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Research Article

Functional Properties of Novel Silver Nanoparticles Synthesized using *Moringa Oleifera*

Anto Cordelia TAD[1]* and Sharmila Mohana Dass[2]

[1]*Centre for Biodiversity Research, Department of Chemical Science, Faculty of science, Universiti Tunku Abdul Rahman, Kampar, Perak Malaysia*

[2]*Department of Chemical Science, Faculty of science, Universiti Tunku Abdul Rahman, Kampar, Perak Malaysia*

***Corresponding Author:** Anto Cordelia TAD, Centre for Biodiversity Research, Department of Chemical Science, Faculty of science, Universiti Tunku Abdul Rahman, Kampar, Perak Malaysia.

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Abstract

Green method to synthesize noble metal nanoparticles is attracting attentions from various fields due to the properties of nanoparticles and non-hazardous effects to the environment. In this study, silver nanoparticles (AgNPs) were synthesized using aqueous silver nitrate solution and mediated by *Moringa oleifera* leaf extract. AgNPs synthesized were characterized using UV-Visible spectrophotometer, SEM, FTIR and XRD. Total phenolic and flavonoids were estimated using standard protocols. The free radical scavenging activity was appraised using DDPH, ABTS, iron chelating and nitrogen oxide scavenging methods. Antibacterial activity was tested against *E. coli* and *S. aureus* species. Appropriate statistical analysis was carried to establish possible correlation between the antioxidants and their activity. The AgNPs produced showed a peak centered at 419 nm by using UV–Vis. High-density AgNPs synthesized by *Moringa oleifera* leaf extract was relatively sphere and cuboidal shape with diameter between 30nm to 60nm. AgNPs were confirmed to have face centered cubic (FCC) crystalline. Terpenoids and tannic acids are the functional groups that aid in bio-reduction of silver ions and stabilize the AgNPs. The novel AgNPs possessed higher phenolic contents (123.00 mg GAE/g dry weight) as compared to leaf extract (130.00 mg GAE/g dry weight). Total flavonoid was higher in leaf extract compared to AgNPs (179.60 mg QE/g dry weight and 93.00 mg QE/g dry weight respectively). Both the leaf extract and AgNPs exhibited highest antioxidant capacity using NO radical method. Correlation between total phenolic content, total flavonoid content and antioxidants capability showed the strong influence of phenolic compounds and flavonoids towards antioxidant activity of both AgNPs and leaf extracts. AgNPs are effective in inhibiting both gram-negative and gram-positive bacteria. Hence *Moringa oleifera* can be successfully used to synthesize silver nanoparticles which can find application in fields of nano medicine, nutraceutical and pharmaceutical industries.

Keywords: Silver Nanoparticles (AgNPs); *Moringa oleifera;* Green Synthesis; Antioxidant Activity; Antimicrobial Activity

Introduction

The environment friendly processes called the green synthesis in chemistry and chemical technologies are fetching increasingly popularity and are important due to worldwide problems related with environmental alarms [1]. Green synthesis is the application of certain ideologies that lessens or abolishes the use or production of hazardous chemicals. Besides that, it also reduces the amount of energy used comparing to chemical synthesizing method [2].

Synthesis of silver nanoparticles (AgNPs) using plant extracts has evolved as an alternative approach to chemical synthesizing method because of its varied benefits [3]. They are modest and simple to prepare, cost-effective, provides high yields, and are environmentally friendly as well. Synthesis of nanoparticles using plant parts or plant extracts is an eco-friendly, clean, non-hazardous, and economical method producing nanoparticles with diverse shape, size, and morphology [4]. This study also confirms that the biosynthesis route is able to produce better sizes and shapes of metal nanoparticles compared to all the other physicochemical methods employed in production of these nanomaterial.

AgNPs are now popularly used in various fields such as medical, food, health screening, consumer, and also in industry. This is because of their unique physical and chemical properties like electrical, optical, and thermal ability, high electrical conductivity besides their biological properties [5]. These unique features and applications of nanoparticles are the reasons for gaining importance, especially in the field of medical imaging, biotechnology, and catalysts. AgNPs are one of the most widely used nanoparticles [6].

Medicinal plants contain abundant phytochemicals with high therapeutic standards provides a better platform to synthesis

AgNPs. This is because they are free from hazardous chemicals and they provide natural capping agents which is the key for AgNPs nanoparticles production [7]. Plants contains these capping agents and reduction agents which also stabilizes the silver ions. Previous studies show us there are a mixture of biomolecules like protein, enzymes, polysaccharides, amino acids, alkaloids, tannins, saponins, terpenoids and phenolic besides vitamins too [2]. These biomolecules are environmentally nonthreatening, yet their structures are chemically complex for the reactions to take place.

Moringa oleifera also known as drumstick tree is native to north-western India but is also known to have equal value in some other countries such as Philippines, Sudan, Ethiopia and also South Africa and is considered as an under-utilized tree. Almost all the parts of the tree such as tender pods, flowers and their leave are benign for human consumption [8]. Besides that, the leaves especially, are well known for their natural healing properties and are widely consumed in many different ways and are said to contain various antioxidant properties. *Moringa oleifera* signify to be a promising substance to synthesis bioactive AgNPs in a greener way which is very environmentally friendly too [9].

Materials and Methods

Experimental animals

Moringa Oleifera leaves were collected from a housing area in Kampar, Perak and used for this study.

Figure 1: Picture of the plant (Moringa Oleifera) used.

Preparation of plant extract

Leaves were washed several times with water to remove the dust particles and then dried in the oven for 48 hours and grinded to form powder. Then plant extract was prepared following Mallikarjuna and Narasimha [10] with some modifications. This was done by mixing 1g of plant powder with 10 ml of distilled water in a conical flask. Then, the solution was incubated for 2 days in room temperature. The solution was subjected to centrifuge at 10 000 rpm in room temperature to separate the insoluble parts of the leaves and the supernatant was collected as the leaf extract and stored in the fridge be used within a week or two.

