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MICROWAVE ASSISTED ECOFRIENDLY SILVER NANOPARTICLE SYNTHESIS USING SIX VARIETIES OF CAPSICUM ANNUUM: EVALUATION OF ANTIOXIDANT, ANTIBACTERIAL AND PHOTOCATALYTIC PROPERTIES

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ABSTRACT

Recent advancements of nanotechnology have given rise to nanomaterial generation which plays a major role in medicine. Silver nanoparticles (AgNPs) have gained great importance due to their high antioxidant, antimicrobial, non-toxic nature and biological functionality which make them ideal for biomedical applications. In this study AgNPs were synthesized using six varieties of Capsicum annuum (C.annuum), to assess antioxidant, antibacterial and photocatalytic properties. The synthesized AgNPs were characterized using, UV-vis spectrum which shows a Plasmon resonance peak between 400 – 450 nm. Higher antioxidant properties were observed in AgNPs than in water extracts. Antibacterial properties were assessed against Escherichia coli and Staphylococcus aureus showed no significant difference between AgNPs and water extracts. Photocatalytic activity of Galkiriyagama AgNPs was assessed using methylene blue, showed that 6,350 ppm and 425 ppm AgNPs can degrade dye molecules completely within 30 and 160 minutes respectively. Thereby, green synthesized AgNPs from C.annuum varieties could be used in medical research to reduce free radical induced diseases, antibacterial resistance as well as to have a better environment without poisonous dyes.

Keywords: Nanotechnology, Green synthesis, Chili, Assays, Photocatalysts

INTRODUCTION

Nanotechnology is understanding and control of matter at dimensions of roughly 1-100 nm, where unique phenomena enable novel applications in diverse areas such as medicine, energy, electronics, food, manufacturing, smart cities, etc. (Shahi et al., 2018). Since Nobel Richard P. Feynman presented nanotechnology from his legendry 1959 lecture as "There's Plenty of Room at the Bottom", various revolutionary researches have been made in the nanotechnology field (Khan, Saeed and Khan, 2019). Based on the properties of nanoparticles, they can be categorized as metal nanoparticles, polymeric nanoparticles and ceramic nanoparticles. Since researchers found out the uniqueness of the physiochemical properties of nanoparticles depend on the high surface area, it was realized that the applications of these nanoparticles seem to be exciting (Laurent et al., 2010).

Among various types of nanoparticles, metal nanoparticles have seized the attention of the researchers due to their unique dynamic properties which do not exist in their bulk form and are determined by their size, shape, structure and composition (Paul and Yadav, 2015). Among metal nanoparticles, Silver nanoparticles (AgNPs) have gained great importance due to their physicochemical properties like high antimicrobial efficiency, antioxidant properties,

biological functionality and non-toxic nature which give rise to wide-ranging applications (Burdușel et al., 2018).

There are two synthesis approaches of metallic nanoparticle synthesis: top-down and bottom-up involving physical, chemical and biological methods. Many physical and chemical methods have been explored for AgNP synthesis like gamma irradiation, laser ablation, evaporation-condensation, lithography and micro-emulsion techniques, UV-initiated photoreduction, etc. (Guzel and Erdal, 2018). Even though the production rate of nanoparticles using physical and chemical methods is more rapid, these methods are expensive and involve high pressure, high temperature and toxic chemicals which make these methods non-ecofriendly. (Shahi et al., 2018; Patra and Baek, 2014). Whereas, biological methods involve nanoparticle synthesis utilizing biological components such as bacteria, fungi and plant extracts. The use of plant extracts to synthesize nanoparticles which is known as green synthesis appears to be the very promising biological method of nanoparticle synthesis due to the absence of pathogenicity (Pantidos and Horsfall, 2014). Additionally, green synthesis is a cost-effective, rapid, simple, stable and eco-friendly method (Singh et al, 2016). Though, several high heat and low heat AgNP biosynthesis methods are available, in the current study microwave assisted AgNP synthesis was carried out.

Recently metal nanoparticle synthesis has been done using plant extracts of different parts of plants such as leaves, flowers, seeds, fruits and barks. These extracts provide phenols, proteins, vitamins and polysaccharides which act as stabilizing and reducing agents (Resendez et al., 2013). Many researches have been conducted using different parts of Capsicum annuum (C.annuum) and have shown that it is appropriate to use in the green synthesis due to its powerful reducing activity (Kachhwaha, 2014; Jha and Prasad, 2011).

