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Woodfordia Fruticosa Flower Extract Mediated Silver Nanoparticles and its Prodigious Potential as Antioxidant, Antibacterial and Photocatalyst

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Abstract

Synthesis and characterization of *Woodfordia fruticosa* flowers mediated silver nanoparticles (*Wf*-AgNPs) was reported and its biological activities, including antioxidant, antibacterial and photo catalytic degradation of methylene blue under direct sunlight irradiation, were evaluated. UV-Vis spectral observation of the synthesized *Wf*-AgNPs showed Surface Plasmon Resonance (SPR) peak at 460 nm. Functional groups of *W. fruticosa* flower extract that is responsible for the reduction of silver ions were identified with Fourier Transform Infra-Red (FTIR) spectroscopy and the face-centered cubic (fcc) phase structure of synthesized *Wf*-AgNPs was determined using X-Ray Diffraction (XRD) analysis. The spherical structure of Wf-AgNPs was observed with SEM analysis and the average particle size was found to be 21 nm as determined by Image J software. The potential antioxidant activity *Wf*-AgNPs was assessed using DPPH, ABTS, nitric oxide scavenging activity and reducing power assay. The significant difference in the antioxidant activity of *Wf*-AgNPs was observed compared to the standard antioxidant, ascorbic acid. The synthesized *Wf*-AgNPs showed effective inhibitory activity against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) bacteria. Wf-AgNPs exhibited better catalytic activity in the degradation of methylene blue dye.

Key words: *Woodfordia fruticosa*, Silver nanoparticles, antioxidant, antibacterial, photo catalytic activity.

1. Introduction

Engineered nanoparticles exhibit imperative properties due to their nanostructure, distribution, and morphology compared to their bulk counterparts (Farré et al. 2008). The synthesis and modifications of particles below the size range of 100 nm present a higher surface area to volume ratio with significant properties for designed applications (Dahlous et al. 2019). These nanoparticles differ from their bulk materials in terms of physical, optical, electromagnetic and mechanical properties (Pradeep and Anshup 2009). Green fabrication of nanoparticles has become apparent as an innovative alternative that plays a vital role in bettering humanity and sustainable environment (Agarwal et al. 2018). Recent developments in science and technology have also made nanotechnology a sturdy tool in developing different types of nanomaterials.

The conventional nano synthesis method involves physical and chemical approaches, electrochemical and photochemical reduction and heat evaporation methods (Dubey et al. 2010). But high production cost, the release of toxic chemicals resulting in environmental toxicity, harmful effects on humans and other living organisms has led to the search for a new eco-friendly alternative approach in the synthetic process of nanoparticles (Mittal, Jain, and

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Sharma 2017). Hence microbial synthesis of nanoparticles utilizing bacteria, fungi, actinomycetes and algae gained momentum. But the biogenic production of nanoparticles using plant extracts is advantageous over microbial synthesis because it is eco-friendly, simple, inexpensive and consumes less energy (Hajebi et al. 2019). Silver has gained paramount attention in the preparation of nanoparticles besides gold, zinc, copper etc., (Chandrappa et al. 2016) due to its remarkable application in drug delivery, such as biotherapeutics, pharmaceuticals, and water treatment [9,10]. Further, the high electrical conductivity of silver nanoparticles enhances their ability as an effective antimicrobial agent [11, 12]. The phytochemicals reduce silver to its elemental form and bind to the surface of the synthesized nanoparticles, making it stable.