Phytochemical screening for leaf extract

Test	Method	Reference
Tannins	Leaf extract with volume about 2ml was added into a test tube followed by a few drops of 5% ferric chloride. The presence of tannins is observed by the formation of precipitate in black or bluish-green colour.	(Banso and Adeyemo, 2006) [11]
Glycosides (Keller-Killiani test)	This test was done by using 2ml plant extract. Glacial acetic acid of volume about 2 mL was added to the plant extract in a test tube followed by the addition of a few drops of ferric chloride. Then, a few drops of concentrated sulphuric acid. When observed, the formation of brown ring at the interface and a violet ring below the brown ring gives positive indication for the presence of glycoside.	(Ayoola, 2003) [12]
Saponins	About 2ml of plant extract and 2ml of distilled water was added into a test tube. Then, the test tube was covered and shaken vigorously. After incubation for 5 minutes at room temperature, observed for the presence or formation of frothing, which indicates the presence of saponins.	(Banso & Adeyemo, 2006) [11]
Alkaloids	Potassium iodide and iodine weighed 2g and 1.27g respectively were dissolved in 5ml distilled water. Then, the solution was diluted to 100 mL with distilled water. Few drops of this solution were added to 2ml of plant extract. After the reaction took place, a brown colour precipitate shows the presence of alkaloids in the solution.	(Iqbal, 2015) [13]
Phenols	The plant extract was added 1ml into a test tube. To that, a few drops of 5% ferric chloride solution were added. The presence of phenols are indicated by the change of colour to dark green in the solution.	(Bargah, 2015)[14]
Flavonoids	The plant extract of 1 mL was taken in a test tube and a few drop of diluted NaOH solution was added. Then, a few drops of sulphuric acid was added. The change of yellow colour to colourless indicates the presence of flavonoids	(Hossain, 2013)[15]
Phytosterols	The plant extract of volume 2ml was taken in a test tube. About 2ml of acetic anhydride was added to the plant extract, followed by 1 ml of concentrated sulphuric acid. The presence of phytosterols can be confirmed by the formation of a brown ring at the junction and the colour change to dark green colour at the upper layer of the solution.	(Roopalatha, 2013) [16]
Terpenoids (Salkowski test)	This test was done by shaking the 1ml of plat extract with 2ml of chloroform. Then, 2ml of concentrated sulphuric acid was added. A positive result for the presence of terpenoids is a reddish brown coloration of the interface.	(Ayoola, 2003) [12]

Table

Synthesis of silver nanoparticles

The synthesis method was modified from Ahmed [2]. Silver nitrate solution (10ml) with the concentration of 8mM was prepared and it was mixed with 2ml of leaf extract. The mixture was incubated in water bath with 50oC for 10 minutes or until color change to brown was observed. The dark brown solution obtained containing AgNPs were centrifuged. And the pellet was re-suspended in distilled water and centrifuged again and freeze dried. The dried powder was collected and kept in an air tight container at room temperature for further analysis.

Characterization of silver nanoparticles

AgNPs formed were characterized and confirmed for their presence using the following techniques. The absorption spectrum of the sample was measured in the range between 300nm and 700nm by using the light UV-Vis spectrophotometer (GENESYS 10). Scanning electron microscope (SEM) (JEOL USA JSM–7610F) was used to examine the particle shape and morphology. The photomicrographs were taken at different amplifications. (Nagaich, 2016). The X-ray diffraction (XRD) pattern of the prepared sample of AgNPs was recorded by using X-ray diffractometer (Siemens D500) to identify the crystalline structure. The Fourier Transform Infrared (FTIR) spectra was used to identify the functional groups with the aid of FTIR spectrophotometer (Perkin-Elmer) using potassium bromide (KBr).

Total phenolic content and Total Flavonoids Content

Total phenolic content

Phenolic content in the sample was determined by using Folin-Ciocalteu reagent as described by Clarke [17] with some modifications. Approximately 100µl of sample was added with 750µl Folin-Ciocalteu reagent. The tubes were left to incubate in dark for 5 minutes. Around 750 µl of 6% of sodium carbonate was added and the mixture was incubated at room temperature for 90 minutes in dark. The absorbance was read at 725nm. Gallic acid standards were used to plot the calibration curve and the phenolic content in the sample was expressed as Gallic acid equivalents (mgGAE).

Total flavonoids content

Total flavonoids content was determined by aluminium colorimetric method as described by Bibi & Ullah [18] with some modifications. For this, 100µl sample is added with 150µl of 5% sodium nitrate solution and incubated for 6 minutes at room temperature. Then, 150µl of 10% aluminium chloride solution was added and incubated again for 6 minutes at room temperature, followed by addition of 800µl 10% sodium hydroxide solution. The mixture was incubated at room temperature for 5 minutes and absorbance was read at 510nm. The calibration curve was plotted by using quercetin solution as the standard. The flavonoid content in the sample was expressed as quercetin equivalents (mgQE).

Determination of antioxidant activity

Radical scavenging activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

Antioxidant potential of AgNPs sample and plant extract against DPPH was determined using method described by Clarke [17] with slight modification. Test sample with 50µl volume was poured in respective tubes with different concentrations. Then, 1ml of DPPH solution was added in each well to make 1050µl final volume. These mixtures were incubated at room temperature for half an hour in dark. After incubation, absorbance of reaction mixture was read at 517 nm in a spectrophotometer. The scavenging activity of the reaction was calculated by the equation given below.

% Scavenging= (Abs of control-Abs of test sample)/(Abs of control) x 100

Ascorbic acid was used as the reference standard with concentration in range from 0 µg/ml to 100 µg/ml. The results were then expressed in EC50 value where the concentrations of each sample needed to scavenge 50% of DPPH radicals.

Iron chelating assay

The chelating activitity of samples were determined by using the method of Dinis [19] with slight modification. An aliquot of 100µl of sample was added into the tubes with different concentrations. Then, 50µl ferrous chloride with 2mM concentration was added and mixed well. These mixtures were then added to 200µl ferrozine with 5mM concentration. These mixtures were mixed and shaken vigorously before incubating at dark for 10 minutes at room temperature.

After incubation, absorbance was read at 562 nm using UV–Vis spectrophotometer. The percentage of inhibition of ferrozine-Fe2+complex was calculated using the formula given below.