C.annuum is the domesticated species of genus Capsicum of family Solanaceae which is commonly known as peppers that have been a common spice all over the world. It is an essential agricultural crop, not only because of its economic importance but also for the numerous medicinal and nutritional values (Iranbakhsh et al., 2018). Researchers have found out that capsaicin and other phenolic compounds that could be isolated from the chili peppers has valuable properties such as, antibacterial, antioxidant and anticarcinogenic (Baytak and Aslanoglu, 2017).

In Sri Lanka, chili is cultivated in intermediate and dry areas such as Anuradhapura, Vavuniya, Puttalama, Kurunagala, Monaragala, Hamabanthota and Ampara. During Maha 2016/17 project, the Field Crops Research and Development Institute (FCRDI), Mahailluppallama, Sri Lanka has grown more than 10 accessions of C.annuum under greenhouse conditions in experimental fields to produce high yields of the crop as well as to combat the major diseases and pest outbreaks (Kumari et al., 2019).

Antioxidants are molecules which stable enough to donate electrons to rampaging free radicals and neutralize those. Free radicals have unpaired electrons which lead to taking electrons from stabilized molecules. The imbalance between antioxidants and free radicals gives rise to many chronic health complications (Salgado et al., 2019). Though, the effectiveness of natural or synthetic antioxidants is restricted due to their poor absorption, degradation during delivery and difficulties to cross the cell membranes. To address the above-mentioned issue, antioxidants are covalently linked with nanoparticles to provide biocompatibility, better stability and targeted delivery of the antioxidants

(Khalil et al., 2019). Hence, AgNPs which have been green synthesized using C.annuum have shown high antioxidant properties in many researches (Iranbakhsh et al., 2018; Samrot, Shobana and Jenna, 2018; Abraham et al., 2016).

As a result of the increased resistance of microorganisms against antibiotics that use in the health care sector, there is a need to develop efficient and novel antibacterial agents. It has been found out that size, shape, charge and surface are the factors of AgNPs which give rise to antibacterial properties. However, the mechanism of action is not yet clearly understood. C.annum has proved to be a good antibacterial agent against pathogenic microorganisms such as Escherichia coli, Candida albicans, Staphylococcus aureus, Streptococcus faecails, and Pseudomonas aeruginosa, etc. (Samrot, Shobana and Jenna, 2018; Otunola, et al., 2017). It has been found out that capsaicin, which is the active compound of Capsicum and capsaicinoids are the compounds which show the antibacterial properties (Marini et al., 2015).

As a result of the industrial revolution, many hazardous organic compounds especially azo dyes and industrial effluents have been directly discarded into the ecosystem over many years. Since the mutagenic, toxic and carcinogenic characteristics of these pollutants many methods like ozonization, ion exchange, flocculation and UV radiation, etc. have been utilized to remove them. However, recently usage of metal NPs like AgNPs as photocatalysts for the degradation of poisonous dyes like methylene blue (MB), Methyl Orange, Rhodamine B and Erichrome Black T, etc. have gained attention due to its low cost, abundance, high oxidative properties, high photostability as well as a simple, rapid and effective mechanism of degradation which, give rise to remediation of the ecosystem (Sharma et al., 2015).

Hence, the aim of this project was to synthesize the AgNPs using water extracts from six varieties of C. annum (MI-02, MI-02, MI-Waraniya, Galkiriyagama, MICH3 and CA8) which are the experimental accessions in Sri Lanka and to assess antioxidant, antibacterial and photocatalytic properties. To evaluate antioxidant properties, Ferric Reducing Antioxidant Power (FRAP), DPPH radical scavenging assay, total antioxidant content (TAC), total phenolic content (TPC) and total flavonoid content (TFC) will be performed. Well diffusion technique was performed to analyze antibacterial properties of samples with Escherichia coli (E.coli) and Staphylococcus aureus (S.aureus). The photocatalytic property of AgNPs was evaluated using MB. Thereby, these compounds could be used in medical research to reduce free radical induced diseases, antibacterial resistance as well as to have a better environment without poisonous dyes.

METHODOLOGY

Sample collection

Six varieties of C.annuum L. dried seed samples; MI-01, MI-02, MI-Waraniya, Galkiriyagama, MICH3 and CA8 were collected from FCRDI, Mahailupallama, Sri Lanka (8°06'42.4"N 80°28'01.2"E) and Department of Agriculture, Seed Division, Gannoruwa, Sri Lanka (7°20'17.7"N 80°36'41.9"E).

Sample preparation.

