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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING CINNAMOMUM VERUM LEAF EXTRACTS AND DETERMINATION OF THEIR ANTIOXIDANT, ANTIMICROBIAL ACTIVITY AND PHOTOCATALYTIC ACTIVITY

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ABSTRACT

With the rapid development of Nanotechnology, its applications have influenced on all sectors of human life and opened up a spectrum of research opportunities. Green synthesis of nanoparticles had gained special attention as they are ecofriendly, non-toxic, and cost effective. In this study, five varieties of Cinnamon verum leaf extracts were used for the synthesis of silver nanoparticles (AgNPs) to study its antioxidant, antimicrobial, and photocatalytic activity (Cinnamon Sri Gemunu, Cinnamon dubium, Cinnamon Sri Wijaya, Cinnamon sinharajanese and Cinnamon revulorum). All these five varieties indicated a colour change to pale brown confirming the presence of AgNPs under optimizing conditions and indicated a distinct peak from 420-480 nm when characterized in UV spectrophotometry. Scanning electron microscope (SEM) analysis resulted in spherical particles of 50 nm, and they all behaved as semiconductors. The antioxidant activity was determined by TPC, TFC, TAC, DPPH and IC50 assays. The results from these assays showed a high antioxidant activity in AgNPs compared to its water extracts. The antimicrobial activity was determined by well diffusion technique using E.coli and S.aureus and the AgNPs and water extract activity did not show a significant difference. At two different concentrations, 266 ppm and 4000 ppm, Photocatalytic activity was determined for AgNPs using methylene blue and AgNPs showed a better degradation of methylene blue at 266 ppm with and without the catalyst. Finally, all the results concluded at Cinnamon verum is a good source of nanoparticle synthesis and can be useful in medical research and to create an ecofriendly environment.

INTRODUCTION

In recent years, the study of Nanotechnology has captivated and emerged in various fields of biology, medicine, manufacturing, and engineering due to their incredible applications (Elmer and White, 2018). Nanoparticles have a dimension of 1-100 nm with unique properties due to ratio of specific size and elevated surface area to volume (Kavitha, Sujatha, and Manoharan, 2017). Among various types of nanoparticles, metal nanoparticles are immensely studied due to special characteristics such as antimicrobial properties, catalytic activity, optical properties, electronic and magnetic properties (Velusamy et al., 2016). Silver nanoparticles are synthesized and given greater importance in this study as they have high antimicrobial properties, less toxic and cost effective (Bharathi and Bhuvaneshwari, 2018).

There are two foundational principles of nanoparticles synthesis; Top-down and bottom-up approaches involving

biological, physical, and chemical methods (Figure 1). Traditionally, chemical etching, laser ablation, sputtering, lithography and ball milling, techniques have been used to synthesize metal nanoparticles. However, considering the rapid increase in the usage of nanomaterials, these procedures are not preferable as they involve high pressure, high temperature, toxic chemicals and expensive (Khandel et al., 2018). A bottom-up method called green synthesis has been introduced to overcome the limitations of these traditional methods.

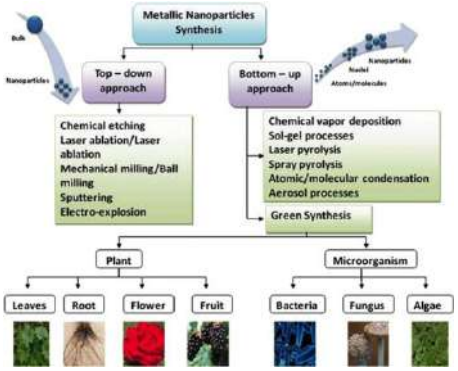


Figure 1: Different approaches of Nanoparticle synthesis (Singh et al., 2018)

The use of biological components such as microorganisms and plant extracts of different parts of the plant for nanoparticle synthesis is known as Green synthesis. This procedure avoids the production of toxic and unwanted products, cost effective, simple, and ecofriendly (Singh et al., 2018). In this study, plant extract was preferred over microorganisms due to its difficulty in growth, maintenance of culture, pathogenic toxicity, and standardization of inoculum size (Srikar et al., 2016). During green synthesis, a metal salt solution is added to the plant extract and a biochemical reduction takes place by the phytochemicals in the plant extract. In this reaction, the metal ions convert from their M+ to a M0 followed by nucleation, growth, and stabilization to form the metal nanoparticles (Figure 2).

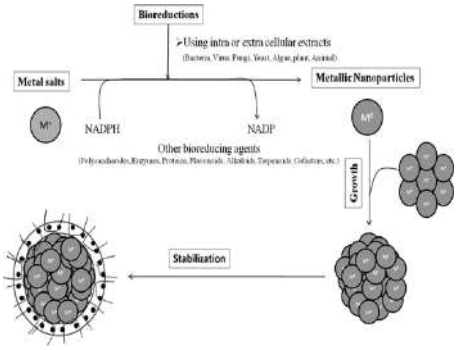


Figure 2: Mechanism of nanoparticle synthesis (Velusamy et al., 2016)

In this study, leaf extracts of Cinnamon verum were used in synthesizing nanoparticles as they have been reported as one of the major bio reactive compounds and show high antioxidant property loaded with polyphenols, high inflammatory property, high antifungal, and antibacterial activity (Ansari et al., 2020). Cinnamomum verum commonly known as true cinnamon tree, is native to Sri Lanka and belongs to the family Lauraceae. Cinnamon is the oldest known spice obtained from the Cinnamon tree bark and is popularly utilized as flavorings, as a condiment and in cooking. In Sri Lanka, different parts of cinnamon plant are mostly made use in Ayurvedic medicine as a remedy to various diseases such as diabetes, heart related diseases, and cancers (Suriyagoda et al., 2021).

Free radicals are highly unstable molecules with an unpaired electron in the outermost energy level. They lead to a reaction with stable molecules causing oxidative damage to the body. Antioxidants are stable molecules that can decrease the oxidative damage by donating electrons to free radicals and neutralizing them (Figure 3) (Hunyadi, 2019).

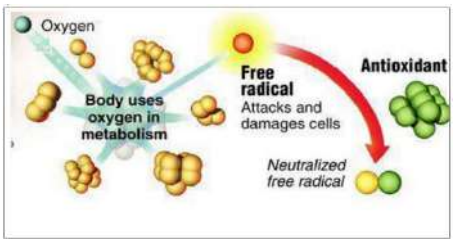


Figure 3: Mechanism of Antioxidant activity (Dottor Gabaldo, 2020)

In the human body, when there is an imbalance between antioxidants and free radicals, the free radicals generate oxidative stress by damaging cells. This causes various diseases such as neurodegenerative, cancer, inflammatory, and cardiovascular diseases (Lobo V et al., 2010). Better stability, biocompatibility, and targeted delivery to the body can be provided by nanoparticles synthesized from antioxidant rich natural sources as they are better radical scavengers (Salgado et al., 2019).