Hence in this investigation, *Woodfordia fruticosa* (Family: Lythracaea) flower extract is used to mediate the synthesis of silver nanoparticles. The plant is an evergreen shrub native to Asia and Africa (Thakur RS et al 1989). In India, the flowers of *W. fruticosa* are used in the preparation of herbal formulations of Ayurveda and Unani medicine systems [14-16]. These flowers are used traditionally for treating dysentery, leprosy, blood diseases, leucorrhoea and menorrhagia (Sharma P.V 1956). Phytochemicals such as tannins, flavonoids, phenolic compounds and terpenoids were reported in *W. fruticosa* (Neha Grover et al 2014). The presence of phenolic compounds in flowers contributes to its antioxidant property. The methanol extract of *W.fruticosa* exhibited effective free radical scavenging activity and better inhibitory activity against both gram-positive bacteria and gram-negative bacteria (Bhatt LR and Seung HB 2005; Neha Grover et al 2014). Recently, the silver nanoparticles synthesized using *Mangifera indica* (Ameen et al. 2019) flower extracts effectively inhibited the growth of bacterial pathogens. The interaction between positive silver ions of AgNPs and the negative cell membrane of bacteria causes cell membrane disturbance and further, the affinity of silver ions to bind and interact with vital enzymes impairs DNA replication (Ameeta A ; Ameeta R; Ahuja M 2013). In another study, Hajebi et al. (2019) synthesized silver nanoparticles using Rapeseed flower extract that exhibited significant antioxidant activity. The antioxidant property of AgNPs might be attributed to the induction of cell apoptosis via ROS generation (Sriram M.I; Kanath S.B; Kalishwaralal K; Gurunathan S 2010). AgNPs effectively degrade the highly stable and complex organic dyes compared to other methods using UV radiation or hydrogen peroxide (Qiao et al. 2005). Photocatalysis of organic dyes with AgNPs under solar radiation is an effective alternative in wastewater treatment (Nezamzadeh-Ejhieh and Banan 2012). Arunachalam et al. [2012] managed to synthesis silver nanoparticles mediated by *Coccinia grandis* leaf extract that showed photocatalytic degradation of Coomasive Brilliant Blue – G250 dye. Therefore, the green synthesis of silver nanoparticles might be advantageous in medical applications as well as in remediation of pollutants from water bodies since it utilises natural compounds (phytochemicals) for the reduction and capping of Ag ions.

The present work is designed with the primary objective of evaluating the efficacy of *W. fruticosa* flower extract as a mediator in the synthesis of silver nanoparticles. To discern the objective of this study, characterization of the synthesized AgNPs was carried out using advanced instrumentations besides determining their potential as antioxidant, antibacterial and photocatalytic agents. To the best of our knowledge, the present investigation will be a pioneer report on the synthesis of silver nanoparticles from *W. fruticosa* floral extract.

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2. Materials and Methods

2.1 Chemicals and Plant material

Chemicals used in this study are analytical grade, purchased from Sigma Aldrich chemicals, USA. Fresh and healthy flowers of *W. fruticosa* were commercially purchased for the synthesis of silver nanoparticles.

2.2. *Woodfordia fruticosa* flower extract

The shadow dried flowers of *W.fruticosa* was rinsed with tap water and then washed thrice with sterile distilled water to remove all the dust particles. About 25 g of this dried flowers was cut into pieces and 250 mL of sterile distilled water was added and boiled for 10 minutes on a hot plate with a magnetic stirrer. The resulting solution was filtered using Whatman filter paper No.1 and stored at 4 °C for further use.

2.3 Phytochemical analysis

About 100 g of dried flower powder was added separately to different solvents (chloroform, methanol, hexane and water) for the extraction of phytochemicals using the soxhlet apparatus for 24 h. The extract collected was filtered and concentrated using a rotary evaporator. The solvent extracts were stored at 5 °C for future experiments. The solvent extracts were subjected to phytochemical screening to identify alkaloids, flavonoids, saponins, tannins and glycosides as per the protocol of Kodota et al. (Kadota et al. 1990).