Samples were prepared by adding a 4 g of crushed C.annuum seed sample with 30 mL of distilled water into beakers separately and boiled at 90 ℃ for 5 minutes The water extracts were filtered first with a muslin cloth and then with Whatman No. 1 filter papers. The water extracts were stored at 4 ℃ until further use (Singh et al., 2016).

Silver nanoparticle synthesis and optimization.

20 mL of 1mM AgNO3 was mixed with 1 mL of the water extract in separate beakers. These solutions were then microwaved at MEDIUM power (511 W) for 5.5 minutes discontinuously. The absorbance was measured from 300-500 nm using distilled water as the blank using a UV-vis spectrophotometer (Perera and Kandiah, 2018). All six samples were prepared as aforementioned and optimized at MEDIUM power (511 W) for 2.5 minutes and 4 minutes. Absorbance was measured for each optimization. Diluted samples were prepared by adding 14 mL of distilled water to 1 mL of each extract and AgNps separately. The diluted samples were stored at 4 ℃ until further use.

Qualitative phytochemical analysis.

Qualitative phytochemical analysis was performed for diluted water extracts according to Roghini and Vijayalakshmi 2018, to identify secondary metabolites.

Following assays were performed using diluted water extracts and AgNPs.

Determination of Total Flavonoid Content (TFC)

1.5 mL of sample and 1.5 mL of 2 % w/v AlCl3 was mixed and incubated for 10 minutes at room temperature. Absorbance was measured at 415 nm in triplicates using distilled water as the blank. The concentration was calculated using Quercetin equivalence (QE) (mg QE / 100g) (Perera and Kandiah, 2018).

Determination of Total Phenolic Content (TPC)

1.6 mL of Na2CO3 and 2 mL of 10% Folin Ciocalteu reagent were mixed with 400 µL of sample and incubated for 1 hour at room temperature. Absorbance was measured at 765nm in triplicates using distilled water as the blank. The concentration was calculated using Gallic acid equivalence (GAE) (g GAE / 100 g) (Perera and Kandiah, 2018).

Determination of Total Antioxidant Content (TAC)

3 mL of sample was mixed with 1 mL of a reagent which was prepared by adding 28 mM Na2SO4, 4mM ([NH4]6Mo7O24.4H2O) and 0.6M H2SO4 (1:1:1) and incubated for 90 minutes at 90 ℃. Absorbance was measured at 695 nm in triplicates using distilled water as the blank. The concentration was calculated using Ascorbic acid equivalence (AAE) (mg AAE / 100 g) (Perera and Kandiah, 2018).

Ferric Reducing Antioxidant Properties (FRAP) assay

Frap reagent was prepared by mixing freshly prepared stock solutions, acetated buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20mM FeCl3.6H2O in a 10:1:1 ratio. 100 µL of the sample was mixed with 2.9 mL of FRAP reagent in a cuvette. Absorbance was measured of each sample at 593 nm per minute time (Singh et al., 2016).

DPPH assay

0.004 g of DPPH was dissolved in 100 mL of methanol. 1 mL of diluted sample was mixed with 2 mL of DPPH solution and kept for 30 minutes incubation in the dark. Absorbance was measured at 517 nm using methanol as the blank (Perera and Kandiah, 2018).

Photocatalytic activity

Galikiriyagama AgNP sample was selected to perform the photocatalytic activity. 1 mL of 0.2 M NaBH4 and 1 mL of 2 mM MB was diluted in 198 mL of distilled water. 1 mL of 6,350 ppm Galkiriyagama AgNPs were added into 100 mL of the above solution and absorbance was measured for 30 minutes. The same procedure was performed for 425 ppm Galkiriyagama AgNPs and absorbance was measured for 160 minutes (Sharma et al., 2015).

Median Inhibition Concentration (IC50)

For 1 mL of series of six concentrations (100%, 80%, 60%, 40%, 20% and 10%), 2 mL of 0.004% DPPH solution was added. Absorbance was measured at 734 nm using methanol as the blank (Perera and Kandiah, 2018).

Well diffusion method for S.aureus and E.coli

Muller-Hinton agar was poured into sterilized, labeled Petri plates under the fume hood and left to solidify. After solidification inoculated saline solutions with S.aureus and E.coli were swabbed on agar and wells were prepared separately. Duplicates of each sample (S1 and S2) and negative control were loaded into wells. Gentamycin was used as the positive control. The Petri dishes were incubated overnight at 37 ℃ and the zone of inhibition was measured using a ruler (Perera and Kandiah, 2018).