% Inhibition of ferrozine-Fe2+comple= (Abs of control-Abs of test sample)/(Abs of control) x 100

Ethylenediaminetetraacetic acid (EDTA) was used as the reference standard with concentrations ranging from 0 µg/ml to 120 µg/ml to plot the calibration curve. The results were then expressed in EC_{50} value which is the concentrations of each sample needed to scavenge 50% of ferrozine-Fe2+ complex.

2, 2'-azino-bis (ABTS) assay

ABTS assay was done following the method stated by Madhanraj [20] with some modifications. The ABTS solution was prepared by mixing 8mg/ml ABTS aqueous solution with 1.32mg/ml potassium persulfate solution and incubated in the dark for 12-16 hours at room temperature prior to the assay. Earlier before starting the experiment, this solution was diluted with potassium persulphate buffer and equilibrated to pH 7.4 to give an absorbance at 734 nm

of 0.700 ± 0.02. This works as the working solution.

Then, 100µl of sample was added into tubes with different concentrations. Into the tubes containing samples, 1ml of ABTS working solution was added followed by incubation in dark for 10 minutes. Then, absorbance was recorded at 734nm [21]. The percentage of ABTS cation radical scavenging activity of the reaction was calculated using the formula given below.

% of Cation radical scavenging ability=(Abs of control-Abs of test sample)/(Abs of control) x 100

Butylated hydroxytoluene (BHT) was used as the reference standard with concentrations in range from 0 µg/ml to 50 µg/ml used to plot the calibration curve. The results were then expressed in BHT equivalent antioxidant capacities values.

Nitric oxide (NO) assay

Nitric oxide generated from sodium nitroprusside was measured using spectrophotometer by using Griess reaction method of Patil., *et al* [4]. Sodium nitroprusside with concentration of 5 mM was prepared in phosphate buffered saline and adjusted to pH 7.4. Then, 200 µl of it was mixed with 800 µl different concentration of the samples and incubated under light for 30 minutes. After 30 minutes of incubation, 50µl Griess reagent consisting of equal volume of 1% sulphanilamide, 5% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride was added. The mixture was incubated at dark for 10 minutes and absorbance reading was measured at 546 nm. The percentage of nitric oxide scavenging activity was calculated as below.

% of Nitric Oxide scavenging activity= (OD of control-OD of test sample)/(OD of control) x 100

Ascorbic acid was used as the reference standard and the results were expressed in EC_{50} value which is the concentrations of each sample required to scavenge 50% of nitric oxide.

Antimicrobial assay

Disk diffusion method to determine the bactericidal activity from Ruparelia & Chatterjee [22] was used with a slight modification. For this, one Gram-positive Bacteria *Staphylococcus aureus* and one Gram-negative bacteria *Escherichia coli* were used. Bacterial cultures were incubated in nutrient broth for 24 hours at 35°C. Then, the bacteria from broth was streaked in Mueller-Hinton agar medium for the growth of bacterial strains and left incubated for 24 hours in 35oC. Few colonies were picked from the streaked plates and mixed with 0.85% saline solution to achieve a turbidity of bacterial suspension similar to when compared to 0.5% McFarland standard. Then, the agar plates were streaked with this saline solution containing respective bacteria using sterile cotton buds. Discs were then impregnated into respective solutions to be tested such as distilled water (negative control), silver nitrate solution, AgNPs solution, and plant extract to be placed in the agar plate containing bacteria and incubated for 24 hours at 35oC for the antibacterial activity to take place. Tetracycline was used as the positive control. Zones of inhibitions were observed and measured using a ruler after 24 hours of incubation.

Results

Phytochemical screening

The aqueous extract of Moringa oleifera leaf extracts were tested for different constituents of secondary metabolites present in them. The results obtained were tabulated in Table 1.

Secondary Metabolites	*Moringa oleifera* leaf ectracts
Tannins	+
Glycosides	+
Saponins	+
Alkaloids	-
Phenols	+
Flavonoids	+
Phytosterols	+
Terpenoids	+

Table 1: Phytochemical screening of Moringa oleifera leaf extract for several secondary metabolites.

(+) = Presence of Phytochemical Constituents

(-) = Absence of Phytochemical Constituents

Synthesis of silver nanoparticles

The change of solution mixture colour to dark brown from light yellow after the mixing of silver nitrate and leaf extract was observed and shown below.

Figure 2: The colour change of the mixture contain 10% *Moringa oleifera* leaf extract and 8mM silver nitrate solution before and after incubation in water bath (50°C).

Characterization of silver nanoparticles

UV-Vis spectrophotometer

The surface Plasmon resonance of AgNPs showed a peak centred at 419 nm by using UV–Vis spectrum when the absorption spectrum was recorded at 300 to 550 nm using UV-Vis spectrophotometer.

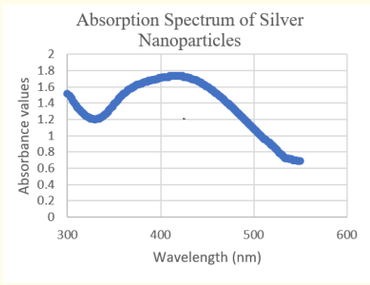

Figure a

Scanning Electron Microscope (SEM)

High-density AgNPs synthesized by Moringa oleifera leaf extract was shown by SEM (Figure 3 and 4). Relatively sphere and cuboidal even AgNPs were formed in diameter ranging from 30 nm to 60 nm. The larger AgNPs in size (114.9nm to 203.0nm) may possibly be due to aggregation of the smaller sized AgNPs [23].

Figure 3 and 4: SEM micrographs of silver nanoparticles synthesised by *Moringa oleifera* leaf extract.

X-Ray Diffraction Method (XRD)

The crystalline structure of AgNPs produced were confirmed by using X-ray crystallography (XRD). The XRD pattern of the main peaks of AgNPs can be seen labelled in Figure 5 below.

Figure 5: XRD pattern of silver nanoparticles produced using *Moringa oleifera* leaf extract.

