RESEARCH ARTICLE

Impact of Ocimum Tenuiflorum Mediated Green Synthesis of Silver Nanoparticles on *In-Vitro* Antioxidant and Antibacterial Activities

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ABSTRACT

The leaf extract of *O. tenuiflorum* was used to synthesize silver nanoparticles (AgNPs) and evaluated for its antioxidant and antibacterial properties. The silver nanoparticle was characterized using the UV-Vis spectrophotometer, SEM, FTIR, and XRD. The total phenolic and total flavonoid contents were determined for both leaf extract and synthesized silver nanoparticles. Antioxidant activities before and after synthesis of silver nanoparticles was assessed by DPPH, ABTS, iron chelating, and NO radical scavenging methods. The antibacterial activity of the leaf extract and AgNP were tested against *Escherichia coli* and *Staphylococcus aureus*. Statistical analysis was carried out to establish possible relations between the antioxidant, antibacterial and antioxidant activities. The formation of a dark brown solution mixture confirms the formation of silver nanoparticles at a wavelength of 450 nm. The AgNPs synthesized were spherical, with the size between 14 to 33 nm. Functional groups such as alcohol, aldehyde, nitrile, primary amines, carbonyl, and aromatic groups were confirmed by FTIR and XRD. Total phenol was higher in leaf extract, while total flavonoids were higher in the AgNps. Silver nanoparticles exhibited strong NO scavenging activity while leaf extract showed better ABTS scavenging activity. Silver nanoparticles inhibited *E. coli* better compared to *S. aureus* bacteria. It can be coined that the leaf extract of *Ocimum tenuiflorum* mediated the green synthesis of silver nanoparticles and possess strong antioxidant and antibacterial potentials that can find application in various biomedical areas.

Keywords: green synthesis, nanoparticles, phytochemicals, UV-Vis, SEM, FTIR, XRD antioxidant activity, antibacterial activity.

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INTRODUCTION

The word "Nano" evolved from nanos, which carries the meaning of dwarf in Greek. Nano (Symbol n) is also known as a unit prefix, which means one billionth or denotes 10^{-9} meter. Nano-meter sized objects are dealt with by technology that is being empowered by nanotechnology. The term was first coined by Professor Norio Taniguchi of Tokyo Science University, who explained the precision production of substances on the nanometer level (Taniguchi, 1974). 'There's plenty of room at the bottom' was a path-breaking lecture, and an idea of nanotechnology was given by an American physicist Professor Richard P. Feynman (Feynman, 1959). The use of silver nanoparticles is increasing swiftly by, and largely due to the advantages promised by means of their incorporation into merchandise. Other than the various applications in science and medicinal drug (Salata , 2004), silver nanoparticles now find application in a growing number of products such as household usages, cosmetics, food packaging, and odor resistant fabrics.

The synthesis, characterization, and application of metal nanoparticles with size usually ranging from 1 to 100 nm are an emerging area of nanoscience and nanotechnology.[1] Numerous synthetic techniques had been evolved for the synthesis of metal nanoparticles which includes photochemical,[2] radiochemical,[3] electrochemical,[4] chemical[5] and biological methods.[6] Amongst all the techniques, a stabilizing agent is present in the chemical reduction of a metal salt to avoid aggregation of the metallic nanoparticle. This is the most regularly carried out technique for the technology of nanoparticles as a strong colloidal dispersion in organic or water solvents.[7] The main disadvantage of the chemical method is that it uses numerous chemical reducing agents which includes hydrazine, N, N-dimethyl formamide, sodium borohydride and polyols in addition to the stabilizers including surfactants, artificial polymers, and dendrimers which causes chemical toxicity

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and severe environmental issues, therefore restricting their application particularly in biomedical applications.[8]

Consequently, it's far vital to increase facile and environmentally gentle synthetic techniques for the research of metal nanoparticles, especially in a massive scale. Currently, there was an increased interest on green chemistry strategies with the objective of lessening environmental endangerments.[9] The choice of a non-toxic reducing agent, a profitable and without problems renewable stabilizing agent and an environmentally benign solvent machine are the three fundamental standards for a 'greener' nanoparticles synthesis.[10] Hence the current study aimed to use green technology in the synthesis of silver nanoparticles and test their efficacy on the antibacterial and antioxidant activities.

MATERIALS AND METHODS

Preparation of Leaf Extract

The *Ocimum Tenuiflorum* leaves were rinsed with running tap water and continued to rinse in distilled water for about three times. The leaves were oven-dried for 24 hours at 37ºC. The dried leaves were powdered with a domestic blender and it was stored in a glass bottle. About 5 g of *Ocimum Tenuiflorum* leaf powder was soaked in 100 mL of distilled water overnight. The next day, the leaf extraction was obtained by using cheesecloth, and on the same cheesecloth, about 50 mL distilled water was added to completely rinse of the aqueous leaf extract from the cheesecloth. This was stored at 4ºC and was used within one week for further analysis. A portion of the aqueous leaf extracts was freeze-dried until the constant weight was reached and stored at 4ºC.

Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles using aqueous leaf extract and silver nitrate (AgNO3) was adapted from (Dhanapal, et al., 2016) with slight modification. 0.01 M aqueous solution of $AgNO_3$ was prepared by dissolving 0.33974 g of $AgNO_3$ salts into 200 mL of distilled water and used for the synthesis of silver nanoparticles. About 10 mL of KT aqueous extract and 90 mL of 0.01 M of $AgNO_3$ was measured and poured into a conical flask. The mixture was stirred for 10 minutes, and then the flask was kept in the dark at room temperature for 3 hours.