Statistical analysis

Using Microsoft® Excel 2013 software, ONE-way ANOVA tables were generated.

Scanning electron microscopy (SEM)

SEM analysis was carried out at Sri Lankan Institute of Nanotechnology (SLINTEC), Homagama, using the Hitachi SU6600 SEM.

RESULTS

Qualitative phytochemical analysis.

Table 1: Phytochemical test results.

Test	MI-01	MI-02	MI-Waraniya	Galkiriyagama	MICH3	CA8
Carbohydrates	✓	✓	✓	✓	✓	✓
Tannins	×	×	×	×	×	×
Saponins	×	×	×	×	×	×
Terpenoids	✓	✓	✓	✓	✓	✓
Anthraquinone	×	×	×	×	×	×
Steroids	×	×	×	×	×	×
proteins	✓	✓	✓	✓	✓	✓

Silver Nanoparticle synthesis.

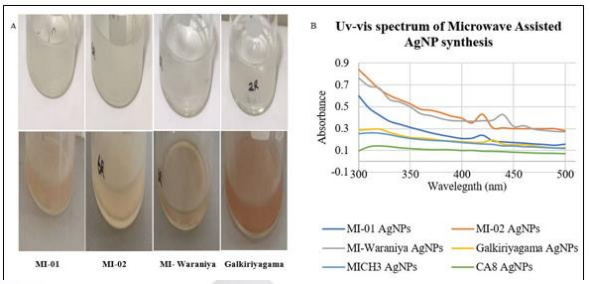


Figure 01: AgNP synthesis A) Colour change (Top) Water extracts with AgNO3 solution before heating (Bottom) after heating. B) UV-Vis spectrum of synthesized AgNPs

The colour of water extracts changed from colorless to light brown which indicates the nanoparticle formation (figure 01: A). UV-vis spectrophotometer was used to further confirmation of nanoparticle formation and results showed peaks between 400 nm and 450 nm which relates to the Plasmon resonance of AgNPs (Figure 01: B). Optimization of AgNP synthesis was performed for different time durations at MEDIUM power (511 W) (table 2). From 5.5 minutes four samples could produce AgNPs, therefore it was taken as the optimized time for further analysis.

Table 2: Optimization of Silver nanoparticles synthesis.

Sample name	2.5 minutes	4.0 minutes	5.5 minutes
MI-01	✓	✓	✓
MI-02	×	×	✓
MI-Waraniya	×	×	✓
Galkiriyagama	✓	✓	✓
MICH3	×	×	×
CA8	×	×	×

Scanning Electron Microscopic (SEM) analysis.

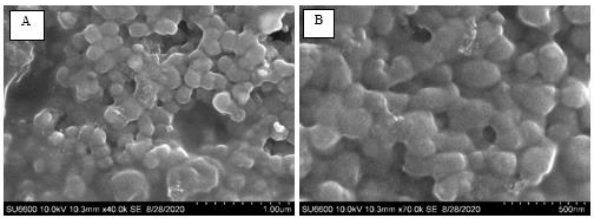


Figure 02: SEM images for Galkiriyagama AgNPs in different magnifications A) 40.0k B) 70.0k 25-75 nm AgNPs can be observed which are spherical in shape.

Total Flavonoid Content (TFC).

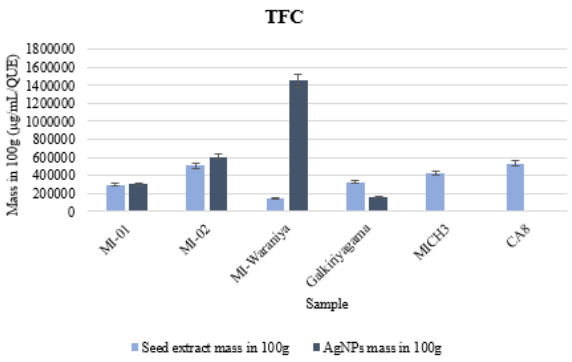


Figure 03: Total flavonoid content of water extracts and AgNPs.

Total Phenolic Content (TPC).

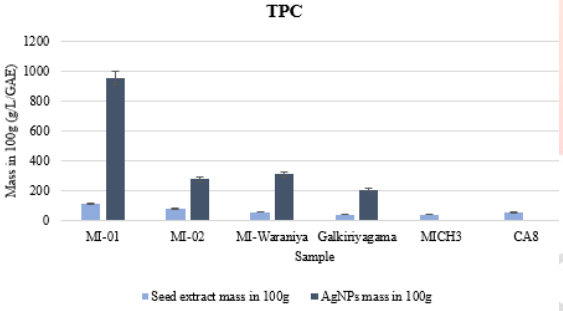


Figure 04: Total phenolic content of water extracts and AgNPs.