Fourier Transformed Infrared Spectra (FTIR)

The FTIR spectra obtained (Figure 6) and the biomolecules responsible for the reduction of silver ions and also to determine the capping agent of the AgNps synthesized are determined

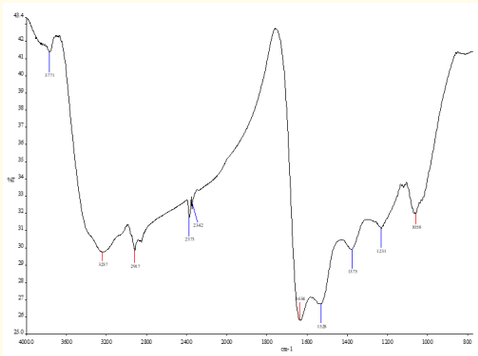

Figure 6: Fourier-Transform Infrared (FTIR) Spectra of the AgNPs synthesized using Moringa oleifera leaf extract.

Total phenolic and total flavonoid content

The standard calibration curve of Gallic acid and Quercetin hydrate were plotted for phenolic content and flavonoid content respectively. The total content of both phenol and flavonoid in 1mg/ml leaf extract and 1mg/ml AgNPs for phenolic content and 5mg/ml leaf extract and 5mg/ml AgNPs for flavonoids content were then calculated.

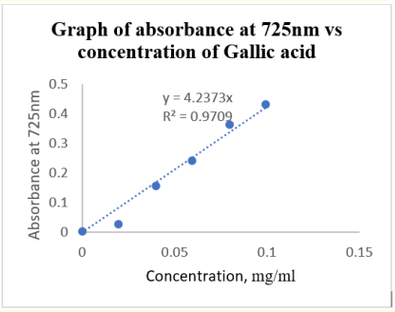

Figure 7: Graph of absorbance at 725nm vs. concentration of Gallic acid.

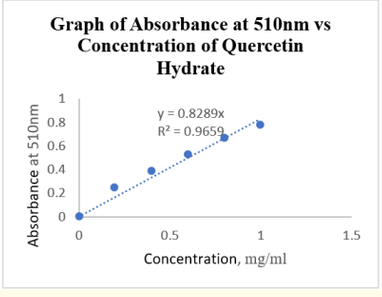

Figure 8: Graph of absorbance at 510nm vs. concentration of Quercetin Hydrate.

Table 2 shows the total phenolic content is 123.00 ± 7.31 (mg GAE/g dry weight ± SE) and 130.00 ± 6.74 (mg GAE/g dry weight ± SE) in *Moringa oleifera* leaf extract and AgNPs synthesized respectively. This shows a higher phenolic content in the AgNps synthesized compared to leaf extract. While, the total flavonoid content is 179.60 ± 1.33 (mg QE/g dry weight ± SE) and 93.00 ± 6.56 (mg QE/g dry weight ± SE) for *Moringa oleifera* leaf extract and AgNPs synthesized respectively. This shows higher flavonoid content in leaf extract compared to AgNPs.

Antioxidant activity

Antioxidant activity of the synthesized AgNPs and also the leaf extract of *Moringa oleifera* were done using antioxidants assays such as DPPH assay, Iron chelating assay, ABTS assay, and Nitric oxide assay. The determination was shown in Table 3.

Bioactive compound	Total phenolic content (mg GAE/g dry weight ± SE)	Total flavonoid content (mg QE/g dry weight ± SE)
Leaf extract	123.00 ± 7.31	179.60 ± 1.33
AgNPs	130.00 ± 6.74	93.00 ± 6.56

Table 2: The total phenolic content (mg GAE/g dry weight ± SE) and total flavonoid content (mg QE/g dry weight ± SE) of *Moringa oleifera* leaf extract and AgNPs synthesized from it.

GAE = Gallic acid equivalent

QE = Quercetin equivalent

Sample	EC50 values of radical scavenging activity (mg/ml) ± SE			
	DPPH Scavenging	ABTS Scavenging	Iron Chelating	NO Scavenging
Ascorbic acid	0.087 ± 0.001	-	-	0.030 ± 0.001
EDTA	-	-	0.080 ± 0.009	-
BHT	-	0.033 ± 0.002	-	-
Plant extract	2.383 ± 0.051	0.282 ± 0.024	0.734 ± 0.067	0.091 ± 0.001
AgNPs	6.976 ± 0.325	2.033 ± 0.255	7.500 ± 1.25	0.180 ± 0.004

Table 3: The antioxidant capacity of *Moringa oleifera* leaf extract and silver nanoparticles synthesized using it expressed in EC_{50} (mg/ml ± SE).

The radical scavenging activity with low concentration of sample needed was highest in NO followed by ABTS, DPPH and lastly in Iron chelation for the AgNPs. The amount of AgNPs in mg/ml required to produce the effect was 0.180mg/ml ± 0.004 for NO, 2.033 mg/ml ± 0.255 for ABTS, 6.976 mg/ml ± 0.325 for DPPH and, 7.500 mg/ml ± 1.25 for iron chelating. While, for the leaf extract, radical scavenging activity was highest in NO then followed by ABTS, iron chelating, and lastly DPPH. The amount of leaf extract needed in µg/ml are 0.091 ± 0.001 for NO, 0.282 ± 0.024 for ABTS, 0.734 ± 0.067 for iron chelating and, 2.383 ± 0.051 for DPPH. From the observation, it was clear that both leaf extract and AgNPs show the highest antioxidant activity using NO radical method.

Correlation between total phenolic content, total flavonoids contetn, and antioxidant activity

Correlation coefficient, r value determination was used to relate the relationship between total phenolic and flavonoid content with the antioxidant capacity of the leaf extracts of *Moringa oleifera* and AgNPs synthesized using it.

Assays	Total Phenolic Content (TPC)		Total Flavonoids Content (TFC)	
	r value	P value	r value	P value
DPPH	0.4662	0.4111 NS	-0.9921	0.0001**
Iron chelating	0.1719	0.7447 NS	-0.8943	0.0162**
ABTS	0.5304	0.2790 NS	-0.9428	0.0048**
NO	0.4099	0.4233 NS	-0.9880	0.0002**

Table 4: Statistical analysis of correlation coefficient, r between phenolic and flavonoid content with other antioxidant assays in plant extracts of *Moringa oleifera* and silver nanoparticles synthesized.