There is a need to develop antimicrobial agents due to increased resistance to microorganisms against antibiotics. Nanoparticles synthesized using plant extracts are used against pathogenic microorganisms. Researchers have proved that Cinnamon extracts is a good antimicrobial agent against Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Corynebacterium xerosis, Bacillus megaterium, Enterobacter cloacae, Pseudomonas aeruginosa, and Streptococcus faecalis by well diffusion technique (Nabavi et al., 2015). Cinnamaldehydes and eugenol are the main active compounds in cinnamon which show these antibacterial properties (Vasconcelos, Croda, Simionatto, 2018). Further, disposing hazardous organic dyes (azo dyes) such as Methylene blue (MB), Methyl orange, Methylene red and Eriochrome black is a crucial problem for many years since the presence of strong azo bonds makes them non degradable, toxic, and carcinogenic (Figure 4). Studies report that using AgNPs as photocatalysts to remove these dyes is a better alternative to common dye removing methods such as ion exchange, ozonation and UV radiation since there is no harmful byproduct formation, effective mechanism, and low cost (Sharma et al., 2015).

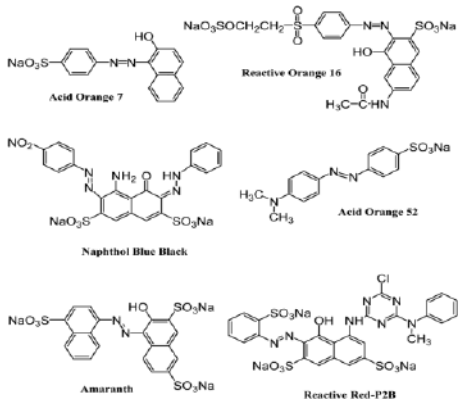


Figure 4: Examples of Azo dyes (Qurrat et al., 2020)

The objective of this research is to synthesize ecofriendly silver nanoparticles using five varieties of Cinnamon verum leaves (Sri Wijaya, sinharajanese, dubium, revolurum and Gemunu) and to determine their antioxidant, antimicrobial, and photocatalytic activity. The antioxidant activity will be studied by Total Flavonoid Content (TFC), Total Phenolic Content (TPC) and Total Antioxidant Content (TAC) assays. Well diffusion techniques will be performed to determine the antimicrobial activity against a Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. The photocatalytic activity will be studied by the removing methylene blue dye. By achieving these goals, the AgNPs can be a useful tool in medical research to minimize diseases caused by free radicals, antibacterial resistance and to make a hazardous azo dyes free environment.

MATERIALS AND METHODOLOGY

Materials

Sample Collection

Samples of Cinnamon verum leaf were collected from the Cinnamon Research Institute situated in Matara, Sri Lanka. The five varieties of leaves used in this study are Sri Gemunu (G), dubium (D), Sri Wijaya (W), sinharajanse (S) and revulorum (N) (Figure 5).

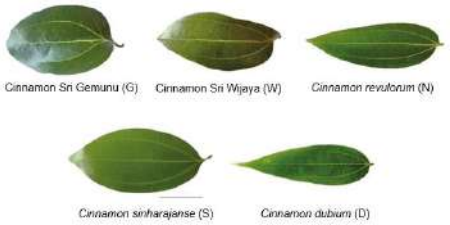


Figure 5: The five varieties of Cinnamon leaf samples (Senaratne and Pathirana, 2020)

Chemical and Reagents

Acetic acid (CH3COOH), Aluminium chloride (AlCl3), Ammonium hydroxide (NH4OH), Ammonium molybdate ([NH4]6 Mo7O24.4H2O), Chloroform (CH3Cl), Copper sulphate (CuSO4), 2, 2-diphenyl-1- picrylhydrazyl (DPPH), Ethanol (C2H5OH), Ferric chloride (FeCl3), Folin–Ciocalteu reagent, Methanol (CH3OH), Hydrochloric acid (HCl), Methyl blue, Millon's reagent, Molisch's reagent, Mueller-Hinton agar powder, Ninhydrin solution, saline, Silver nitrate (AgNO3), Sodium borohydride (NaBH4), Sodium carbonate (Na2CO3), Sodium hydroxide (NaOH), Sodium nitrate (NaNO3), Sodium sulphate (Na2SO4) and Sulphuric acid (H2SO4).

Apparatus

Fume hood, spectrophotometer, analytical weighing scale, refrigerator, micropipettes, hot air oven, incubator, water bath, and autoclave.

Consumables

Micropipettes (100µL, 1000µL) Glass beakers (50 mL, 100 mL and 250mL), conical flasks (250 mL), measuring cylinders, Whatman no.1 filter paper, falcon tubes (15 mL and 50 mL), watch glasses, spatula, mortar and pestle, gauze, sterile pipette tips, cuvettes, aluminum foil, plastic petri plates, cotton swabs, test tubes, boiling tubes, and filter funnels.

Methodology

Good lab practice and the use of personal protective equipment was followed throughout all experiments. COSHH and bioCOSHH forms were submitted before beginning this study.

Sample Preparation

The samples were air dried for 5 days until all the moisture was removed and cut into fine pieces. 2 g of each type of cut leaves were placed separate beakers. Then distilled water of 50 mL was added to each beaker and incubated for 30 minutes at 60 °C while keeping the beaker mouth covered with foil. Whatman filter paper No. 1 was used in filtering the extract using a funnel into falcon tubes of 50mL. The extracts were labelled and stored until further use at 40C.

Synthesis of silver nanoparticles

Leaf extract of 1 mL from each type was mixed with 9 mL of 1 mM silver nitrate solution. These solutions were then covered separately in foil and optimized at 900C, 600C for 15 minutes, 30 minutes, 45 minutes, and 60 minutes, with one set left at room temperature for 24 hours. From 320- 500 nm, the absorbances were measured using distilled water as a blank.

Phytochemical analysis

The phytochemical tests were performed according to the Table 1.

Table 1: Methodology of the phytochemical screening tests.