Characterization of Silver Nanoparticles

Five percent of leaf extract in 100 mL of distilled water was added with 90 mL of AgNO3. The color change and absorption spectrum of the silver nanoparticles solution was observed every 30 minutes during the 3 hours of incubation. The absorption spectrum was recorded using a UV-Vis spectrophotometer (GENESYS 10) from 300 nm to 800 nm and the wavelength of the absorption peak was recorded. The sample size was analyzed using a scanning electron microscope (JEOUL USAJSM – 7610F) at magnification of 40,000X. The samples were characterized with FTIR instrument (Perkin-Elmer). The spectrum was taken from 4000.0 cm^{-1} to 400.0 cm^{-1}. The peaks that fell into this range will be interpreted based on the functional groups that it represents. The freeze-dried silver nanoparticle powder was transferred into the sample holder of the X-Ray Diffractometer (Siemens D500). The spectrum was recorded at 2θ range between 10º to 80º.

Determination of Total Phenolic Content

Freeze-dried leaf extract and synthesized silver nanoparticle powders were used to determine the total phenolic content by using Folin-Ciocalteu colorimetric assay, as described previously with modification.[11,12] About 100 µL of the leaf extract powder (1 mg/mL) and silver nanoparticle powder (1 mg/mL) was added up with 750 µL of Folin-Ciocalteu reagent (2 mL Folin-Ciocalteu reagent + 18 mL distilled water) and it was kept in the dark for 5 minutes at room temperature. Then 750 µL of 6 % sodium bicarbonate solution was added and mixed well by using the vortex. It was allowed to incubate for 90 minutes at room temperature. For the blank, the samples were replaced with distilled water. The absorbance of the mixture was recorded at 725 nm. A calibration curve was prepared using 0 to 0.10 mg/mL gallic acid. Total phenolic content of the leaf extract and synthesized silver nanoparticle were expressed in terms of Gallic acid equivalent (GAE) (mg/g and or mg/L). This assay was performed in triplicate, and the absorbance is expressed as mean ± standard errors (SE).

Determination of Total Flavonoid Content

The total flavonoid content of leaf extract and synthesized silver nanoparticle was determined by the aluminum chloride colorimetric method, as described previously with modification.[12,13] About 150 µL of 5 % of $NaNO_2$ was added into each 2.0 mL microcentrifuge tube containing the 100 µL of leaf extract powder (3 mg/mL) and synthesized silver nanoparticle powder (1 mg/mL) separately and mixed properly with the help of vortex. The microcentrifuge tubes were incubated at room temperature for 6 minutes. To that, 150 µL of 10% aluminum chloride solution was added mixed thoroughly and incubated at room temperature for another 6 minutes. Then, 800 µL of 10 % sodium hydroxide solution was added and mixed well, and incubated again at room temperature for about 15 minutes. For the blank, the samples were replaced with distilled water. The absorbance of the mixture was recorded at 510 nm. The calibration curve was plotted by using 0 to 1.0 mg/mL quercetin hydrate as the standard solution (The total flavonoid contents of the leaf extract and synthesized silver nanoparticles were expressed in terms of quercetin equivalent (QE) (mg/g or and mg/L). The test was done in triplicates and expressed as mean ± standard error (SE).

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The DPPH radical scavenging activity of leaf extract and synthesized silver nanoparticle was determined by method used by Thaipong et al., 2006; Lim & Quah, 2007 and Wong et al., 2012.[12,14,15] DPPH stock solution was prepared by dissolving 24 mg DPPH into 100 mL of ethanol. Then, DPPH working solution was prepared by adding and mixing 10 mL of the stock solution with 45 mL ethanol. In 50 µL of varying concentration of leaf extract (0 to 5 mg/mL) and synthesized

silver nanoparticle (0 to 30 mg/mL), 1 mL of DPPH working solution was added and mixed well with the help of vortex. It was left in the dark at room temperature for 30 minutes. The absorbance of each sample was measured at 517 nm. For the blank, varying concentrations of leaf extract and silver nanoparticle were added with 1 mL of ethanol instead of adding 1 mL of DPPH working solution. The activity of DPPH radical scavenging (%) was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100$$

Where, Acontrol = absorbance of control reaction (without leaf extract and synthesized silver nanoparticle). Asample = absorbance in the presence of a leaf extract and synthesized silver nanoparticles. Ascorbic acid (0 to 0.10 mg/mL) was used as a reference. Results are presented in EC50 values, which represents a concentration of leaf extract and silver nanoparticle required to scavenge 50 % of the DPPH radicals.

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) Radical Cation Scavenging Ability Assay

The ABTS radical cation scavenging activity of leaf extract and synthesized silver nanoparticles was determined based on the methods described by Wong, et al. (2012).[12] The ABTS stock solution (8 mg/mL) was prepared by dissolving 0.4 g of ABTS in 50 mL of distilled water. Potassium persulfate, $K_2S_2O_2$ (1.32mg/mL) was prepared by dissolving 0.066 g of potassium persulfate in 50 mL distilled water. An equal volume was measured from each prepared solution and then mixed and kept in the dark (amber bottle) at room temperature for 12 to 16 hours, allowing them to react. Then, solution A (2.72 g potassium dihydrogen phosphate (KH2PO4) dissolved with 200 mL of distilled water) and solution B (3.48 g potassium hydrogen phosphate (K2HPO4) dissolved with 200 mL of distilled water) was prepared. 100 mL of 100mM, pH 7.4 potassium phosphate buffer was prepared by measuring 19 mL of solution A (200 mM KH2PO4) and 81 mL of solution B (200 mM K2HPO4) and poured together into a 150 mL Scott bottle. The ABTS working solution was prepared by diluting 250 µL of the $ABTS^+$ stock solution with 10 mL of 100 mM, pH 7.4 potassium phosphate buffer to obtain an absorbance of 0.700 $\pm$ 0.005 at 734 nm. Then, 100µL of varying concentration of leaf extract (0 to 0.30 mg/mL) and synthesized silver nanoparticle (0 to 1.0 mg/mL) were added with 1 mL of ABTS working solution. The mixture was allowed to incubate at room temperature in the dark for 10 minutes. For the blank, the varying concentration of leaf extract and silver nanoparticle was added with 100 µL of 100 mM, pH7.4 potassium phosphate buffer. The absorbance was read at 734 nm. The ABTS working solution was prepared freshly on that day just before the analysis and was kept in amber bottle. The ability of ABTS radical cation scavenging (%) was calculated by using the formula given below:

$$\text{ABTS radical scavenging ability (\%)} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100$$