P value <0.05 is set at significant: significant (**),(NS) Not significant at 95%

Antibacterial assay

AgNPs (5mg/ml) demonstrated antimicrobial activity against chosen pathogenic microorganisms, while the leaf extract (5mg/ml) of *Moringa oleifera* did not show inhibition. Two types of bacteria, one Gram-negative (*E. coli*) and one Gram-positive (*S. aureus*) were used to test zone of inhibition. Among them, *E. coli* showed a larger zone of inhibition compared to *S. aureus*. This might be due to the difference in their cell wall composition. Silver nitrate solution was tested and similar size zone of inhibition in both Gram-negative and Gram-positive bacteria to AgNPs. Tetracycline was used as the positive control and distilled water was used as the negative control.

Figure 9(a): Disk diffusion test of positive control (Tetracycline) on Gram-positive *S. aureus* bacteria.

Figure 9 (b): Disk diffusion test of positive control (Tetracycline) on Gram-negative *E. coli* bacteria.

Figure 9 (c): Disk diffusion test of negative control on Gram-positive *S. aureus* bacteria.

Figure 9 (d): Disk diffusion test of negative control on Gram-negative *E. coli* bacteria.

Sample	Zone of Inhibition (mm) ± SE			
	Positive Control		Negative Control	
	S.aureus	E.coli	S.aureus	E.coli
Silver nitrate	10.00 ± 0.58	12.33 ± 0.33	8.33 ± 0.33	10.67 ± 0.33
AgNPs	9.33 ± 0.33	11.67 ± 0.33	7.67 ± 0.33	10.67 ± 0.33
Leaf extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Tetracycline	22.00 ± 0.58	24.00 ± 0.58	-	-

Table 5: Zone of inhibition in antimicrobial activity of AgNPs, Leaf extract, silver nitrate and Tetracycline.

Paired Parametric t-test (E.coli vs S.aureus)	Silver nanoparticles	Silver nitrate
Mean of differences ± SE	2.6667 ± 0.3333	2.333 ± 0.3333
t-value	5.0600	3.6391
p-value	0.0002**	0.0022**

Table 6: Statistical analysis of antimicrobial test with silver nanoparticles and silver nitrate.

P < 0.05 was set as significant: Highly significant*

Discussion

Phytochemical screening

The qualitative phytochemical screening of *Moringa oleifera* leaf extract showed the presence of several secondary metabolites such as tannins, glycosides, saponins, phenols, flavonoids, phytosterols, and terpenoids as shown in Table 1. These findings further confirm the formation of AgNPs by the aid of phyto constituents present in the leaf extract of *Moringa oleifera* [24]. Besides that, they are also known to possess various medicinal properties and importance too. For example, according to research, tannins have the ability to act as an antibacterial agent [25]. It is also known to have anti-tumour and anti-viral activities as well [26]. Their mechanism of action work via microbial protein precipitation which makes the unavailability of nutritional protein for them. Glycosides present in plants called cardiac glycosides are well known for their importance in treatment of congestive heart failure and cardiac arrhythmia besides having antibiotic and antitumor activity [27]. While, for steroids derived from plants are known to have cardio energizer effect and also have antibacterial and insecticidal properties as well [28].

These compounds were found to be responsible for the biological characteristics of the plant in producing the AgNPs [8]. Most of the parts from the plant are being used for traditional medicinal uses. The leaves of the *Moringa oleifera* plant especially known for their wound healing properties and also for other properties such as hypotensive, antitumor and cardio protection [29]. They are also highly toxic for many different pathogenic microorganisms but not for humans.

Characterization of silver nanoparticles

The change in the colour of the mixture to dark brown was because of the excitation of the Surface Plasmon Resonance (SPR), usually, AgNPs will be having absorbance values in the visible range of 400–440 nm as reported by Ahmed [2]. Besides that, the peak observed also indicates the reduction action of silver nitrate into AgNPs. The formation of AgNPs in our case can be observed via the colour change when leaf extract and silver nitrate solution were mixed and incubated in water bath. This observation was due to changes in the morphology of AgNPs formed with time. This reaction was caused by the excitation of surface plasmon due to reduction reaction taking place in the mixture [30]. The formation of AgNPs were further confimed using UV–Visible spectroscopy which showed peak at 419nm which was in the range of AgNPs literature evidences. The spectrum is important to show the role of silver nitrate solution and the existence of ingredients such as the reducing agent and capping agents in the leaf extract that is responsible for the formation of AgNPs [31].

The SEM image of AgNPs produced could be viewed because of the interaction of hydrogen bond and electrostatic interaction in between the capping agents bound to the AgNPs produced [32]. This result further confirms that *Moringa oleifera* leaf extracts contains matters that are able to act as reducing agent and capping agent that produces the AgNPs.

The diffracted intensities of XRD were collected from 20° to 80°. From the pattern, four strong Bragg reflections, which gives the angles for the scatter from a crystal lattice were observed at 38.45°, 44.6°, 64.8° and 77.6° which relates to the planes of (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of silver respectively [33]. The pattern of synthesized AgNPs from *Moringa oleifera* leaf extract found to possess a face cantered cubic (FCC) structure. While, those unidentified peaks occur due to the capping agent of plant extract stabilising the silver nanoparticles formed [34]. The crystalline size of the AgNPs produced was calculated by Debye-Scherrer's formula:

$$D = K\lambda/\beta cos\theta$$

From the formula, where D stands for the average crystallite size, K is the Scherer's constant (K = 0.94), λ is the X-ray wavelength (1.54056 Ang), β is the full-width at half-maximum of diffraction line in radians and θ half diffraction angle. The average crystallite size was found to be 24 nm which also tallies with a study by Theivasanthi & Alagar [35].