Total Antioxidant Content (TAC).

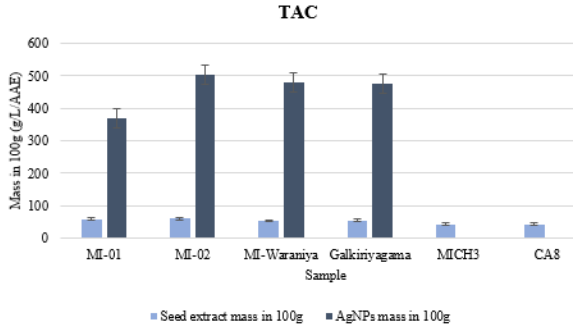
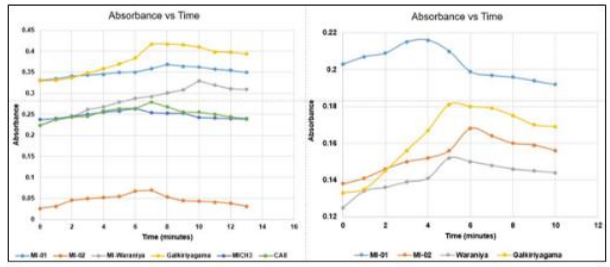


Figure 05: Total antioxidant content of water extracts and AgNPs

FRAP assay.



DPPH radical scavenging assay.

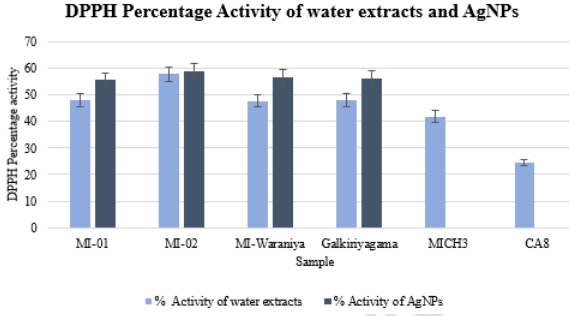


Figure 07: DPPH percentage activity of water extracts and AgNPs.

Inhibitory concentration (IC50) of DPPH

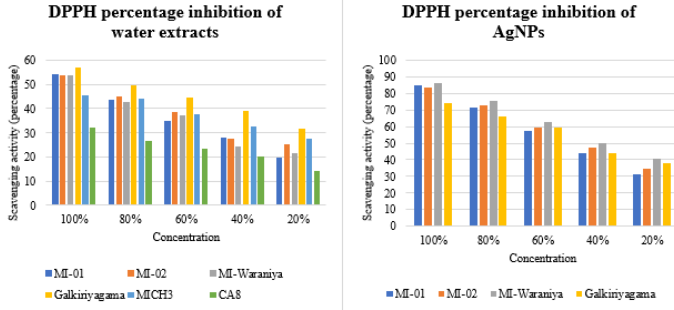


Figure 08: DPPH percentage inhibition of water extracts (left) and AgNPs (right).

Table 3: Inhibitory concentration (IC_{50}) of samples.

Sample	Extract	AgNPs
MI-01	92.54	48.58
MI-02	91.88	44.32
MI-Waraniya	93.97	38.02
Galkiriyagama	78.14	46.74

IC_{50} was lower in AgNPs which indicates the higher antioxidant capacity

Determination of photocatalytic activity.

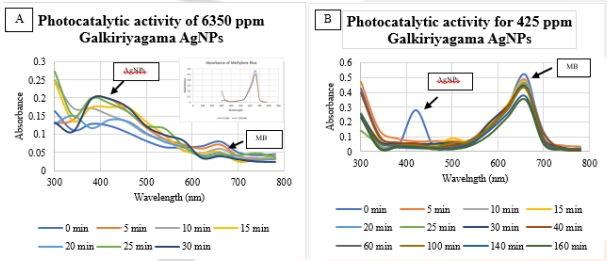


Figure 09: Photocatalytic activity of A) 6,350 ppm B) 425 ppm Galkiriyagama AgNPs.

The photocatalytic degradation of MB by 6,350 ppm and 425 ppm Galkiriyagama AgNPs has been completed within 30 minutes and 160 minutes respectively. Which indicates by the peak at 660 nm.