Phytochemical	Methodology	Expected positive color	Reference
Carbohydrates	0.5 ml extract was treated with 0.25 ml of Molisch's reagent and few drops of conc H_2SO_4 were added	Purple color formation	Banu and Catherine, 2015
Amino acids	0.5 ml extract was treated with few drops of Ninhydrin solution and heated for 10 mins at 90°C	Purple color formation	Deshmukh and Theng, 2018
Saponins	0.5 ml extract was treated with 0.5 ml distilled water and shaken for 3 minutes	Thick layer of foam formation	Varadharajan, Janarthanan and Krishnamurthy, 2012
Tannins	0.5 ml extract was treated with 1.25 ml of 3% Ferric chloride	Greenish black color formation	Ahmed *et al.*, 2020
Proteins	0.5 ml of extract was treated with few drops of Millon's reagent	White precipitate formation	Banu and Catherine, 2015
Quinones	0.5 ml of extract was treated with 0.5 ml conc H_2SO_4	Red color formation	Varadharajan, Janarthanan and Krishnamurthy, 2012
Glycosides	0.5 ml extract was treated with 1 ml glacial acetic, few drops of 3% Ferric chloride and 0.5 ml conc H_2SO_4	Brown ring formation at interface	Ahmed *et al.*, 2020
Terpenoids	0.5 ml extract was treated with 0.5 ml chloroform and 0.5 ml conc H_2SO_4 dropwise	Reddish brown color formation	Ahmed *et al.*, 2020

Preparation of diluted samples

In a 15 mL falcon tube, 1 mL of each extracted sample was added to 14 mL of distilled water. Similarly, 1 mL of nanoparticles was added to 14 mL of distilled water and the falcon tube was covered in foil. These samples were stored until further use at 4 °C.

Determination of Total Flavonoid Content (TFC)

1.5 mL of the diluted sample was added to 1.5 mL of 2 % aluminum chloride and incubated at room temperature for 10 minutes. At 415 nm, the absorbances were measured in triplicates and the blank was distilled water. µg Quercetin equivalents per 100 g was used to express the concentration of TFC (Kandiah and Chandrasekaran, 2021).

Determination of Total Phenolic Content (TPC)

0.5 mL of the diluted sample was added to a mixture of 2 mL of 7.5 % sodium carbonate solution and 2.5 mL of 10% Folin-Ciocalteu reagent and incubated at 40 °C for 30 minutes. At 765 nm, the absorbances were measured in triplicates and the blank was distilled water. mg gallic acid equivalents per 100 g was used to express the concentration of TPC (D Fernando and Kandiah, 2023).

Determination of Total Antioxidant Content (TAC)

1 mL of prepared solution (0.6 M sulphuric acid, 28 mM sodium sulphate and 4 mM ammonium molybdate in 1:1:1) was added to 3 mL of diluted sample and incubated at 90°C for 90 mins. At 695 nm, the absorbances were measured in triplicates and the blank was distilled water. mg ascorbic acid equivalents per 100 g was used to express the concentration of TAC (Kandiah and Chandrasekaran, 2021).

Determination of DPPH Scavenging activity

1 mL of diluted sample was added to 2 mL of 0.004% DPPH solution and incubated at room temperature for 30 minutes. At 517 nm, the absorbances were measured in triplicates and the blank was methanol. The following equation was used to calculate the % DPPH activity;

% activity = (A control – A sample) X 100

A control

Determination of Median Inhibition Concentration (IC50)

1 mL each of concentrations 100%, 80%, 60%, 40% and 20% were prepared using the samples and distilled water and 2 mL of 0.004% DPPH solution was mixed to each concentrated sample. Incubation was done for 30 minutes at room temperature. At 517 nm, the absorbances were measured, and blank was methanol.

Determination of Photocatalytic activity

a. Photocatalytic activity using UV

0.5 mL of 4000 ppm GAgNP (Gemunu AgNP) was added to 50 mL of 2 mM

Methylene blue, and the solution was kept under UV. The absorbance of the solution was measured from 320-800 nm every 30 minutes and the blank was distilled water. The same method was repeated for 266 ppm GAgNPs.

b. Photocatalytic activity using sunlight

0.5 mL of 4000 ppm GAgNP (Gemunu AgNP) was added to 50 ml of 2 mM Methylene blue, and the solution was kept under sunlight. The absorbance of the solution was measured from 320-800 nm every 30 minutes and the blank was distilled water. The same method was repeated for 266 ppm GAgNPs.

c. Photocatalytic activity using sunlight with catalyst

0.5 mL of 4000 ppm GAgNP (Gemunu AgNP) was added to 1 mL of 0.2 M NaBH4 and 50 ml of 2 mM Methylene blue, and the solution was kept under sunlight. The absorbance of the solution was measured from 320-800 nm every 5 minutes and the blank was distilled water. The same method was repeated for 266 ppm GAgNPs.

Determination of Antimicrobial activity

Two types of bacteria; Staphylococcus aureus and Escherichia coli were analyzed using the agar well diffusion method. Muller-Hinton agar was prepared according to the instructions in the bottle label. Then it was autoclaved and poured into labelled petri plates in the presence of Bunsen flame. The plates were left to cool and solidify. After solidification, S.aureus and E.coli inoculated in saline solution was spread evenly on the agar plates under the fume hood. Three wells were made on the agar for the two duplicates of the sample (S1 and S2) and the negative control. Saline was loaded to the negative control (−) and the sample was loaded to both to S1 and S2 wells. The positive control (+) was Gentamicin (Figure 6). After incubating the plates for 24 hours at 370C, with the help of a ruler the diameter of the Zone of inhibition was measured in cm.

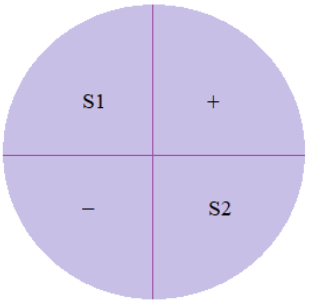


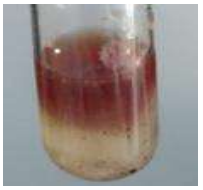
Figure 6: Demonstration of labeling the petri plates

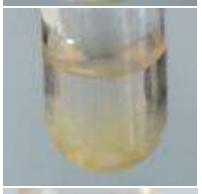
RESULTS AND DISCUSSION

Results

Phytochemical screening

Table 2: Phytochemical test results for the 5 varieties of Cinnamon verum leaf.