Where Acontrol = absorbance of control reaction (without leaf extract and synthesized silver nanoparticle). Asample = absorbance in the presence of a leaf extract and synthesized silver nanoparticles. Butylated hydroxytoluene (BHT) (0 to 0.025 mg/mL) was used as standard and the results were expressed as BHT equivalent antioxidant capacities value and EC50 which is the concentration required to scavenge 50 % of ABTS radicals.

Iron Chelating Activity Assay

The iron-chelating activity assay performed following the methods suggested by Chai & Wong, (2012), Hassan & Jamaludin (2012).[16] 0.039 g of ferrous chloride tetrahydrate (FeCl2.4H2O) was dissolved in 100 mL distilled water to prepare 2 mM ferrous chloride (FeCl2) solution. 5 mM ferrozine solution was freshly prepared by dissolving 0.123 g ferrozine in 50 mL distilled water. Varying concentration of leaf extract (0 to 1.0 mg/mL) and AgNp (0 to 10 mg/mL) was prepared. 200 µL of varying concentrations of both samples were mixed well by vortex after the addition of 200 µL of 2mM ferrous chloride. Then, 400 µL of 5 mM ferrozine solution was added to all the sample mixture. The mixture was left for incubation at room temperature in the dark for 10 minutes. For the blank, varying concentrations of leaf extract and AgNP were added with 200 µL of distilled water. The absorbance was measured at 562 nm. The percentage of metal-chelating activity was calculated using the following formula:

$$\text{Metal chelating activity (\%)} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100$$

Where Acontrol = absorbance of control reaction (without leaf extract and synthesized silver nanoparticle). Asample = absorbance in the presence of a leaf extract and synthesized silver nanoparticle. Ethylenediaminetetraacetic acid, EDTA (0 to 0.012 mg/mL) was used as the standard. The data collected were expressed in EC50 as the minimum concentration required to inhibit 50% of ferrozine-Fe2+ complex.

Nitric Oxide (NO) Scavenging Activity Assay

The nitric oxide (NO) scavenging activity of the leaf extract and AgNP was determined following method given by Wong, et al. (2012). The Griess reagent was prepared by adding and mixing 2 mL of 1 % sulphanilamide (0.02 g sulphanilamide dissolved in 2 mL of distilled water), 2 mL 0.1 % N-(1-naphthyl) ethylenediamine dihydrochloride (0.002 g N-(1-naphthyl) ethylenediamine dihydrochloride dissolved in 2 mL of distilled water), and 2 mL of 5% phosphoric acid (0.12 mL of 85% phosphoric acid was diluted with 1.88 mL of distilled water). Phosphate-Buffer Saline (PBS) was prepared by adding the following reagents:8 mL of distilled water + 0.08 g of sodium chloride, NaCl + 0.014 g sodium hydrogen phosphate, Na_2HPO_4 + 0.0024 g monopotassium phosphate (KH_2PO_4). 5.68 mM sodium nitroprusside solution (pH 7.4) was prepared with 0.0169 sodium nitroprusside dihydrate dissolved in 10 mL PBS. About 800 µL of varying concentration of leaf extract (0 to 0.5 mg/L) and AgNP (0 to 0.25 mg/mL) was added with 200 µL of sodium nitroprusside and the mixture was incubated at room temperature under light source (10 cm length between a sample and the light source) for 30 minutes followed by addition of 50

µL of Griess reagent to all samples and incubated in the dark for 10 minutes. For the blank, 200 µL PBS and 50 µL of Griess reagent was added to all the varying concentration of leaf extract and AgNps. The mixtures were well vortexed before transferring them into cuvette for the measuring absorbance. The absorbance was read at 546 nm. The activity of -NO radical scavenging (%) was calculated using the formula:

$$\text{NO scavenging activity (\%)} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100$$

Where, Acontrol = absorbance of control reaction (without leaf extract and synthesized silver nanoparticle). Asample = absorbance in the presence of a leaf extract and synthesized silver nanoparticle. Ascorbic acid (0 to 0.05 mg/mL) was used as the reference standard. The results were expressed in as EC50 value.

Antibacterial Assay

Disc diffusion method[17] with slight modification was used to assess the antibacterial property of the leaf extract and AgNP against *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive) bacteria. The bacteria cultures of *E. coli* and *S. aureus* were incubated in nutrient broth at 35ºC for 24 hours. Then, Mueller-Hinton (MH) agar was prepared and autoclaved. After autoclave, the agar was collected from the oven, and the pour plate was done. The Petri dishes were sealed properly with a parafilm and kept in the refrigerator to allow proper solidification of agar to take place. The next day, each bacteria culture from the nutrient broth were streaked (four-quadrant streak) on the MH agar to isolate a pure strain from a single species of bacteria. The plates were incubated at 35ºC for 24 hours. The next day, 2-3 single colonies from both bacteria were picked by using the loop from the streak plate and were mixed with 0.85 % saline solution to compare the turbidity of bacterial suspension with 0.5 % McFarland standard. Then, the MH agar plates were streaked using sterile cotton swab with saline solution containing *E. coli* and *S. aureus* separately. The discs were immersed with distilled water (negative control), leaf extract (5 mg/mL), 0.01M silver nitrate solution, and AgNPs (5mg/mL) separately using a sterile forceps on the MH agar plates that were already streaked by respective bacteria. The plates were all sealed well with parafilm and allowed to incubate at 35ºC for 24 hours. Tetracycline was used as a positive control. The experiment was done under aseptic conditions. After 24 hours of incubation, the zone of inhibition was measured in millimeters (mm) by using a ruler.

RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles

The colour change from clear yellow to dark brown was the primary indication of the successful synthesis of silver nanoparticles. The colour change indicates the Ag^{+} has been reduced to Ag^{0} by the presence of biomolecules in the leaf extract.[18] Similar color change were observed in the leaf extracts *Artemisia argyi,* marigold flower, *Calliandra haematocephala,* and *Coleus aromaticus*.[19-21]

UV-Vis Spectroscopy

The maximum absorption shown in the UV-Vis spectrum was at 450 nm, which confirms the formation of silver nanoparticles. The reductions of silver ion to form the synthesized silver nanoparticles were confirmed by UV-Vis spectra by measuring

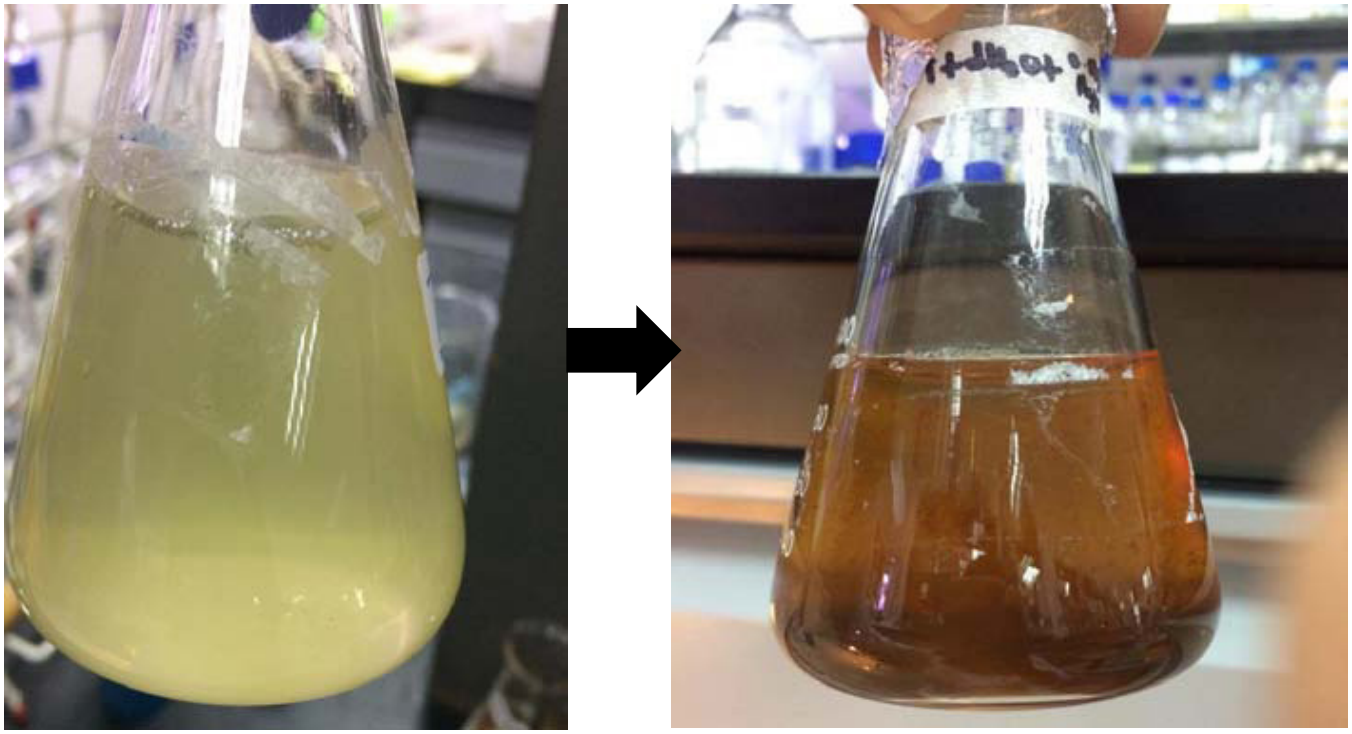

Fig. 1: Colour change after the addition of AgNO3 to the leaf extract

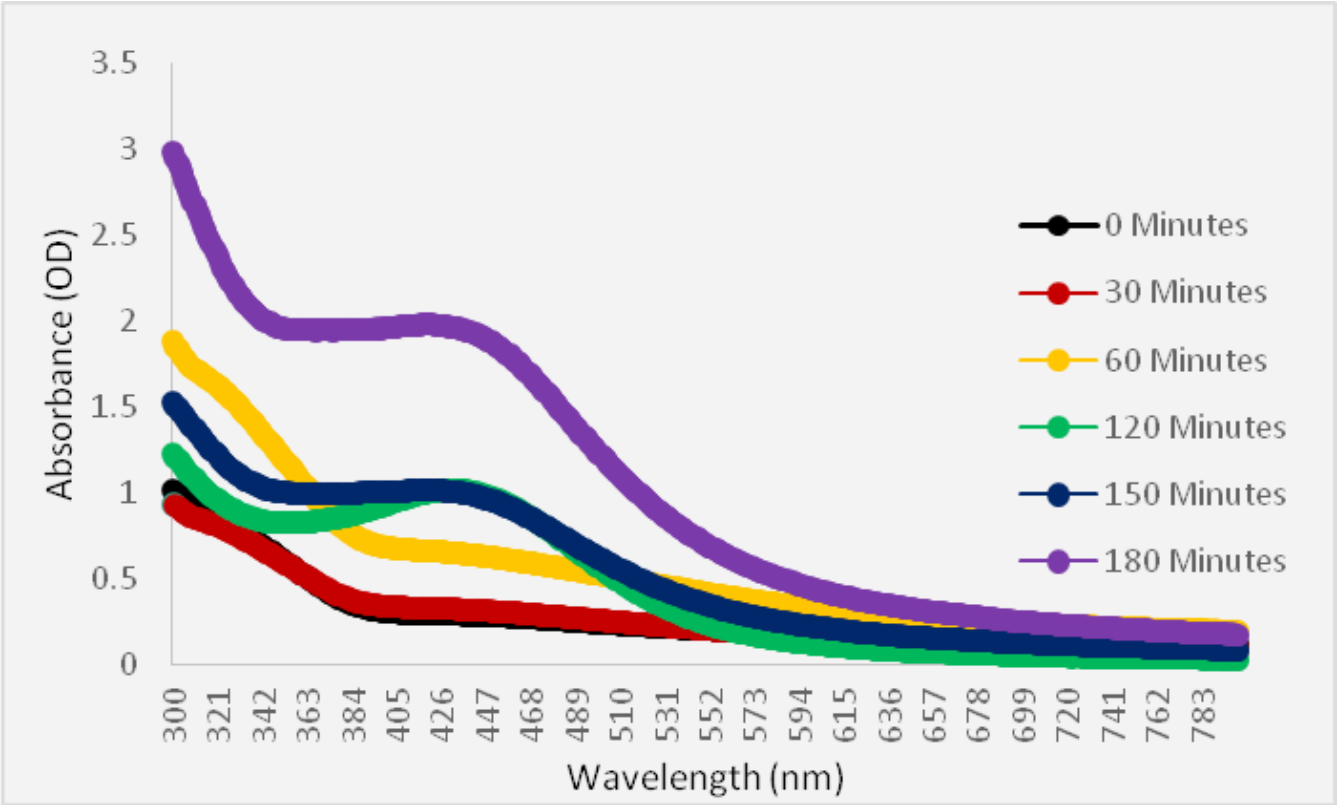

Fig. 2: The formation of silver nanoparticles as measured by UV-Visible Spectrophotometer

every 30 minutes. The UV-Vis range from 420 to 450 nm is an indication of the presence of surface plasmon resonance (SPR) of synthesized silver nanoparticles.[17,22,23] The silver nanoparticle synthesis from 10 minutes to 180 minutes indicates no significant modifications in the shift of SPR band and it is assumed that no changes were found in the size of nanoparticles.[24] After 2 hours, the complete formation of silver nanoparticles was obtained when the intensity decreases in the UV-Vis spectrum.[25]

Scanning Electron Microscope (SEM)

Characterization of synthesized silver nanoparticles aids in understanding the morphology of the particles.[25] The particle size of the synthesized silver nanoparticles was found to be in the range of 14 nm to 33 nm, which fits into the range of nanoscale. The particles were spherical in shape based on the SEM analysis. The image also indicates that synthesized silver nanoparticles were well dispersed in various size ranges. The SEM analysis was found to be in line with UV-Vis spectrum data.