The FTIR peak observed at 3771 cm^{-1} can be related to the presence of amide (N-H) group. Then, two more very broad peaks of the AgNPs were observed at 3237 cm^{-1} and 2972 cm^{-1} signifies the stretching vibration of hydroxyl group (OH) bond, alkane group (C-H), and also amide (N-H) bonds respectively. Two sharper peaks were identified at 2373 cm^{-1} and 2342 cm-1 signifying stretch vibration of nitriles (C≡ N) bonds. A broader and stronger peak was observed at 1634 cm^{-1} signify the aromatic (C=C) stretch and amine (C=N) bond. Then, peak at 1528 cm^{-1} and 1375 cm^{-1} relates the presence of the aromatic stretch (C=C) stretch. Peaks at 1232 cm^{-1} and 1058 cm-1 signify the amines (C-N) stretching in the molecule being tested. The presence of these bonds or functional groups indicates proteins and phenolic group's besides terpenoids and tannic acids existence which shows the capability to reduce silver ions and stabilize the AgNPs being formed [36,37]. Few studies by [38-40] say that the existence of proteins in the synthesized AgNPs is responsible to act as reducing and stabilizing agents for the nanoparticles formation. This is further explained by the strong affinity of the phytochemicals to bind metal ions such as silver ions in our study. The carbonyl groups of amino acids together with the peptides of proteins present have the ability to encapsulate nanoparticles that leads to the stabilization of nanoparticles synthesized. This further prevents the aggregation of those AgNPs produced.

Total phenolic and total flavonoid content

Redox potential in the phenolic compounds are their vital characteristic to act as an antioxidant [41]. Their hydroxyl groups are the matter that facilitates their free radical scavenging ability. Hence, the total phenol concentration may possibly be used as a source for the screening of antioxidant activity. Flavonoids are well known as a plant's secondary metabolites. Flavonoids in plant plays an important role in their antioxidant activity. This is because the antioxidant activity depends on the existence of free hydroxyl (OH) groups, mainly 3-OH [41]. Hence, both phenolic and flavonoid compounds are vital for the antioxidant activity. The high level of total phenolic content was found in AgNPs produced. While, higher level of total flavonoids content was determined in leaf extract. Theoretically, the total phenolic content should be higher in leaf extracts compared to AgNPs produced [42]. Hence, this slight difference might be due to the presence of other unknown compounds or impurities in sample.

Total antioxidant activity of silver nanoparticles

The antioxidant capacity of the *Moringa oleifera* leaf extract and AgNPs synthesized was determined by using DPPH scavenging, Iron chelating, ABTS scavenging, and Nitric oxide (NO) scavenging assays. From Table 3, the antioxidant capacity of plant extract was observed to be higher than AgNPs synthesized with lower concentration needed to provide half maximal response for all the antioxidant assays. This finding indicates us that the plant extract is more responsible for higher antioxidant capacity compared to AgNPs [43]. The most effective antioxidant assay to test for the antioxidant capacity of the *Moringa oleifera* leaf extract and AgNPs synthesized among the four is nitric oxide (NO) scavenging assay. This is because it shows the least concentration of both leaf extracts and AgNPs needed to provide half maximal response (EC50). Studies by Bhakya & Muthukrishnan [44] suggested that interaction between AgNPs and leaf extract with nitric oxide happen easily under anaerobic conditons and at room temperature because the NO radical will be very unstable with high electronegative environment that can easily accept electrons from the AgNPs or leaf extract. NO method is widely implicated in cancer, inflammation and many other pathological conditons similarly to reactive oxygen species (ROS) as well. The property of plant and silver products here are able to oppose the effect in formation of nitric oxide and prevents excessive formation of NO in human body which leads to many ill effects [45]. All the other test also strongly acclaim the potential application of AgNPs to be used as natural antioxidants for health defense against various oxidative stress associated with all kind of different degenerative diseases [46]. It is essential to evaluate antioxidant capbility of AgNPs formed beforehand being used in medical practices.

Correlation between total phenolic content, total flavonoids contetn, and antioxidant activity.

The antioxidant activity and its relation to total phenol and flavonoid contents of Moringa oliefera leaf extract and AgNPs synthesized were analyzed. From Table 4, it was observed that the EC50 of DPPH, Iron chelating, ABTS, and No scavenging activity showed positive correlation with phenolic compounds (r= 0.4662, 0.1719, 0.5304, and 0.4099) respectively and negative correlation with flavonoids (r=-0.9921, -0.8943, -0.9428, -0.9880) respectively. These findings relate to the strong influence of total phenolic and total flavonoid content to the antioxidant activity. There are many findings by researchers that show us that the flavonoids and phenolic contents in a plant directly involve or vital in their anti-oxidative action [47-49]. The strong negative and positive correlation shows us that they influence largely in the antioxidant capacity of the leaf extract and also AgNps synthesized. The improved antioxidant activity can be linked or related to the greater expression with the ability to adsorb by antioxidant functional groups onto the surface of AgNPs [50].

Antibacterial activity

Silver nitrate solution used to produce AgNPs with concentration of 8mM and the AgNPs produced shows almost same zone of inhibition to the Gram-positive (*S. aureus*) bacteria with 8.33 ± 0.33mm and 7.67 ± 0.33mm respectively. While, when tested using a Gram-negative (*E. coli*) bacteria, it was observed an increase in the zone of inhibition of both silver nitrate solution and the AgNPs produced with 10.67 ± 0.33 mm for both as shown in Table 5. Same situation was observed when tested using same two kind of bacteria with positive control. The zone of inhibition of silver nitrate solution and AgNPs produced in Gram-positive bacteria was lower with 10.00 ± 0.58 mm and 9.33 ± 0.33 mm respectively compared to zone of inhibition in Gram-negative bacteria with 12.33 ± 0.33mm and 11.67 ± 0.33 mm respectively as can be observed from Table 5 as well. This was further confirmed by the positive control antibiotic tetracycline used where it shows higher zone of inhibition in gram negative bacteria with 24.00 ± 0.58 mm compared to zone of inhibition in gram positive bacteria with 22.00 ± 0.58 mm. While, the leaf extract shows no zone of inhibition which is due to very low concentration (5mg/ml) used.

The difference in zone of inhibition shown in Gram-negative and Gram-positive bacteria might be due to the difference in their cell wall composition. The Gram-positive bacteria cell wall is composed of a thick peptidoglycan layer. This thick layer has a more rigid structure because it consists of linear polysaccharide chains that are cross linked by short peptides. Thus, making it hard for the penetration of AgNPs. While, using Gram-negative bacteria with thinner cell wall was easier for the penetration of AgNPs and higher zone of inhibition was observed [51,52].

