated that gram-negative bacteria are more prone to destruction by AgNPs than gram-positive bacteria. This is due to the walls of gram-negative bacteria being relatively thin which contributes to higher diffusion rates of AgNPs into their cytoplasm (Slavin et al., 2017). This has also been observed by Wang and Du in other species of Lilium (Luo et al., 2018). However, this is in contrast to the findings of this study as ZOI produced by AgNPs against Escherichia coli is not significantly higher than ZOI produced by AgNPs against Staphylococcus aureus (Figure 27). Additionally, ZOI produced by WAgNPs against both Escherichia coli and Staphylococcus aureus were not significantly greater than the ZOI produced by W water extracts (Figure 16 and 18) (Table 10 and 11) and was there no significant difference between all samples of Escherichia coli and Staphylococcus aureus (Table 12). This observation emphasizes the need to investigate further.

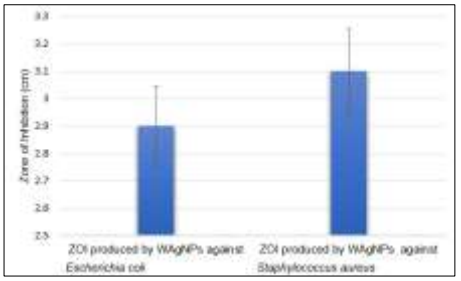

Figure 27. Zone of Inhibitions produced by WAgNPs against the bacteria

CONCLUSION

In conclusion, AgNP synthesis was successful at RT using aqueous extracts of white trumpet Lilium spp. TEM analysis on this species confirmed the presence of spherical AgNPs with average diameter of 20 nm. AgNPs synthesized by this species performed the best in all antioxidant assays. Despite the insignificant difference of ZOI exhibited by AgNPs against Escherichia coli and Staphylococcus aureus, both WAgNPs and water extracts exhibited good ZOI of approximately 2 cm against both microbes. Degradation of EBT by synthesized WAgNPs semiconductors was successful and 4000 ppm achieved a higher rate constant. Hence, green synthesis of AgNP from white trumpet lilies could be used to facilitate the creation of a greener environment and lead a better quality of life.

Future studies based on this research could incorporate the optimization of AgNP biosynthesis by varying concentrations of AgNO3, different concentrations of plant extract, reaction time and pH along with nanoparticle synthesis using Lilium spp. collected from different regions of Sri Lanka as physical and chemical properties of the soil, water and air affect the content of phytochemicals available within the plant.

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