Phytochemical	**G**	**N**	**D**	**S**	**W**	**Positive result colour change**
Carbohydrates	✓	✓	✓	✓	✓	

Amino acids	✓	✓	✗	✓	✓	
Saponins	✓	✓	✓	✓	✓	
Proteins	✓	✓	✓	✓	✓	
Quinones	✓	✓	✓	✓	✓	
Glycosides	✓	✓	✓	✓	✓	
Tannins	✓	✓	✓	✓	✓	
Terpenoids	✓	✓	✓	✓	✓	

All samples showed positive results for Carbohydrates, Saponins, Proteins, Quinones, Glycosides, Tannins and Terpenoids. However, sample dubium (D) was negative for Amino acids while other samples were positive.

Silver Nanoparticle Synthesis

Nanoparticles were synthesized using Cinnamon verum leaf water extracts. The optimized temperature is 600C for 30 mins.

Figure 7: AgNPs synthesis A. Water extracts with AgNO3 solution before heating B. synthesized AgNPs after heating

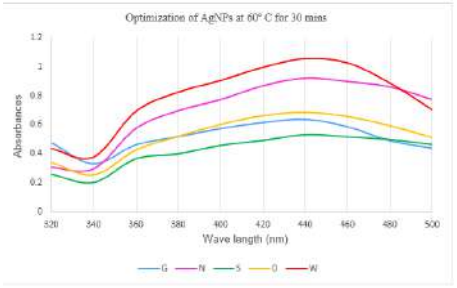


Figure 8: The optimization graph at 600C for 30 mins

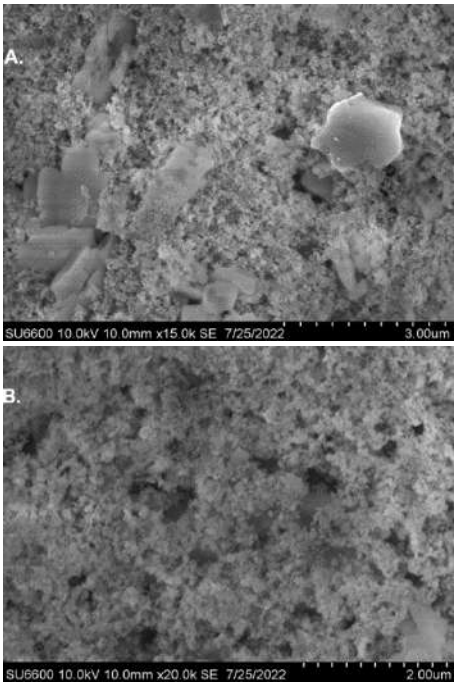
Table 3: The optimization of AgNP synthesis

Samples	60 °C				60 °C				Room temperature
	15 mins	30 mins	45 mins	1 hour	15 mins	30 mins	45 mins	1 hour	24 hours
N	✓	✓	✓	✓	✓	✓	✓	✓	✓
S	✓	✓	✓	✓	✓	✓	✓	✓	✓
D	✓	✓	✓	✓	✓	✓	✓	✓	✓
W	✓	✓	✓	✓	✓	✓	✓	✓	✓
O	✓	✓	✓	✓	✓	✓	✓	✓	✓

Colour change was observed in the samples indicating the reduction of silver ions to silver. Spectrophotometric analysis indicated clear peaks from 420 nm t0 480 nm in all 5 samples at the three different temperatures.

SEM analysis

Characterization of AgNPs was performed using SEM analysis (Figure 9)



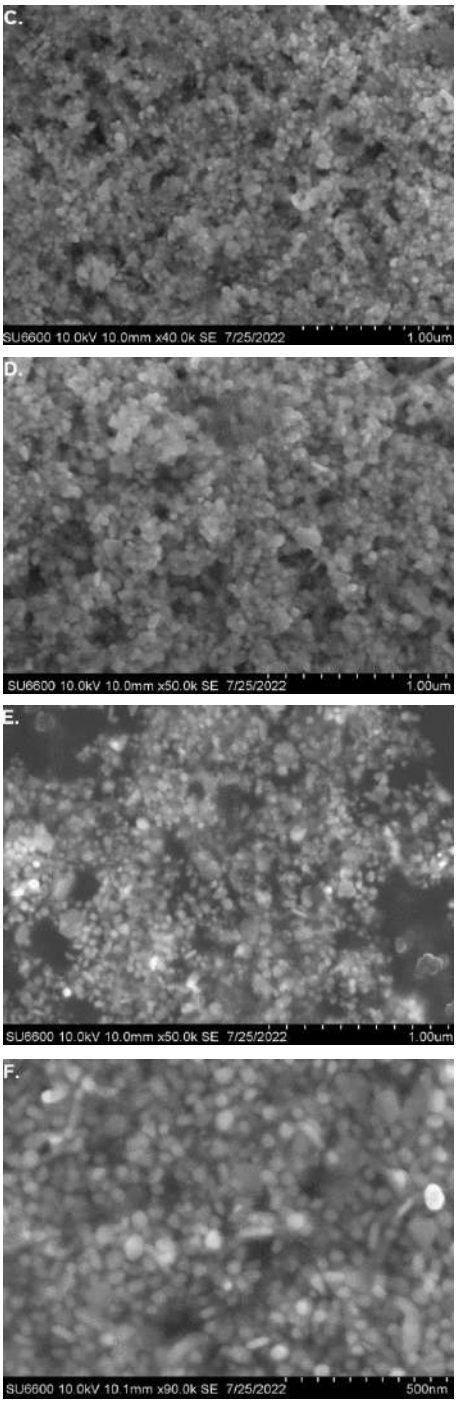


Figure 9: SEM images of G AgNP at different magnifications A) 10.0 kV 10.0 mm X 15.0 k B) 10.0 kV 10.0 mm X 20.0 k C) 10.0 kV 10.0 mm X 40.0 k D & E) 10.0 kV 10.0 mm X 50.0 k F) 10.0 kV 10.1 mm X 90.0 k

SEM images showed the presence of spherical AgNPs of 50 nm

Total Flavonoid Content (TFC)

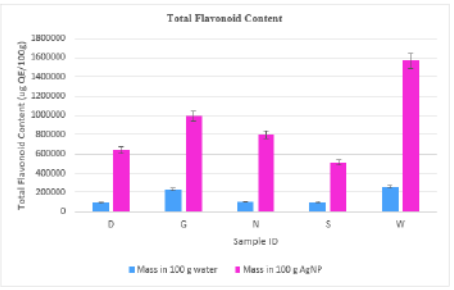


Figure 10: Total Flavonoid Content of water extracts and AgNPs.