From the statistical analysis done, significant difference of 2.6667 ± 0.3333 and 2.333 ± 0.3333 mm was seen for AgNPs and silver nitrate solution respectively. Also, a high significance between the bacteria used was recognized between the efficiency of AgNPs and antimicrobial ability. This proves that silver ions and also AgNPs possess strong bactericidal effect of silver ions and ability of their polymer subunit that can disrupt the bacterial membrane [53]. Besides that, they do have a broad spectrum of antimicrobial activities as well. Studies by Feng & Wu [52] also showed that some forms of silver are capable to be used in treatment against burns, severe chronic osteomyelitis which is an inflammation of bone or bone marrow, urinary tract infections, and also central venous catheter infections.

Conclusion

This research showed that high-density AgNPs could be synthesized using *Moringa oleifera* leaf extract. Proteins and phenolic group's besides terpenoids and tannic acids reduced silver ions and stabilized the AgNPs being formed. The higher level of total phenolic content was determined in AgNPs as compared to leaf extract. While for the total flavonoids content, plant extracts showed higher amount compared to AgNPs produced. Both the plant extract and AgNPs produced shows the highest antioxidant capacity using NO radical method. Correlation between total phenolic content, total flavonoid content and antioxidants capability showed the strong influence of phenolic compound and flavonoids in the antioxidant activity of AgNPs and leaf extracts as well. AgNPs and silver nitrate showed antimicrobial ability as they are being effective to both gram-negative and gram-positive bacteria.

The study threw new insights into the bio reduction properties of *Moringa oleifera* in synthesizing silver nanoparticles in most economic and eco-friendly method. These silver nanoparticles show better stability and can find application in nano medicine, nutraceutical and pharmaceutical fields. They can also be incorporated into various consumer products to exhibit better antibacterial properties. However further research is required to establish possible results at cellular and molecular levels.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Bibliography

1. Dua R and Shrivastava S. "Green Chemistry and Environmentally Friendly Technologies: A Review". *Middle-East Journal of Scientific Research* 11.7 (2012): 846-855.

2. Ahmed S. "Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract". *Journal of Radiation Research and Applied Sciences* 9.1 (2016):1-7.

3. Zhang, Y., *et al*. "Synergetic Antibacterial Effects of Silver Nanoparticles@Aloe Vera Prepared via a Green Method". *Nano Biomedicine and Engineering* 2.4 (2010): 252-257.

4. Patil S., *et al*. "An Investigation of Antioxidant and Cytotoxic Properties 0f Green Synthesized Silver Nanoparticles". *Indo American Journal of Pharmaceutical Sciences* 2.10 (2015): 1453-1459.

5. Kumar K. "Green Synthesis of Silver Nanoparticles Using Hydnocarpus pentandra Leaf Extract: In-vitro Cyto-Toxicity Studies against MCF-7 Cell Line". *Journal of Young Pharmacists* 10.1 (2018): 16-19.

6. Yildiztekin M. "Green Synthesis and Characterization of Silver Nanoparticles from Crocus mathewii; A Disremembered Turkish Flowering Plant". *Indian Journal of Pharmaceutical Sciences* 79.4 (2017): 536-543.

7. Selvan D., "Garlic, green tea and turmeric extracts-mediated green synthesis of silver nanoparticles: Phytochemical, antioxidant and in vitro cytotoxicity studies". *Journal of Photochemistry and Photobiology B: Biology* 180 (2018): 243-252.

8. Moodley JS., *et al*. "Green synthesis of silver nanoparticles from Moringa oleifera leaf extracts and its antimicrobial potential". *Nanoscience and Nanotechnology* 9.1 (2018).

9. Prasad T and Elumalai EK. "Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity". *Asian Pacific Journal of Tropical Biomedicine* 1.6 (2011): 439-442.

10. Mallikarjuna K and Narasimha G. "Green Synthesis of Silver Nanoparticles Using Ocimum Leaf Extract and Their Characterization". *Digest Journal of Nanomaterials and Biostructures* 6.1 (2011): 181 - 186.

11. Banso A and Adeyemo S. "Phytochemical screening and antimicrobial assessment of Abutilon mauritianum, Bacopa monnifera and Datura stramonium". *Biokemistri* 18.1 (2006): 39-44.

12. Ayoola G. "Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria". *Tropical Journal of Pharmaceutical Research* 7.3 (2003): 1019-1024.

13. Iqbal E. "Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam". *Journal of King Saud University - Science* 27.3 (2015): 224-232.

14. Bargah Rk. "Preliminary test of phytochemical screening of crude ethanolic". *Journal of Pharmacognosy and Phytochemistry* 4.1 (2015): 7-9.

15. Hossain MA. "Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris". *Asian Pacific Journal of Tropical Biomedicine* 3.9 (2013): 705-710.

16. Roopalatha U. "Phytochemical Analysis of Successive Reextracts of The Leaves of Moringa Oleifera Lam". *International Journal of Pharmacy and Pharmaceutical Sciences* 5.3 (2013).

17. Clarke G. "High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and Total Phenolics Content Indicates Redundancy in Use of All Three Assays to Screen for Antioxidant Activity of Extracts of Plants from the M". *Antioxidants* 2.1 (2013): 1-10.

18. Bibi G and Ullah N., "Phytochemical evaluation of naturally growing Aster thomsonii plant species". *Journal of Pharmacy* 2 (2003): 33-39.

19. Dinis CP. "Action of Phenolic Derivatives (Acetaminophen, Salicylate, and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Peroxidation and as Peroxyl Radical Scavengers". *Archives of Biochemistry and Biophysics* 315.1 (1994):161-169.

20. Madhanraj R. "Antioxidant Assay of Gold and Silver Nanoparticles from Edible". *Free Radicals and Antioxidants* 7.2 (2017): 137-142.

21. Moteriya P. "Characterization, synergistic antibacterial and free radical scavenging efficacy of silver nanoparticles synthesized using Cassia roxburghii leaf extract". *Journal of Genetic Engineering and Biotechnology* 15.2 (2017): 505-513.

22. P Ruparelia J and Chatterjee AK. "Strain specificity in antimicrobial activity of silver and copper nanoparticles". *Acta Biomaterialia* 4.3 (2008): 707-716.

23. Jaini D. "Synthesis of Plant-Mediated Silver Nanoparticles using Papaya Fruit Extract and Evaluation of their Anti-Microbial Activities". *Digest Journal of Nanomaterials and Biostructures* 4.3 (2009): 557 - 563.