Antibacterial Assay.

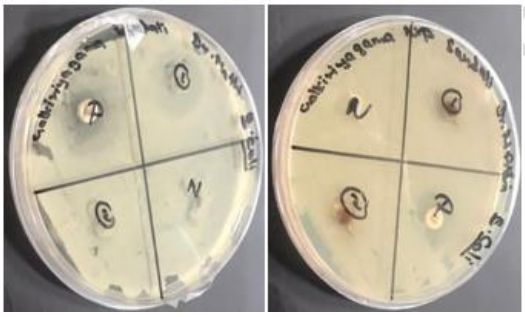
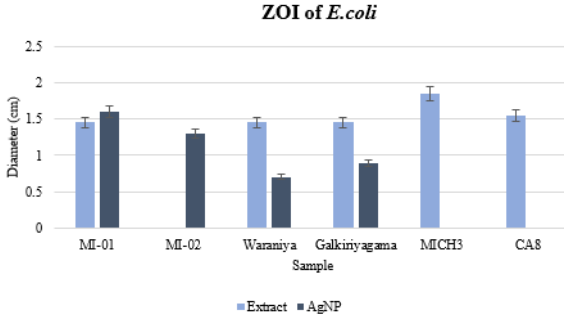


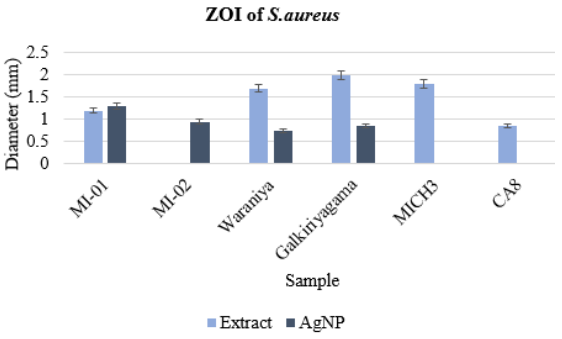
Figure 10: Zones of inhibition (ZOI) of E.coli in Galkiriyagama water extract (left) and AgNPs (right).



The ZOI of MI-01 AgNPs for E.coli was higher than its water extract.

Figure 12: Zones of inhibition (ZOI) of S.aureus in Galkiriyagama water extract (left) and AgNPs (right).

The ZOI of MI-01 AgNPs for S.aureus was higher than its water extract.



DISCUSSION

This project was carried out to synthesize AgNPs using six varieties of C.annuum and to assess antioxidant, antibacterial and photocatalytic properties. AgNPs have been utilized in many fields due to its unique physiochemical properties. Green synthesis of AgNPs has been a promising approach compared to other methods since it is ecofriendly, rapid, cost effective, comparatively reproducible, sustainable and easy to perform (Veerasamy et al., 2011). Additionally, the method which was used in the current study is scalable, biocompatible and medical applicable since the water was used as the reducing medium (Singh et al, 2016).

In the current study microwave assisted green synthesis was utilized. During the synthesis, Ag+ gets reduced to Ag0 which gets stabilized by phenolics, proteins, polysaccharides, vitamins and terpenoids which are biomolecules present within water extracts. Moreover, compared to conventional approaches, microwave assisted synthesis has been found to be a promising approach due to less energy consumptions and lesser reaction times as particle formation rate is accelerated by microwave irradiation (Perera and Kandiah, 2018; Zhao et al., 2014). The microwave assisted green synthesis of AgNPs was successful, since characteristics of AgNP formation; colour change (figure 01: A) and distinct peaks between 400 nm- 450 nm in UV-vis spectrometry that relates to the Plasmon resonance of AgNPs (figure 01: B) were observed.

Prior to the aforementioned method conventional method of AgNP green synthesis was performed, where 1 mL of seed extract with 9 mL of 1 mM AgNO3 was incubated at 90 ℃ for 60 minutes, 24 hours, 48 hours and 72 hours. However, a colour change or distinct peaks in the Uv-vis spectrum were not observed.

SEM analysis was carried out for the characterization of AgNPs. It was observed that the synthesized AgNPs were 25 – 75 nm and spherical in shape with aggregations (figure 02). Similar studies have been reported 30 – 80 nm also 50 -70 nm C.annuum AgNPs with slight aggregations in spherical shape (Samrot, Shobana and Jenna, 2018).