2.4 Biosynthesis of *W. fruticosa* mediated silver nanoparticles (*Wf*-AgNPs)

The nanoparticles was prepared as per the protocol of Ananth and Thangamathi (Ananth and Thangamathi 2018). Briefly, 10 mL of the flower extract was added to 90 mL of 1mM $AgNO_3$ solution and mixed vigorously with a magnetic stirrer for 30 minutes and allowed to rest at room temperature to reduce silver ions. The change in the color of the reaction mixture indicates the synthesis of AgNPs. The synthesized nanoparticle solution was then subjected to centrifugation at 10,000 rpm for 10 minutes. Pellets obtained after decanting the supernatant was dispersed in deionized water thrice to remove the uncoordinated biological molecules. Finally, the pellets were dried in a lyophilizer.

2.5 Characterization of *Wf*-AgNPs

The characterization of synthesized nanoparticles was done according to Ananth and Thangamathi, (2018). A small aliquot of the synthesized nano solution was initially screened for the reduction of silver ions by UV-Vis spectrophotometer (Jasco UV-650, Japan) between wavelengths ranging from 300-800 nm at regular time intervals. The identification of functional groups present on the surface of the synthesized nanoparticles was done using Fourier Transform Infrared spectroscopy (FTIR). The X-Ray diffraction (XRD) was carried out using X'Pert Pro X-ray diffractometer operated at a voltage of 40kv and a current of 30 mA with Cu Kα radiation. The structure and the elemental composition of *W.fruticosa* synthesized AgNPs was confirmed by FE-SEM-EDX, Carl Zeiss – Σigma model, Germany). *Wf*-AgNPs was also examined using TEM operated at a voltage of 200 kV. The crystallinity was analyzed using SAED. Droplets of *Wf-AgNPs* were placed on a carbon-coated copper grid and dried in an oven at 60 °C. Particle size distribution and the stability of the

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synthesized AgNPs was determined by Malvern Zetasizer (model- Nano-ZS90, Malvern Instruments)

2.6 *In vitro* antioxidant assay

The antioxidant property of the synthesized nanoparticles (*Wf*-AgNPs) was determined with DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), nitric oxide scavenging assays and total reducing power assay using UV-Vis Spectrophotometer. L-Ascorbic acid and double distilled water were used as positive standard and control, respectively for all the antioxidant assays used as control (Syed et al. 2020)(Hashemi and Ebrahimzadeh 2014) (Hajebi et al. 2019).

2.7 Antibacterial activity of *W. fruticosa* AgNPs

Gram-negative bacteria, *Escherichia coli* (MTCC1610) and Gram-positive bacteria, *Staphylococcus aureus* (MTCC96), were obtained from Microbial Type Culture Collection, Chandigarh India. The antibacterial activity of *W.fruticosa* crude flower extract and synthesized AgNPs was performed by the well diffusion method (Bauer et al., 1966). These bacterial strains were inoculated in the nutrient broth medium and cultures were adjusted to 0.5 McFarland standards (1×10^8 CFU mL^{-1}) and spread on to sterilized Mueller Hinton agar plates. The plates were dried for 15 min before being used for the sensitivity test. Discs were impregnated with different concentrations of AgNPs (20, 40, 60, 80 µg/ mL) and crude extract (20 µL), respectively and placed on Mueller Hinton agar surface. Standard antibiotic Kanamycin was used as the positive control. The plates were then incubated at 37 °C for 24 h and the zone of inhibition was measured.

2.8 SEM analysis of antibacterial activity

Briefly, 100 µl of log-phase bacterial cultures (~ 10^8CFU/mL) treated with *Wf*-AgNPs (2.5 µg/mL) for 3 h at room temperature was centrifuged for 3 min at 1000g. The harvested cells were washed with 0.1M TRIS buffered saline (pH 7.3) to remove excess culture media and resuspended in 2.5 % glutaraldehyde (500 µl) in 0.1 M TBS. The suspension was incubated for 30 min at room temperature, washed and re-suspended in 0.1 M TBS. 50 µl of the samples were placed on a poly-L-lysine coated glass coverslip. After 1 h of resting at room temperature, the samples are dehydrated using 95% ethanol and sputter-coated with gold. The samples were observed using SEM (Hitachi S-350) and the images were captured using Quartz PCI software (version 8).