Fourier Transformed Infrared Spectroscopy (FTIR)

Since leaf extract have reducing agent and capping agent in the form phenolic and flavonoids,it was confirmed by FTIR analysis of the synthesized silver nanoparticles using *O. tenuiflorum*. Figure 4 shows FTIR analysis that identifies the presence of various functional group. The peaks were interpreted (Table 1) for functional group region and fingerprint region. The peak at 3750 cm-1 was due to the presence of alcohol group (-OH) stretching with medium and sharp peak. Then, peak at 3422 cm-1 indicates the presence of alcohol groups (-OH) stretching with strong and broad peak. A medium peak with C-H stretching indicates the presence of alkane group at 2923 cm^{-1}. A sharp and strong peaks at 2373 cm^{-1} and 2345 cm–1 indicates the presence of Nitrile group (C≡N) vibrational stretch. At 1623 cm^{-1}, a strong and broad peak was observed and it was due the overlapping of primary amines group (C-N) to carbonyl group (C=O) stretch and aromatic group (C=C) stretch. This also suggest that there were higher chances for the silver nanoparticles for binding with the protein present in leaf extract (Prakash, et al., 2013). The medium peak at 1388 cm–1 was due to the presence of aldehyde group (C-H) bending. The amine group (C-N) stretch was found at 1247 cm^{-1} and 1052 cm^{-1} with a medium peak. Based on the functional groups that were present in the synthesized silver nanoparticles, it can be said that these groups were mainly involved in as reducing agent and also acts as a stabilizing for the synthesis of silver nanoparticles. Similar FTIR spectrums were obtained by (Martínez-Castañón, et al., 2008; Raja, et al., 2015).[18,26]

X-ray Diffraction (XRD)

The peaks of the XRD data were analysed using the JCPDS (file no. 04-0783) and they were also analysed using the (Match! software). The peaks at 32.334º, 33.46º, 27.91º and 76.82º correspond to the lattice parameters (111), (200), (220), and (311) respectively. These lattice parameters report the