Silver nanoparticle samples show higher Flavonoid content than the water extracts. Sample W AgNP shows the highest Flavonoid content. To compare the significant difference between water extracts and AgNPs, ANOVA was performed.

Table 4: ANOVA of water extracts and AgNPs

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	5	787760.4	157552.1	6.62E+09		
Column 2	5	4505208	901041.7	1.7E+11		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.38E+12	1	1.38E+12	15.63708	0.00421	5.317655
Within Groups	7.07E+11	8	8.84E+10			
Total	2.09E+12	9				

The P value is less than 0.05, therefore there is a significant difference between the water extracts and the AgNPs.

Total Phenolic Content

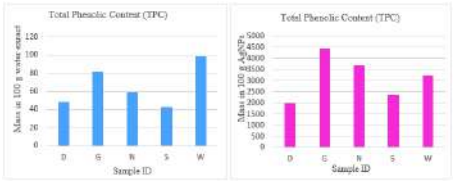


Figure 11: Total Phenolic Content of water extracts and AgNPs.

Silver nanoparticle samples show high Phenolic content than its water extracts. Sample G AgNP shows the highest Phenolic content. To compare the significant difference between water extracts and AgNPs, ANOVA was performed.

Table 5: ANOVA of water extracts and AgNPs

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	5	330.7142857	66.14286	538.9923		
Column 2	5	15739.28571	3147.857	1007153		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	23742407.35	1	23742407	47.12235	0.000129	5.317655
Within Groups	4030768.214	8	503846			
Total	27773175.56	9				

The P value is less than 0.05, therefore there is a significant difference between the water extracts and its AgNPs.

Total Antioxidant Content

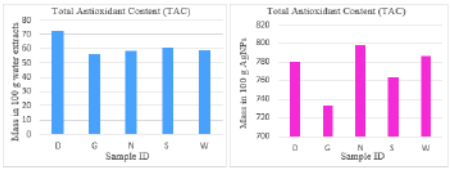


Figure 12: Total Antioxidant Content of water extracts and AgNPs.

Silver nanoparticle samples show higher Antioxidant content than the water extracts. To compare the significant difference between water extracts and AgNPs, ANOVA was performed.

Table 6: ANOVA of water extracts and AgNPs

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	5	307.8136364	61.62273	42.7531		
Column 2	5	3861.363636	772.2727	634.4267		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1262913.906	1	1262914	3729.922	5.74E-12	5.317655
Within Groups	2708.719008	8	338.5899			
Total	1265622.625	9				

The P value is less than 0.05, therefore there is a significant difference between the water extracts and its AgNPs.

DPPH Assay

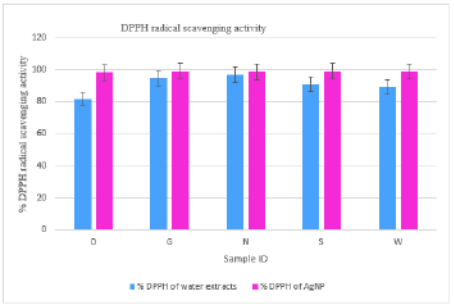


Figure 13: DPPH scavenging activity of water extracts and AgNPs.

AgNPs have a higher DPPH percentage activity than water extracts.

IC_{50}

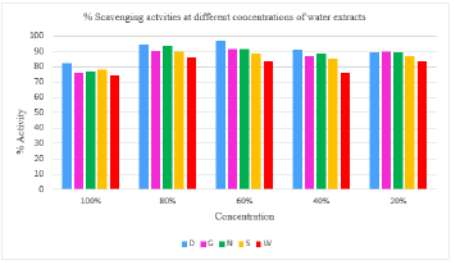


Figure 14: Inhibitory concentrations of water extracts

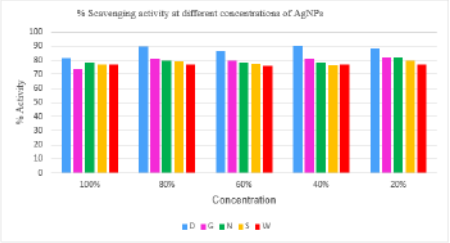


Figure 15: Inhibitory concentrations of AgNPs

Table 7: Minimum inhibitory concentrations (MIC) of each sample

Minimum Inhibitory Conc (MIC)		
Sample	IC50 of water extract (%)	IC50 of AgNPs (%)
G	5.856721173	3.737479
N	3.41810227	4.351989
D	7.580696514	22.11802
S	3.175611305	3.224766
W	6.837326332	3.924955

Photocatalytic activity of G AgNPs

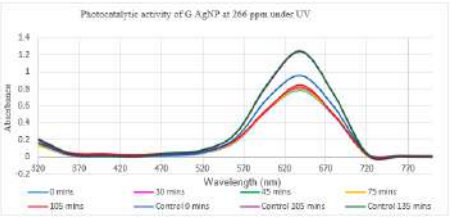
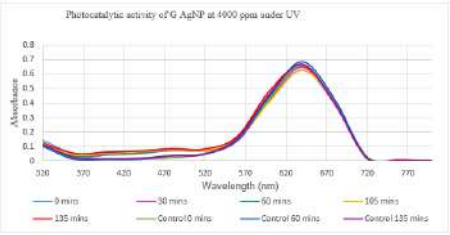


Figure 16: Photocatalytic degradation of MB by 266 ppm G AgNPs under UV.

There is a slight degradation in 266 ppm under UV



There is a no visible degradation in 4000 ppm under UV

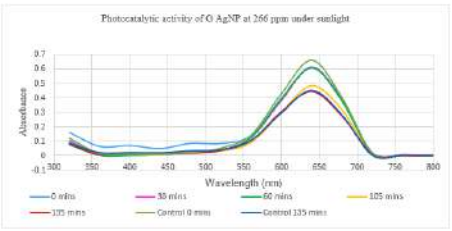


Figure 18: Photocatalytic degradation of MB by 266 ppm G AgNPs under sunlight.

There is a degradation visible in 266 ppm under sunlight

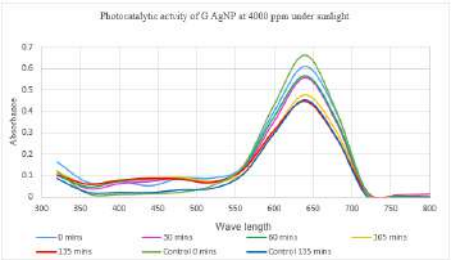


Figure 19: Photocatalytic degradation of MB by 4000 ppm G AgNPs under sunlight.