UV-vis absorbance spectroscopy was used to analyze the optical properties of AgNPs. The conductivity of AgNPs was analyzed by measuring the energy difference that occurs due to electron transitions between the top of the valence band and the bottom of the conduction band which is the bandgap. If the band energy, minimum energy that is required for an electron transition is > 4 eV or < 3 eV, the materials can be classified as insulators or semiconductors respectively. Hence, using Planck's equation (1), C.annuum AgNPs were identified to have a higher potential to act as semiconductors as the bandgap energy of all samples was between 2.80 eV-2.95 (Sundeep et al. 2017).

$$E=hC/\lambda$$

(E= Bandgap energy, h= Planck's constant (6.626 ×⟦10⟧^(-34) Js), C= Speed of light (3 × ⟦10⟧^8 ⟦ms⟧^(-1)), λ= Cutoff wavelength of AgNPs (410 nm – 430 nm))

To assess antioxidant properties of water extracts and synthesized AgNPs, antioxidant assays were performed. These assays have not been performed previously for C.annuum varieties used in the current study.

TFC was estimated by AlCl3 which forms acid-stable complexes with C4 keto groups and either C3 or C5 hydroxyl groups of flavonols and flavones. Additionally, it forms acid-labile structures with ortho-dihydroxyl groups in A- or B- rings of flavonoids. These complexes have the maximum absorption at 415 nm (Bhaigyabati, Devi and Bag, 2014). In the current study, overall higher

TFC was observed in AgNPs however, in Galkiriyagama, higher TFC was observed in water extracts. This could be due to degradation of flavonoids by microwave irradiation or 90 ℃ used for sample preparation or due to binding of non-flavonoid molecules to AgNPs (Jimoh, Afolayan and Lewu, 2019; Rizki et al., 2017). TFC among AgNPs and water extracts was MI-Waraniya > M-02 >MI-01> Galkiriyagama and CA8 > MI-02 > MICH3 > Galkiriyagama > MI-01 > MI-Waraniya respectively (figure 03).

TPC was estimated using folin-ciocalteu reagent that forms a stable blue complex with phenolic compounds that can absorb radiation and quantify at 765 nm (Hatami et al., 2014). Higher TPC was observed in AgNPs than in water extracts. TPC among AgNPs and water extracts was, MI-01> MI-02 = MI-Waraniya > Galkiriyagama and MI-01 > MI-02 > MI-Waraniya = CA8 > Galkiriyagama = MICH3 respectively (figure 04).

TAC was estimated by assessing the reduction of Mo (VI) into Mo (V) at acidic pH and by antioxidants which form a green phosphate/Mo (V) complex that can be measured at 695 nm (Kumar and Jain, 2015). Comparatively, higher TAC was observed in AgNPs than in water extracts. TAC among AgNPs and water extracts are MI-02 = MI-Waraniya = Galkiriyagama > MI-01 and MI-01 = MI-02 = Galkiriyagama = MI-Waraniya > MICH3 = CA8 respectively (figure 05). The Higher TFC of MI-Waraniya correlates with its higher TAC value.

According to generated ONE-way ANOVA, significance difference was observed for both TPC (p-valueTPC < 0.05 (pTPC = 0.027529) and TAC (p-valueTAC < 0.05 (pTAC = 1.48E-07) between AgNPs and water extracts. Though a significant difference was not observed for TFC as p-valueTFC > 0.05 (pTFC = 0.310932).

FRAP assay was done to measure the antioxidant potential in samples by measuring the reduction of Fe (III) to Fe (II) by antioxidants in the presence of TPTZ ligand resulting formation of a blue complex with maximum absorption at 593 nm (Sacchi et al., 2019). In FRAP assay, acetate buffer with 3.6 pH was used to produce FRAP reagent to maintain ion solubility. The reaction at low pH decreases the ion potential that derives atom transfer and increases the redox potential which is the dominant mechanism. Higher reducing power was observed in AgNPs (4-6 minutes) than in water extracts (6-10 minutes) (figure 06). A similar study has been reported higher antioxidant power in C.annuum AgNPs (Singh et al., 2016).

DPPH assay is based on stable DPPH free radicals at RT, which produces a violet solution in methanol and which gives rise to a yellow solution when get reduced by antioxidants. This is measured at 517 nm. In DPPH assay methanol or ethanol is used to keep the hydrophobic hydroxyl radicals and phenolic test compounds soluble while offering sufficient buffering capacity (Garcia et al., 2012). Higher DPPH percentage activity was observed in AgNPs than in water extracts which correlates with higher TAC in AgNPs. Among AgNPs percentage activity was equal whereas in water extracts it was MI-02 > MI-01 = MI-Waraniya = Galkiriyagama > MICH3 > CA8 (figure 07). However, another study has been reported that the percentage activity was higher in water extracts than in AgNPs (Shahi et al., 2018).