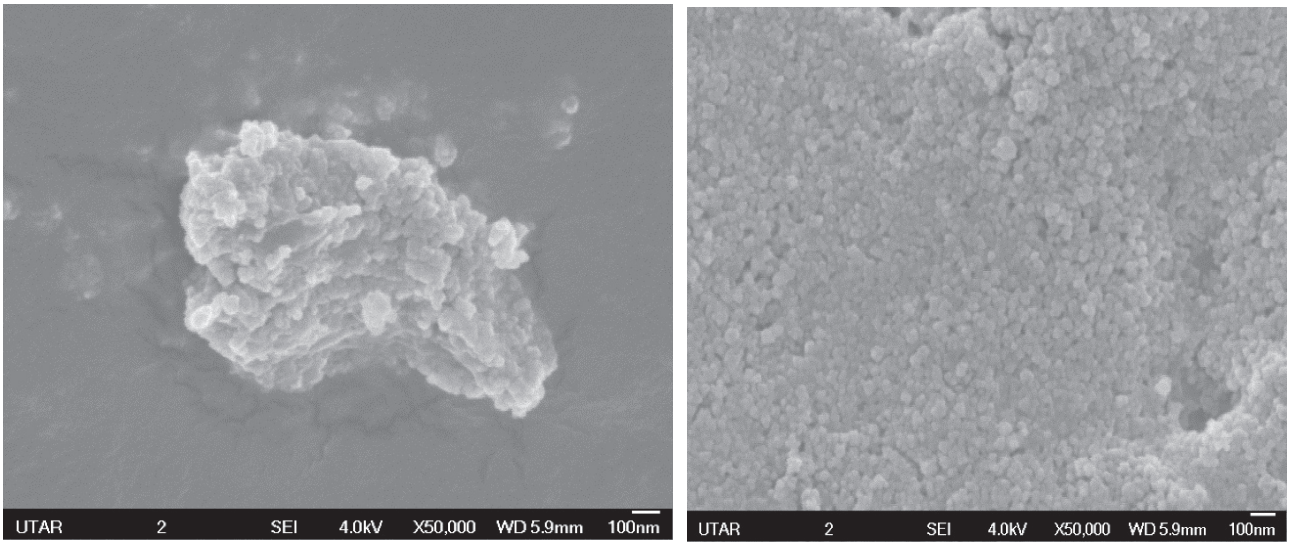

Fig. 3: SEM of Silver nanoparticles

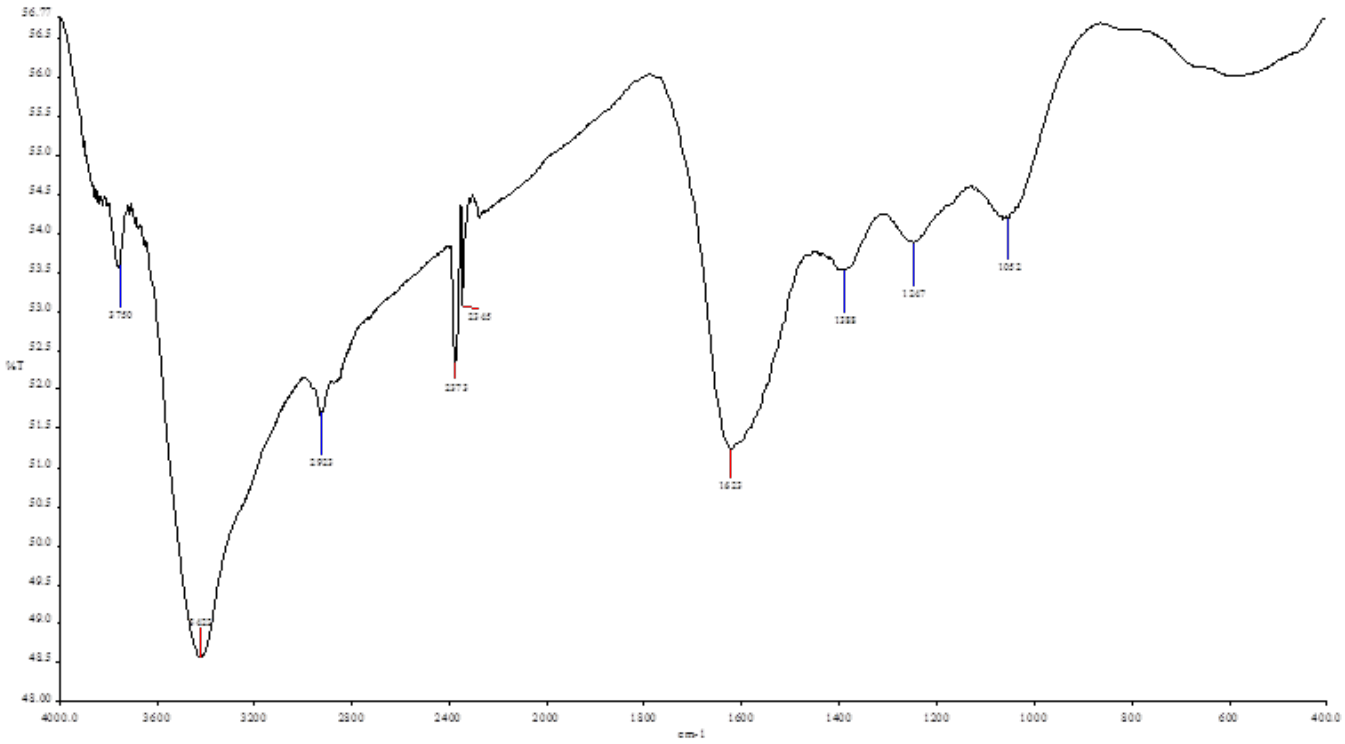

Fig. 4: FTIR spectrum of Silver nanoparticles

presence of the face cantered cubic of the silver crystals. The presence of these peaks indicates the presence of the organic compounds that has the capability to stabilize and reduce the silver ions. The presence of impurities states that there are no any spurious diffraction. The highest degree of crystallinity of silver nanoparticles is being reflected as the peaks intensity. Moreover, the broader the diffractions of the peak, the smaller is the size of crystallites.