There is a degradation visible in 4000 ppm under sunlight similar to the degradation at 266 ppm.

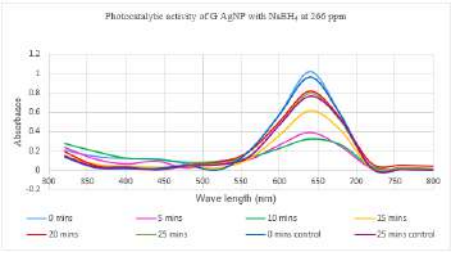


Figure 20: Photocatalytic degradation of MB by 266 ppm G AgNPs with catalyst under sunlight.

There is a significant degradation visible in 266 ppm with NaBH4 catalyst at 10 minutes.

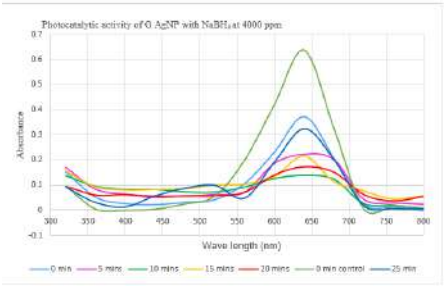


Figure 21: Photocatalytic degradation of MB by 4000 ppm G AgNPs with catalyst under sunlight.

There is a significant degradation visible in 4000 ppm with NaBH4 catalyst at 10 minutes.

Antibacterial activity

S.aureus

Figure 22: Zone of Inhibition (ZOI) for Staphylococcus aureus in G water extract (left) and AgNPs (right).

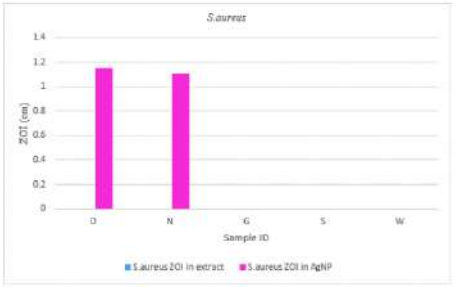


Figure 23: ZOI of S.aureus for water extract and AgNPs.

The ZOI of the AgNPs are higher than its water extract. The water extracts did not show any significant ZOI in any of the samples. To compare the significant difference between water extracts and AgNPs against S.aureus, ANOVA was done (Table 8).

Table 8: ANOVA of water extracts vs AgNPs against S.aureus.

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	5	0	0	0		
Column 2	5	2.25	0.45	0.38		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.50625	1	0.50625	2.664474	0.141256	5.317655
Within Groups	1.52	8	0.19			
Total	2.02625	9				

The P value is more than 0.05, therefore there is no significant difference between the water extracts and its AgNPs.

E.coli

Figure 24: Zone of Inhibition (ZOI) for E.coli in G water extract (left) and AgNPs (right)

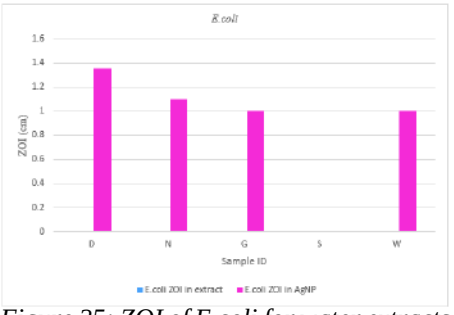


Figure 25: ZOI of E.coli for water extracts and AgNPs.

The ZOI of the AgNPs are higher than its water extract. The water extracts did not show any significant ZOI in any of the samples. To compare the significant difference between water extracts and AgNPs against E.coli, ANOVA was done (Table 9).

Table 9: ANOVA of water extracts vs AgNPs against E.coli.

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	5	0	0	0		
Column 2	5	4.45	0.89	0.268		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.98025	1	1.98025	14.77799	0.004918	5.317655
Within Groups	1.072	8	0.134			
Total	3.05225	9				

The P value is less than 0.05, therefore there is a significant difference between the water extracts and its AgNPs.

DISCUSSION

Nanotechnology is emerging with leading contributions in diverse applications and one of the most successful approaches of nanoparticle synthesis is green synthesis due to its simplicity, eco friendliness and cost effectiveness (Garibo et al., 2020). Green synthesis of AgNPs using five varieties of Cinnamon verum was in this study. Further, its antioxidant, antibacterial and photocatalytic properties were determined. During the AgNP synthesis, three reactant components are important, they are; metal precursor, reducing and stabilizing agents. In most cases phytochemicals present in the cells act as a stabilizing and reducing agent (Srikar et al., 2016). Initially, water is used as the solvent rather than organic substances to maintain an environment friendly research without the violating the principles of green chemistry as some substances such as ethylene and polyvinyl alcohols are toxic (Vishwanath and Negi, 2021). Silver was the chosen metal for this study due to its therapeutic potential and a well-known antimicrobial action since ancient times. Further AgNPs are less toxic and cost effective compared to other metals (Srikar et al., 2016).

Phytochemical analysis indicated the presence of antioxidants such as carbohydrates, proteins, saponins, quinones, glycosides, tannins and terpenoids in each sample (Table 2). AgNPs synthesis was optimized at different temperatures such as 600C, 900C and room temperature with different time intervals. This is because temperature is a major factor in NP synthesis (Singh et al., 2020). All the samples formed AgNPs at all the temperatures, this may be due to the rapid formation and growth and less time consumption for bioreduction. The stability of AgNPs was maintained for more than 5 months when stored at 4° C. During AgNP synthesis a colour change to brown indicated the formation of AgNPs (Figure 7). This is a result of the surface plasma resonance (SPR) where plasmons on the outer surface of AgNPs undergoes excitation when light incidents on it (Jung et al., 2018). This is further confirmed by the highest absorption peaks within the 420-480 nm range in the spectrophotometric analysis (Figure 8). During the optimization, all the samples showed NPs for all time duration but however, the samples at 600C for 30 mins were chosen for further analysis as it showed a better UV/V spectrum compared to the others and an intense colour change. SEM analysis was done for the characterization of AgNPs. The observations revealed that the AgNPs were 50 nm in size and spherical in shape with aggregations. Studies have reported that the size of Cinnamon verum AgNPs vary in the range of 30 – 150 nm (Gauthami et al., 2015).