2.9 Photocatalytic activity

The photocatalytic activity of *Wf*-AgNPs was studied by the degradation of methylene blue under sunlight. 100 mL of dye was added to 10 mg/L of AgNPs and stirred for 30 min in the dark before exposing to direct sunlight irradiation. Control was maintained containing only the dye. At specific intervals (every 1 h), 2 mL of the suspension was centrifuged at 10,000 rpm for 10 min to obtain a clear supernatant and the optical density was measured using a UV-visible spectrophotometer in the wavelength range from 300 to 800 nm (Roy, Sarkar, and Ghosh 2015). The λ_{max} of methylene blue was at 660 nm. The concentration of dye after photocatalytic experiments was evaluated by measuring the absorbance of dye. The photocatalytic degradation efficiency (R) of Wf-AgNPs was calculated using the following equation (Meena, VAYA, and Das 2016).

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$$R= \{(A_0-A_1)/A_0\} \times 100$$

where, A_0 = absorbance of dye at time *0*; A_1 = absorbance of dye at time *t.*

2.10 Statistical Analysis

Experimental samples were run in triplicates. The values are given as mean ± standard deviation. The tool used was SPSS (Version 21). Variations among the experimental groups were determined by ANOVA followed by T-test. *p*-values were calculated for each data and interpretation was carried out for each sample. *p*-value < 0.05, 0.01 or 0.001 were considered statistically significant.

3. Result and Discussion

3.1 Phytochemical screening

Chloroform, methanol, hexane and water were used as a solvent for the phytochemical screening of *W.fruticosa* flower extracts. The aqueous extract showed the presence of cardiac glycosides, saponins and tannins. Alkaloids, saponins, flavonoids, terpenoids and tannins were identified with chloroform extract. Methanol extract confirmed the presence of cardiac glycosides, alkaloids, flavonoids, tannins and terpenoids, while hexane extract was found to contain only tannins (Table 1). The study conducted by Syed et al. (2020) revealed alkaloids and tannins in chloroform extract and saponins, tannins and glycosides in aqueous extract of *W.fruticosa* flowers. Similarly, our results with methanol extract of *W.fruticosa* were in concordance with the findings of Topno and Sinha (Topno and Sinha 2018).

3.2 Synthesis and characterization of *Wf*-AgNPs

The green chemistry approach in the synthesis of nanoparticles, particularly employing plants, is advantageous over other biogenic methods because plants can be used in large scale synthesis. Addition of *W.fruticosa* flower extract to aqueous silver nitrate solution (1mM) turned the solution to dark brown color (Fig. 1a). The change in the color of the reaction mixture indicated the formation of AgNPs. The color developed gradually intensified with an increase in incubation time. After 180 min of incubation, the color of the solution remained stable, indicating the saturation of AgNPs synthesis. The change in the color of the synthesized nano solution was due to Surface plasmon resonance (SPR) of AgNPs. The biomolecules of *W.fruticosa* flowers were responsible for the reduction of silver to its elemental forms. These phytochemicals act as reducing and stabilizing agents in the process of AgNPs synthesis. The UV spectral analysis exhibited SPR peaks at 460 nm measured at regular time intervals (Fig. 1b). Maximum absorbance was obtained after 240 min of incubation at room temperature. A similar trend in SPR peak with λ_{max} at 460 nm was observed with the AgNP synthesis using *Ioxra coccinea* flower extracts (Bin Hamid, et al., 2020). In another study, AgNPs synthesized using *Lantana camara* flower extract exhibited an absorption peak at 470 nm after 168 h of reaction (Kumar et al. 2015). FTIR analysis revealed the function groups of biomolecules that are involved in the reduction and capping of nanoparticles. Fig. 1c shows six prominent peaks in the range between 3504 cm^{-1} to 695 cm^{-1}. The prominent peak at 3504 cm^{-1} confirmed O-H stretch of primary aromatic amines (Isaac, Sakthivel, and Murthy 2013). The peak at 1622 cm^{-1} indicated the C=O bond of amides. The band at 1384 cm^{-1} represented the C-H bend of aldehydes (Hajebi et al. 2019). The peak at 1205^{-1} was assigned to C-O stretch of phenolic compounds. The –C-O-O stretch of peroxides was evident with the peak at 826 cm^{-1} and the C-Br band of aliphatic bromo compounds was represented with the peak at 695 cm^{-1} (Ameen et al. 2019). From the FTIR