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Total Phenolic and Total Flavonoid Content

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Leaf extract contain higher TPC compared with AgNPs (74.08 ± 7.68 and 60.55 ± 1.15), while AgNPs possessed higher TFC compared to leaf extract (78.80 ± 3.03 and 83.68 ± 2.90) respectively. *O.tenuiflorum* leaf extract was found to have higher phenolic content and antioxidant properties than white Vana tulsi (a wild type) in previous studies.[27] The phenolic metabolites play an important role natural antioxidants and it can affected by difference in value of total phenolic content in leaf.[28] Silver nanoparticles also contain higher total flavonoid content than the leaf extract as reported by Abdel-Aziz, et

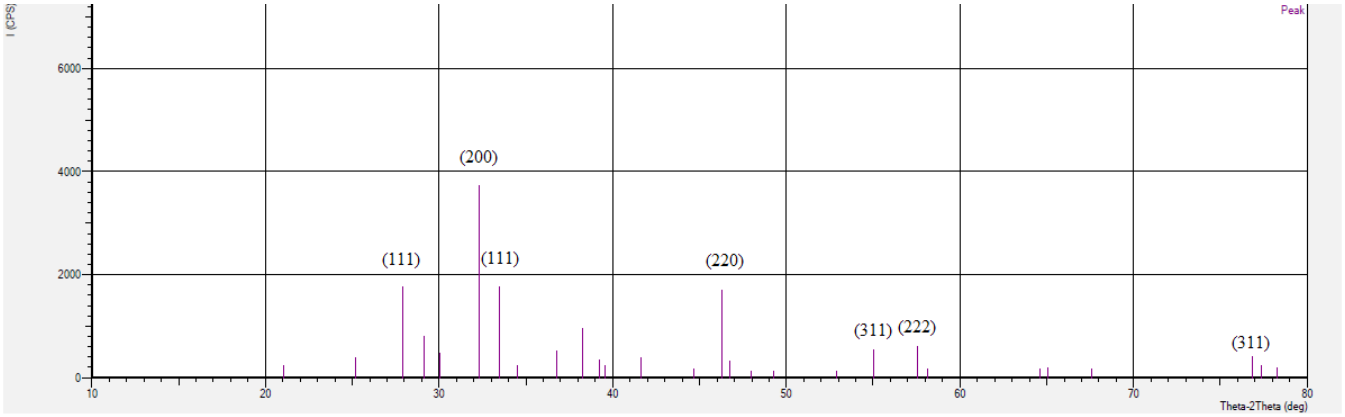

Fig. 5: XRD spectrum of Silver nanoparticles

Table 1: The experimental values of XRD analysis of the silver nanoparticles

Peak No.	*2 Thetha (Degree)*	*d-value (A)*	*Intensity (counts)*	*FWHM (degree)*	*hkl*	*d-spacing (nm)*
6	32.3362	2.76632	2239	0.44800	111	0.277
7	33.4614	2.67583	1050	0.56950	200	0.268
3	27.9148	3.19361	1047	0.44070	220	0.320
26	76.8200	1.23985	237	0.74000	311	0.124

Table 2: Tabulated data of Total Phenolic Content and Total Flavonoid Content

Bioactive compounds	*Leaf Extract*	*Silver Nanoparticles*
Total phenolics ± SE (mg GAE/g dry matter)	74.08 ± 7.68	60.55 ± 1.15
Total flavonoids ± SE (mg QE/ g dry matter)	78.80 ± 3.03	83.68 ± 2.90

Table 3: EC50 values of radical scavenging activity

Sample	*EC50 values of radical scavenging activity (mg/mL)*			
	DPPH Scavenging	*ABTS Scavenging*	*Iron Chelating*	*NO Scavenging*
Ascorbic acid	0.09 ± 0.00	-	-	0.03 ± 0.00
EDTA	-	-	0.01 ± 0.00	-
BHT	-	0.02 ± 0.00	-	-
Leaf Extract	5.01 ± 0.12	0.18 ± 0.01	1.08 ± 0.07	0.35 ± 0.01
Silver Nanoparticles	29.95± 0.61	0.82 ± 0.02	0.73 ± 0.03	0.29 ± 0.01

Table 4: Correlation coefficient Analysis between phenolic, flavonoids and different parameters.

	Correlation coefficient				*P-value*			
	TPC		*TFC*		*TPC*		*TFC*	
EC50 Value	*Leaf Extract*	*AgNP*	*Leaf Extract*	*AgNP*	*Leaf Extract*	*AgNP*	*Leaf Extract*	*AgNP*
DPPH	0.218	0.146	0.067	0.761	0.860	0.907	0.958	0.450
	NS	NS	NS	NS	NS	NS	NS	NS
ABTS	0.980	-0.917	-0.997	–	0.127	0.261	0.0564	0.198
	NS	NS	NS	NS	NS	NS	NS	NS
Iron Chelating	0.423	0.999*	0.148NS	-0.765	0.722	0.0126*	0.905	0.445
	NS	NS	NS	NS	NS	NS	NS	NS
NO	0.795	0.815	-0.934	-0.232	0.416	0.393	0.232	0.851
	NS	NS	NS	NS	NS	NS	NS	NS

* Significant at $p < 0.05$. NS- Not Significant

al. (2013)[29] and Patra and Baek, (2016).[30] It can be concluded that flavonoids are well expressed when they are adsorbed by the silver nanoparticle surfaces, which forms an efficient complex between the flavonoids and the functional groups of chemical reagents.[19]

Table 5: Zone of inhibition (mm) of *S. aureus* and *E. coli* with with positive control

	Zone of inhibition	
	Mean ± SE	
Sample	*S. aureus*	*E. coli*
AgNO3	13.33 ± 1.20	13.00 ± 0.58
AgNP	11.00 ± 0.58	12.67 ± 0.88
Tetracycline (+ve control)	26.33 ± 0.33	21.67 ± 0.33
Leaf Extract	10.00 ± 2.52	00.00 ± 0.00

Total Antioxidant Capacity of Silver Nanoparticles

The results of antioxidant capacity of the *O. tenuiflorum* leaf extract and synthesized silver nanoparticles show that the synthesized silver nanoparticles exhibited superior antioxidant capacity for NO scavenging activity compared to DPPH, ABTS and Iron chelating activity. The higher radical scavenging activity of the synthesized silver nanoparticles can cause combination with highly unstable NOradicals and the released photon energy from light illumination.[31] For leaf extract, it has higher ABTS scavenging activity compared to DPPH, Iron Chelating and NO scavenging activity.