The optical properties of AgNPs such as conductivity was analyzed by measuring the band gap energy using the absorbance spectrum. The minimum energy needed for electrons to move from the valence band (VB) to the conduction band (CB) is called Band gap energy. The calculated band energy in electron volts (eV) is used to classify AgNPs into semiconductors (<3 eV) or insulators (>4 eV) (Sundeep et al., 2017). All the AgNPs were found to be semiconductors according to the following Planck's equation (Table 10).

$E = hC/\lambda$

where; E= band gap energy

h= Planck's constant (6.6269×10^{-34} Js)

C= speed of light (3×10^{8} ms^{-1})

λ = maximum absorption wavelength

Table 10: Classification of conductivity for AgNPs.

Sample ID	Band Gap energy (eV)	Classification
G	2.820438	semiconductor
N	2.820438	semiconductor
S	2.820438	semiconductor
D	2.820438	semiconductor
W	2.807675	semiconductor

Another major focus of this study is the analysis of antioxidant properties of water extracts and its AgNPs. However, previous studies do not show any reports of these assays for the native varieties of Cinnamon verum used in this study. TFC was studied using the AlCl3 in a colorimetric method. In this method, the C-4 keto group or C-5 or C-3 hydroxyl groups of flavones and flavonols form stable acid complexes with AlCl3 ions and is measured at 415 nm (Figure 26) (Ahmed and Iqbal, 2018). AgNPs indicated a high TFC compared to its water extracts. The highest was in Sri Wijaya AgNP sample and lowest in sinharajanese AgNP (Figure 10). ONE-way anova analysis (Table 4) indicated a significant difference between the water extracts and its AgNPs (P value was 0.00421). Previous research studies also confirm that cinnamon leaf, bark, and bud extracts show high TFC levels than the water extracts (Yang et al., 2012).

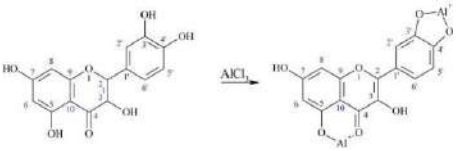


Figure 26: Mechanism of Total Flavonoid activity (Martono et al., 2019).

TPC was studied using folin-ciocalteu reagent. Folin-ciocalteu is a mixture of phosphotungstic acid and phosphomolybdic acid and it reduces to form a stable blue complex of oxides of tungsten and molybdenum after oxidation with phenolic compounds (Figure 27). This is measured at 765 nm (Hatami et al., 2014). AgNPs indicated a high TPC compared to its water extracts. The highest TPC was in Gemunu AgNP and the lowest in dubium AgNP (Figure 11). ONE-way anova analysis (Table 5) indicated a significant difference between the water extracts and its AgNPs (P value was 0.000129). Previous research show that Cinnamon barks have high phenolic content (Wijewardhana, Gunathilaka, and Navaratne, 2019). Another study also revealed that Cinnamon verum species (C.zeylanica) indicated the highest phenolic content compared to the other species of cinnamon such as C. burmanni, C. pauciflorum, C. cassia, and C. tamala (Prasad et al., 2009).

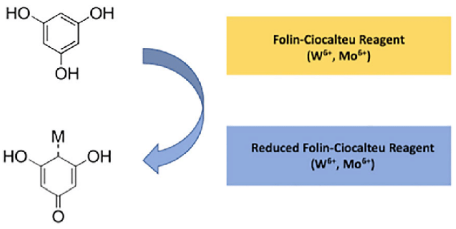


Figure 27: Mechanism of Total Phenolic activity (Ford et al., 2019).

TAC was studied using Phosphomolybdenum method. A green phosphate complex is formed after the reduction of Mo (VI) into Mo (V) by antioxidants (Figure 28) (Apak et al., 2016). This is measured at 695 nm. AgNPs indicated a higher TPC compared to its water extracts. All the AgNPs showed a high level of TAC (Figure 12). ONE-way anova analysis (Table 6) indicated a significant difference between the water extracts and its AgNPs (P value was 5.74E-12). Studies show that cinnamon extracts have a high antioxidant capacity (Ashfaq et al., 2020; Kallel et al.,2019).

Figure 28: Mechanism of Total Antioxidant activity (Madhumitha and Fowsiya, 2017).

DPPH radical scavenging assay was studied to determine the scavenging ability of antioxidants in the sample to quench the stable DPPH free radical by donating an electron (Figure 29). In the presence of an antioxidant, the DPPH reduces and gradually changes colour from violet to pale yellow or colourless (Ahmed and Iqbal, 2018; Sagar and Singh 2011). This is measured at 517 nm. In DPPH assay methanol is used as it is a better solvent for DPPH (Njoya, 2021). Both AgNPs and water extracts showed high DPPH percentages. However, the activity of AgNPs was higher than water extracts (Figure 13).

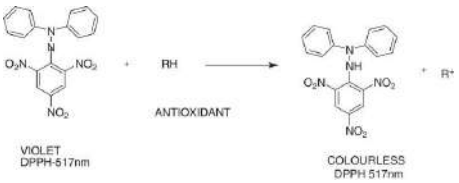


Figure 29: Mechanism of DPPH assay (Nithya and Madhavi, 2017)

IC50 were calculated in both water extracts and AgNPs to find the concentration of sample needed for the inhibition of 50% of the radical (Table 7). The IC50 higher and lower values varied among AgNPs and water extracts. Sample N, D and S showed higher IC50 in AgNPs while sample W and G showed higher IC50 in water extracts.

The statistical correlation of all antioxidant assays from Pearson correlation factor (PCF) were higher than 0.800 which is a strong statistical correlation (Figure 30). TPC-TAC correlation is 0.917 therefore TPC contributes more to TAC than TFC.

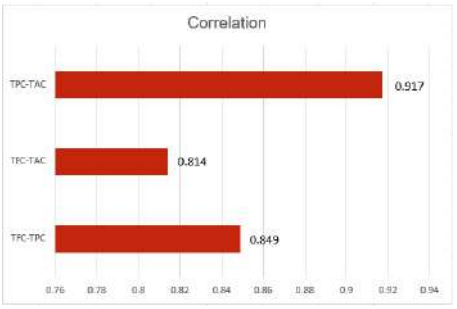


Figure 30: Statistical correlation between antioxidant assays.

The next main goal of this study was the photocatalytic property of AgNPs, the

photodegradation of azo dyes under UV and sunlight with and without the catalyst. When exposed to sunlight or UV, the AgNPs absorb the photons. The electrons undergo excitation from the valence band to conduction band due to SPR effect. Oxygen in water undergoes reduction and converts into free radicals. Photogenerated holes in AgNPs react with MB dye molecules and with water to form hydroxyl radicals. These generated free radicals collectively degrade the azo dye by breaking down complex azo bonds (Figure 31) (Singh and Dhaliwal, 2018).