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analysis results, it could be suggested that amines, amides, carboxylic, aldehyde, alkane, phenolic and aliphatic bromo compounds were the functional organic molecules that are responsible for the synthesis of AgNPs. The XRD analysis confirms the elemental composition and the crystalline nature of the nanoparticles. The XRD spectrum displayed in Fig. 1d shows diffraction peaks at 38.25, 44.48, 65 and 77.68 corresponds to (111), (200), (220) and (311) reflection planes of face centered cubic phase of AgNPs (JCDPS No.04-0783). This result was consistent with the diffraction pattern obtained with synthesized AgNPs mediated by Rapeseed flower (Hajebi et al. 2019). Figure (5) shows the agglomerated AgNPs observed under SEM. The synthesized AgNPs were irregular and mostly spherical in shape. The elemental composition of the synthesized AgNPs was confirmed with energy dispersive x-ray spectroscopy (EDX). The results showed the presence of silver and occupying significant proportions. The AgNPs synthesized in this study showed aggregated morphology and were non-uniform in size distribution (Fig 2a and b). Similarly, SEM analysis of AgNPs synthesized using *Butea monosperma* flower extract was agglomerated spherical structures (Ananth and Thangamathi 2018). The DLS and Zeta potential measurements present the average size and the charge of the synthesized nanoparticles respectively. From the DLS analysis it could be confirmed that the average size distribution of the nanoparticles was 18.17 nm (Fig. 2c). DLS is more significant in determination of particle size as it measures the hydrodynamic size of the nanoparticles. The negative charge of the synthesised AgNPs was determined with zeta potential studies and was found to be -27.8 (Fig. 2d). The zeta potential value obtained in this study revealed that the synthesized AgNPs had higher stability compared to a previous report (Babu et al., 2021). The negative charge of the AgNPs might be due to phenolic compounds that capped the surface of AgNPs. Fig (3a) shows the TEM images of slightly agglomerated uniform-sized nanoparticles that are most spherically produced by reducing silver by phytochemicals of *W.fruticosa* flowers. High-resolution Fig (3a and 3b) displays the TEM images that clearly reveal the morphology, distribution and dimensions of the synthesized AgNPs. The nanoparticles were observed to be polydispersed and spherical (Arokiyaraj et al. 2017). A thin pale layer on the surface of the AgNPs might be due to the capping of phytochemicals present in *W.fruticosa* flower extract and are responsible for the stabilization of the nanoparticles (Venugopal et al. 2017). Fig (3c) represents the selected area electron diffraction (SAED) pattern which indicates the crystalline nature of nanoparticles.

3.3 Antioxidant assay

The antioxidant activity of *Wf*-AgNPs was evaluated with DPPH radical scavenging assay, ABTS radical scavenging assay, Nitric oxide radical scavenging assay and total reducing power assays. The high unstable nature of free radicals leads to ROS generation that interacts with other molecules in the biochemical reactions and causes cellular damage (Rehana et al. 2017). Quenching of these free radicals is instigated by the bio-reductive groups of phytochemicals present on the surface of the AgNPs (Nunes et al. 2018). Fig. 4 describes the free radical scavenging property of synthesized AgNPs. Though the synthesized AgNPs exhibited significant scavenging activity, it was comparatively low with reference to standard ascorbic acid used in the present investigation. Results indicate that the antioxidant property of AgNPs was dose-dependent. The DPPH showed a positive correlation with different concentrations of biogenic AgNPs that were used in the present study. High free radical scavenging activity (64.25%) was observed with the maximum concentration of AgNPs (100µg/mL) used in the present study. The change in color from purple to yellow when *Wf*-AgNPs was treated with DPPH indicates the completion of scavenging activity, which was measured spectrophotometrically at 517 nm. The present study observed the DPPH free