Fig. 6: S. aureus (left) and E. coli (right) with negative control

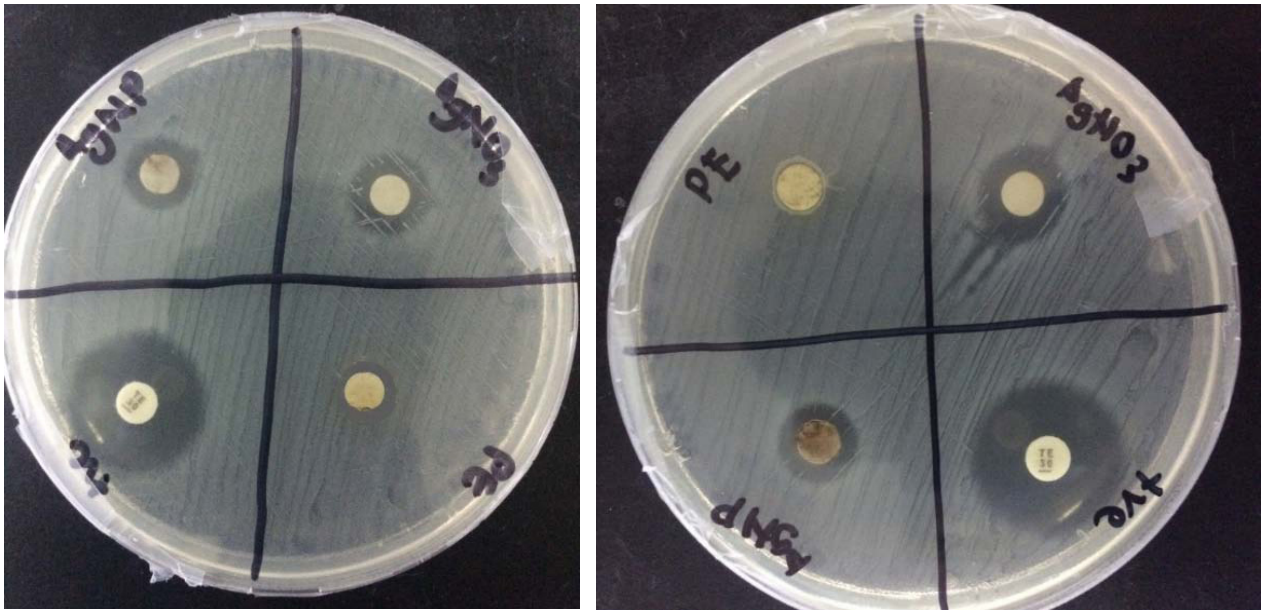

Fig. 7: *S. aureus* (left) and *E.coli* (right) with positive control

Relationship between the antioxidant capacity and TPC and TFC of both synthesized silver nanoparticles and leaf extract of *O. tenuiflorum* was established trough correlation coefficient. Negative correlation between TPC and TFC was seen with EC50 of iron chelating in silver nanoparticles (r = -0.999805) indicating higher radical scavenging at lower concentration of polyphenols and flavonoids. The relationship between TPC and TFC with EC50 was supported Chai & Wong, (2012);[16] Marta, et al., (2013)[32] and Irda, et al., (2013).[33] Much other type of metabolites or phytochemicals may also interfere with the antioxidants activity and not just affected by the phenolic and flavonoid contents alone. Studies have also reported that peptides and polysaccharides may play an important role in influencing the antioxidant capacity.[34]

Antibacterial Assay

The Gram-negative bacteria shows higher inhibition activity towards the silver nanoparticles compared to the gram-positive bacteria. This results can be possible because of the difference in the structure of the cell wall between gram-negative and gram-positive.[35] The data shows very less antibacterial properties were found on the Gram-positive. Gram-positive bacteria consist of a thick layer of cell wall which are composed of peptidoglycan, thus making it difficult for the silver nanoparticles to penetrate by the rigid structure.[36] The green synthesized silver nanoparticles using different leaf extracts showed almost the same antibacterial properties on previous studies.[22,37]

CONCLUSION

The phytochemical present in *O. tenuiflorum* leaf extract played an important role in the green synthesis, which acts as a reducing agent and capping agent. The *O. tenuiflorum* leaf extract possessed a higher TPC compared to TFC, while synthesized silver nanoparticles showed that it has higher TFC compared to TPC. This leaf extract successfully synthesized spherical shaped silver nanoparticles with size range between 14 to 33 nm. The particles synthesized were confirmed to have a face cantered cubic crystalline structure. Finally, the leaf extract showed potent antibacterial properties on Gram-positive bacteria but silver nanoparticle showed better antibacterial properties on both Gram-negative and Gram-positive. Due to its bio-reduction, stability, antioxidant and antibacterial properties, these AgNPs can be well utilized in medical and pharmacological fields. However, detailed study on in-vivo activities are required to establish the role of AgNPs at cellular and molecular levels.

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