According to the literature, the lower the IC50 higher the antioxidant capacity (Cruz et al., 2020). Lower IC50 was observed in AgNPs than in water extracts indicating the higher antioxidant capacity in AgNPs (table 03). The highest antioxidant capacity of MI-Waraniya correlates with its higher TFC and TAC.

The mechanism of the degradation of MB by AgNPs; when surface electrons of AgNPs receive photons from UV-vis light, the collective oscillation of electrons from valance band to conduction band occurs due to surface Plasmon resonance effect. Oxygen gets convert into free radicals (O2●) which present in water due to the absorption of plasmonic excitation of surface electrons. Holes created in AgNPs get filled by electrons of MB dye molecules that are absorbed on to the surface of AgNP. Which leads to the oxidation of dye molecules. Moreover, generated O2● reacts with H+ ions that are produced as a result of water splitting and may result in the generation of other free radicals like OH● and HO2●. These free radicals degrade dye molecules into azo dye intermediates by breaking down complex organic structures (Singh and Dhaliwal, 2018).

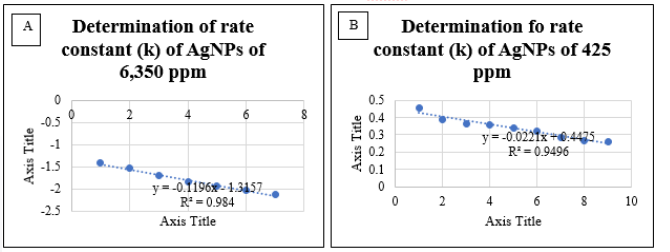
In the current study, the photocatalytic activity assay was carried out to assess the action of Galkiriyagama AgNPs in the degradation of MB. In both 6,350 ppm and 425 ppm AgNP samples, complete MB degradation was observed within 30 minutes and 160 minutes respectively (figure 09). Additionally, a reduction of AgNP peak at 410 nm was observed which indicates the precipitation of AgNPs. The rate constant of photocatalytic degradation was calculated using the rate constant equation (2),

$$\ln[C/C_0 = -kt]$$

(C = Methylene blue concentration, C_0 = Initial Methylene blue concentration, k = Rate constant and t = time).

A higher rate constant (k) was observed in 6,350 pm AgNP sample (k=0.1196) than in 425 pm AgNP sample (k=0.0221). Hence, based on the k, it can be concluded that the photocatalytic activity is highly efficient in 6,350 ppm AgNPs (Figure 14). A similar study has been reported that within 180 minutes completely degradation of MB by Nepta leucophylla 25 ppm AgNPs (Singh and Dhaliwal, 2018).

Figure 14: Determination of Rate constant (k) of AgNPs A) 6,350 pm B) 425 pm.



In the current study, well diffusion technique was performed to screen the antibacterial properties of water extracts and AgNPs against E.coli and S.aureus. Figure 11 and figure 12 show that the ZOI for water extracts are higher than the AgNPs against both E.coli and S.aureus. This could be due to plasmid or chromosomal resistance genes or negative regulation of porins which leads to AgNP resistance in bacteria (Gugala et al., 2018). However, MI-02 only shows ZOI for nanoparticles against both E.coli and S.aureus. The mechanism is not well understood. Though, Kim et al., 2011 have reported that this could be due to increased permeability of cell membrane that leads to leakage of proteins and lactate dehydrogenase (LDH) reduction occur as a result of Reactive Oxygen species (ROS) formation.

CONCLUSION

Microwave assisted AgNP synthesis using C.annuum was successful. SEM analysis on Galkiriyagama AgNPs revealed that spherical shaped 25-75 nm AgNPs were present. Higher antioxidant properties were observed in AgNPs than in water extracts. There was no significant difference between AgNPs and water extracts against E. coli and S.aureus. However, MI-02 only indicated ZOI for nanoparticles against both E. coli and

S.aureus. Galkiriyagama 6,350 ppm AgNPs have a higher ability to degrade MB dye molecules completely within 30 minutes. Thereby, green synthesized AgNPs from C.annuum varieties could be used in medical research to reduce free radical-induced diseases and to have a better environment without poisonous dyes.

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