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radical scavenging activity of *Wf*-AgNPs ranged from 18% to 68% with 10 – 100 µg/mL concentrations (Fig. 4a). The IC_{50} value of AgNPs was 79.38 µg/mL (Table 3). A similar IC_{50} value of 70 µg/mL was obtained with AgNPs of *Caesalpinia pulcherrima* flower extract (Moteriya and Chanda 2017).

Fig. 4b shows the *Wf*-AgNPs antioxidant activity against ABTS free radicals. The antioxidant activity increased with an increase in the concentration of AgNPs. 54% of ABTS scavenging activity was observed with 100 µg/mL of *Wf*-AgNPs. But contrast to the present findings, 50% of inhibition of ABTS free radical was obtained with 0.3 µg/mL concentration of AgNPs synthesized by rapeseed pollen extract (Hajebi et al. 2019). Nitric oxide plays a vital role as regulatory molecules in the immune, nervous and cardiovascular systems. The *Wf*-AgNPs exerts NO scavenging activity by inhibiting nitrite formation and competing with oxygen and oxides of nitrogen. Due to less stability in the high electronegative environment, NO is reduced by readily accepting electrons from *Wf*-AgNPs. Fig. 4c displays the percentage of the nitric oxide inhibition by *Wf*-AgNPs. 42% of inhibition was recorded at 60 µg/mL concentration of *Wf*-AgNPs. The present study results were comparatively low to the inhibitory activity (51.35% at 60 µg/mL) exerted by *Plumeria alba* flower extract mediated AgNPs. This difference in the percentage of ABTS and nitric oxide inhibition potential of flower extract synthesized AgNPs might be attributed to the difference in phytochemical composition.

The total reducing power of *Wf*-AgNPs and the reference compound ascorbic acid was presented (Fig. 4d). Different concentrations of *Wf*-AgNPs (20, 40, 60, 80 and 100 µg/mL) were used for the reduction of Fe^{3+} to Fe^{2+}. The reduction of Fe ions was indicated by the formation of Perl blue color measured using a UV-visible spectrophotometer at 700 nm. Though *Wf*-AgNPs exhibited significant reduction activity, it was relatively lower than ascorbic acid. The antioxidant activity was observed to be dose-dependent. The maximum percentage of reduction activity (68%) was observed with 100 µg/mL of *Wf*-AgNPs. The antioxidant property of AgNPs was due to their hydrogen donating capacity, which breaks the free radical chain (Sohal et al. 2019). Similar observation with *Plumeria alba* flower extract mediated AgNPs exhibited significant reduction activity with concentration ranging between 25-100 µg/mL (Mata, Reddy Nakkala, and Rani Sadras 2015).

3.4 Antibacterial activity

The antibacterial efficacy of *Wf*-AgNPs was evaluated against *E.coli* and *S.aureus*. The results indicated that different concentrations of *Wf*-AgNPs (100, 200, 300 and 400 µg/mL) and crude extract (20 µl) displayed an effective zone of inhibition measuring 20 mm, 22 mm, 23 mm and 24 mm respectively against *E. coli.* Similarly, 17 mm, 18 mm, 20 mm and 21 mm inhibition zones were observed against *S.aureus* (Table 2). The standard antibiotic, kanamycin used in the study, produced 20 mm zone of inhibition against both the bacterial strains. The possible mechanism behind the antibacterial effect of *Wf*-AgNPs might be due to the interaction of silver ions with the bacterial cell membrane proteins, leading to disturbances in membrane permeability and cell death (Lok et al. 2006). The results obtained with *Wf*-AgNPs against bacterial isolates agreed with earlier reports on the antibacterial activity of AgNPs synthesized using plant extracts (Corciova et al. 2018). The *Wf*-AgNPs exhibited good antibacterial activity compared to AgNPs of *Tilia cordata* flower extract, which recorded moderate antibacterial activity against *E.coli* and *S.aureus* measuring 10 mm and 11 mm zone of inhibition (Corciova et al. 2018). SEM observation of *Wf*-AgNPs treated bacterial strains showed disturbances of the bacterial membrane and morphological changes, including filamentation and cell lysis (Fig. 5a and b). Brudzynski and Sjaarda (Brudzynski