Figure 31: Mechanism of photocatalytic degradation by AgNPs (Altaf et al., 2021)

In this study, MB was the model dye while sodium borohydride was used as the catalyst (NaBH4) and the photocatalytic activity assay was done to determine the action of Gemunu AgNPs in the degradation of MB. NaBH4 transfers electron to MB and acts as a reducing agent (Hu et al., 2018). In both 4000 ppm and 266 ppm G AgNP samples, complete MB degradation was observed within 10 minutes when catalyst was used (Figure 20 and 21). However, slight degradation was observed in 266 ppm G AgNP under sunlight and UV. The rate constant of photocatalytic degradation was calculated using the following rate constant equation;

Ln (C/C0) = -kt

where; C= Methylene blue concentration

C0= Initial Methylene blue concentration

k = Rate constant

t = time

The rate constants were obtained for both the concentration according to the gradient of the graphs (Figure 32 and 33). A higher rate constant (k) was observed in 266 pm AgNP sample (k=0.1155) than in 4000 pm AgNP sample (k=0.0994). Therefore, it can be concluded that the photocatalytic activity is highly efficient in 266 ppm AgNPs.

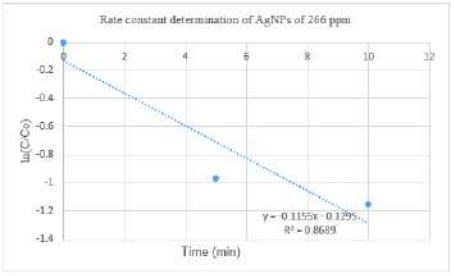


Figure 32: Rate constant determination of 266 ppm AgNPs

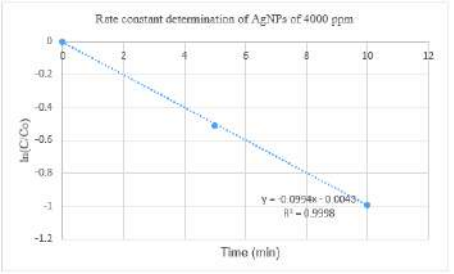


Figure 33: Rate constant determination of 4000 ppm AgNPs.

AgNPs and cinnamon plants are well known to have a higher degree of antimicrobial properties. In the current study, well diffusion technique was performed to determine the antibacterial properties of water extracts and AgNPs against E.coli and S.aureus. AgNPs exhibit their antibacterial ability by inhibiting the synthesis of cell membrane, disrupting the energy transduction, producing of toxic ROS, inhibiting enzymes, and reducing the production of DNA (Figure 34) (Shaik et al., 2019).

Figure 23 and 25 show ZOI only in AgNPs against E.coli and S.aureus. The water extracts do not show a visible antimicrobial activity, and this could be due to a higher concentration of bacteria and bacterial regrowth (Gugala et al., 2018). However, sample dubium and revolurum show ZOI for NP against both E.coli and S.aureus while sample sinharajanese do not show ZOI to either bacteria. Previous studies show satisfactory results for antibacterial activity of cinnamon extracts specifically on E.coli (Khalid et al., 2015). Only the single factor ANOVA for E.coli showed a significant difference (Table 8 and 9).

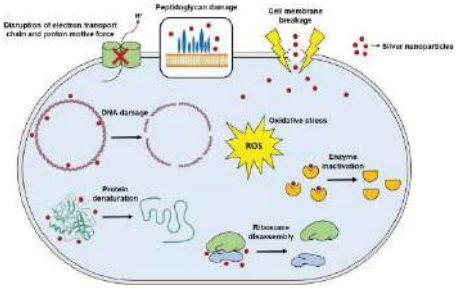


Figure 34: Antibacterial mechanisms of AgNPs (Roy et al., 2018).

In conclusion, Green synthesis of AgNP using all five varieties of Cinnamon verum leaf extracts was successful. SEM analysis showed spherical NP of size 50 nm that act as semiconductors. Antioxidant properties were higher in AgNPs than in water extracts. Antioxidant capacity correlates with the phenolic compounds in sample. Antimicrobial activity of AgNPs against E.coli was more effective than S.aureus. There was no significant difference between water extracts and its AgNPs against E. coli and S.aureus. The water extracts did not indicate ZOI for against both E. coli and S.aureus. G AgNPs with a concentration of 266 ppm (k=0.1155) had more ability to degrade MB dye molecules than the 4000 ppm (k=0.0994). This research concludes that green synthesized AgNPs from Cinnamon varieties could be used in biomedical research to develop tools for a better life.

Future work

• AgNPs could be further characterized to obtain better resolution using techniques such as Transmission Electron Microscopy (TEM), X-ray powder diffraction, Dynamic Light Scattering (DLS) and Aerodynamic Particle Sizer (APS). TEM analysis is a highly preferred method to directly measure nanoparticle size and morphology.

• Antioxidant activity could be further analyzed using Ferric Reducing Antioxidant Power (FRAP), Oxygen Radical Absorption Capacity (ORAC), Hydroxyl Radical Antioxidant Capacity (HORAC), Total Peroxyl Radical Trapping Antioxidant Parameter (TRAP), and Total Oxyradical Scavenging Capacity (TOSC) assays.

• NP could be synthesized using other metals such as Zinc, Gold, Titanium, Copper and Nickel and different solvents such as acetone, methanol and ethanol and their different properties could be compared and studied.

• Photocatalytic activity of AgNPs could be tested using different concentrations of Methylene blue and also by using various azo dyes such as Methylene orange, Methylene red, Eriochrome black and Bromocresol green.

• Antibacterial activity could be determined for a range of bacteria including Klebsiella pneumonia, Bacillus megaterium, Pseudomonas aeruginosa, Enterobacter cloacae, Corynebacterium xerosis and Streptococcus faecalis. Antifungal, antiviral, and anticancer properties could also be studied for the synthesized AgNPs.

• AgNPs could also be used to detect melamine adulteration in milk using

colorimetric method. This can be helpful in assuring food safety and human health.

• Since only 5 varieties of cinnamon native to Sri Lanka was used in this study, we could obtain other cinnamon varieties from different countries for AgNP synthesis. As secondary metabolites vary due to environmental conditions, we could study more about the antioxidant properties.

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