24. Arunachalam KD. "One-step green synthesis and characterization of leaf extract-mediated biocompatible silver and gold nanoparticles from Memecylon umbellatum". *International Journal of Nanomedicine* 8 (2013): 1307-1315.

25. Akiyama H., *et al*. "Antibacterial action of several tannins against Staphylococcus aureus". *Journal of Antimicrobial Chemotherapy* 48.4 (2001): 487-491.

26. Kumari M and Jain S. "Tannins: An Antinutrient with Positive Effect to Manage Diabetes". *Research Journal of Recent Sciences* 1.12 (2012): 1-8.

27. Křen V and Řezanka T. "Sweet antibiotics - the role of glycosidic residues in antibiotic and antitumor activity and their randomization". *FEMS Microbiology Reviews* 32.5 (2008): 858-889.

28. Bagrov AY., *et al*. "Endogenous Cardiotonic Steroids: Physiology, Pharmacology, and Novel Therapeutic Targets". *Pharmacological Reviews* 61.1 (2009): 9-38.

29. Prasad T and Elumalai EK. "Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity". *Asian Pacific Journal of Tropical Biomedicine* 1.6 (2011): 439-442.

30. Sithara R. "Economical synthesis of silver nanoparticles using leaf extract of Acalypha hispida and its application in the detection of Mn (II) ions". *Journal of Advanced Research* 8.6 (2017): 561-568.

31. Ananda K., *et al*. "Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity". *Applied Nanoscience* 6.3 (2016): 399-408.

32. Al-Kalifawi EJ. "Green Synthesis of Silver Nanoparticles Using Leaf Extract of Al-Rawag tree (Moringa oleifera Lamarck) Cultivated in Iraq and Efficacy the Antimicrobial activity". *Mesopotamia Environmental Journal ISSN* 2410, A (2016): 39-48.

33. Jyoti K. "Characterization of silver nanoparticles synthesized using Urtica dioica Linn. Leaves and their synergistic effects with antibiotics". *Journal of Radiation Research and Applied Sciences* 9.3 (2016): 217-227.

34. Annamalai A and Christina V. "Green synthesis and characterisation of AgNPs using aqueous extract of Phyllanthus maderaspatensis L". *Journal of Experimental Nanoscience* 9.2 (2014): 113-119.

35. Theivasanthi T and Alagar M. "Electrolytic Synthesis and Characterizations of Silver Nano powder". *Nano Biomedicine and Engineering* 4 (2012): 58-65.

36. Umoren SA. "Green Synthesis and Characterization of Silver Nanoparticles Using Red Apple (Malus domestica) Fruit Extract at Room Temperature". *Journal of Materials and Environmental Science* 5.3 (2014): 907-914.

37. Balashanmugam P and Kalaichelvan PT. "Biosynthesis characterization of silver nanoparticles using Cassia roxburghii DC. Aqueous extract, and coated on cotton cloth for effective antibacterial activity". *International Journal of Nanomedicine* 10.1 (2015): 87-97.

38. Reddy R and Morais AB. "2-Diphenyl-1-Picrylhydrazyl Free Radical Scavenging Assay and Bacterial Toxicity of Protein Capped Silver Nanoparticles for Antioxidant and Antibacterial Applications". *Asian Journal of Chemistry* 25.16 (2013)" 9249-9254.

39. Ajitha B., *et al*. "Green synthesis and characterization of silver nanoparticles using Lantana camara leaf extract". *Materials Science and Engineering*: C 49 (2015): 373-381.

40. Alshaye NA and Elobeid MM. "Characterization of Biogenic Silver Nanoparticles by Salvadora persica Leaves Extract and its Application against Some MDR Pathogens E. coli and S. aureus". *Research Journal of Microbiology* 12.1 (2017): 74-81.

41. A Baba S and Malik SA. "Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume". *Journal of Taibah University for Science* 9.4 (2015): 449-454.

42. Burri SC. "Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used". *Journal of Functional Foods* 38 (2017): 119-127.

43. Aziz MS and Shaheen MS. "Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using Chenopodium murale leaf extract". *Journal of Saudi Chemical Society* 18.4 (2014): 356-363.

44. Bhakya S and Muthukrishnan S. "Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity". *Applied Nanoscience* 6.5 (2016): 755-766.

45. Nandhakumar E and Parameswaran I. "In vitro Antioxidant Activities of Methanol and Aqueous Extract of Annona squamosa (L.) Fruit Pulp". *Journal of Acupuncture and Meridian Studies* 6.3 (2013): 142-148.

46. Mohanta YK. "Biosynthesis of Silver Nanoparticles from Protium serratum and Investigation of their Potential Impacts on Food Safety and Control". *Front Microbiol* 8 (2017): 626.

47. Baharara J., *et al*. "Antioxidant and anti-inflammatory activity of green synthesized silver nanoparticles using Salvia officinalis extract". *Annals of Tropical Medicine and Public Health* 10.5 (2017): 1265-1270.

48. Mozarina M and Almeida B. "Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil". *Food Research International* 44.7 (2011): 2155-2159.

49. Malta LG. "Assessment of antioxidant and antiproliferative activities and the identification of phenolic compounds of exotic Brazilian fruits". *Food Research International* 53.1 (2013): 417-425.

50. Dhanapal ACTA and Ping HH. "Investigation of Green Synthesized Silver Nanoparticles Using Aqueous Leaf Extract of Artemisia Argyi for Antioxidant and Antimicrobial Potentials". *International Journal of Pharmaceutical Quality Assurance* 8.4 (2017): 190-199.

51. Gomathi M. "Green synthesis of silver nanoparticles using Datura stramonium leaf extract and assessment of their antibacterial activity". *Resource Efficient Technologies* 3.3 (2017): 280-284.

52. Feng QL and Wu J. "A mechanistic study of the antibacterial effect of silverions on Escherichia coli and Staphylococcus aureus". *Journal of Biomedical Materials Research* 52.4 (2000): 663-668.

53. Singh A and Jain D. "Green Synthesis of Silver Nanoparticles using Argemonemexicana Leaf Extract and Evaluation of their Antimicrobial Activities". *Digest Journal of Nanomaterials and Biostructures* 5.2 (2010): 483-489.

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