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and Sjaarda 2015) reported the SEM observations on bacterial cell membrane damage due to the antibacterial effect of honey.

3.5 Photo catalytic degradation of organic dyes

The photocatalytic ability of *Wf*-AgNPs was evaluated using Methylene Blue as a model system under solar irradiations. Previous literature reported that the photocatalytic technique as most effective for the degradation of dyes [27, 47]. The electrons present on the surface of AgNPs become excited after being hit by the photons of sunlight irradiations (Yu L; Xi J; Li M; Chan HT; Su T 2012). The excited electrons are readily accepted by dissolved oxygen present in the reaction mixture and are converted into oxygen anion radicals. These free radicals break the organic molecules of dye into its simpler forms to degrade the dyes (Ameeta A ; Ameeta R; Ahuja M 2013). The degradation of methylene blue was visually observed regarding the change in the color of the dye from deep blue to a colorless solution. Results indicate that the intensity of the absorbance band decreased with respect to exposure to direct sunlight irradiations. The maximum absorption peak was measured at 660 nm (Fig. 6). Kumari et al (Kumari et al. 2016) reported 100% degradation of methylene blue in 6 h of sunlight irradiation using *Cordia dichotoma* leaf extract mediated AgNPs. Similarly, a decrease in absorption peak for methylene blue dye by AgNPs after 5 hours of exposure to sunlight irradiation was reported by Roy et al., (2015).

4. Conclusion

The present study is a pioneer and a simple procedure in synthesizing silver nanoparticles using *W.fruticosa* flower extract. This bio reductive synthesis of AgNPs is an eco-friendly, renewable and cost effective approach for producing metallic nanoparticles in an aqueous solution at room temperature. Characterization of synthesized nanoparticles revealed spherical shapes that are face-centered cubic phase structure and crystalline in nature. The bio reductive groups of phytochemicals present on the surface of the nanoparticles were confirmed with FTIR analysis. These functional groups are involved in the reduction and capping of the synthesized nanoparticles that provide its stability. The synthesized *Wf*-AgNPs exhibited good antioxidant, antibacterial and photocatalytic activity. Hence the silver nanoparticles using *W.fruticosa* can be effectively used in biomedical applications and in water treatment processes. Further studies can be carried out to determine the mechanism of antibacterial activity of AgNPs, whether due to inhibition of cell wall synthesis or disturbance in cell membrane integrity due to physical damage. Further, the mechanism of photocatalytic degradation of industrial dyes has to be investigated.

Conflict of Interest
The authors declare that they have no conflict of interest

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Table 1. Phytochemical composition of *W.fruticosa* flower

Phytochemicals	Solvent extracts			
	Chloroform	Methanol	Hexane	Water
Alkaloids	+	+	-	-
Flavonoids	+	+	-	-
Saponins	+	-	-	+
Tannins	+	+	+	+
Terpenoids	+	+	-	-
Cardiac Glycosides	-	+	-	+

Table 2. Antibacterial activity of *W.fruticosa* flower extract and synthesized *Wf*-AgNPs

Concentration (µg/mL)	Zone of inhibition (mm)	
	Staphylococcus aureus	*Escherichia coli*
20	17	20
40	18	22
60	20	23
80	21	24
Crude extract (20 µl)	20	16
Kanamycin (20 µl)	14	20

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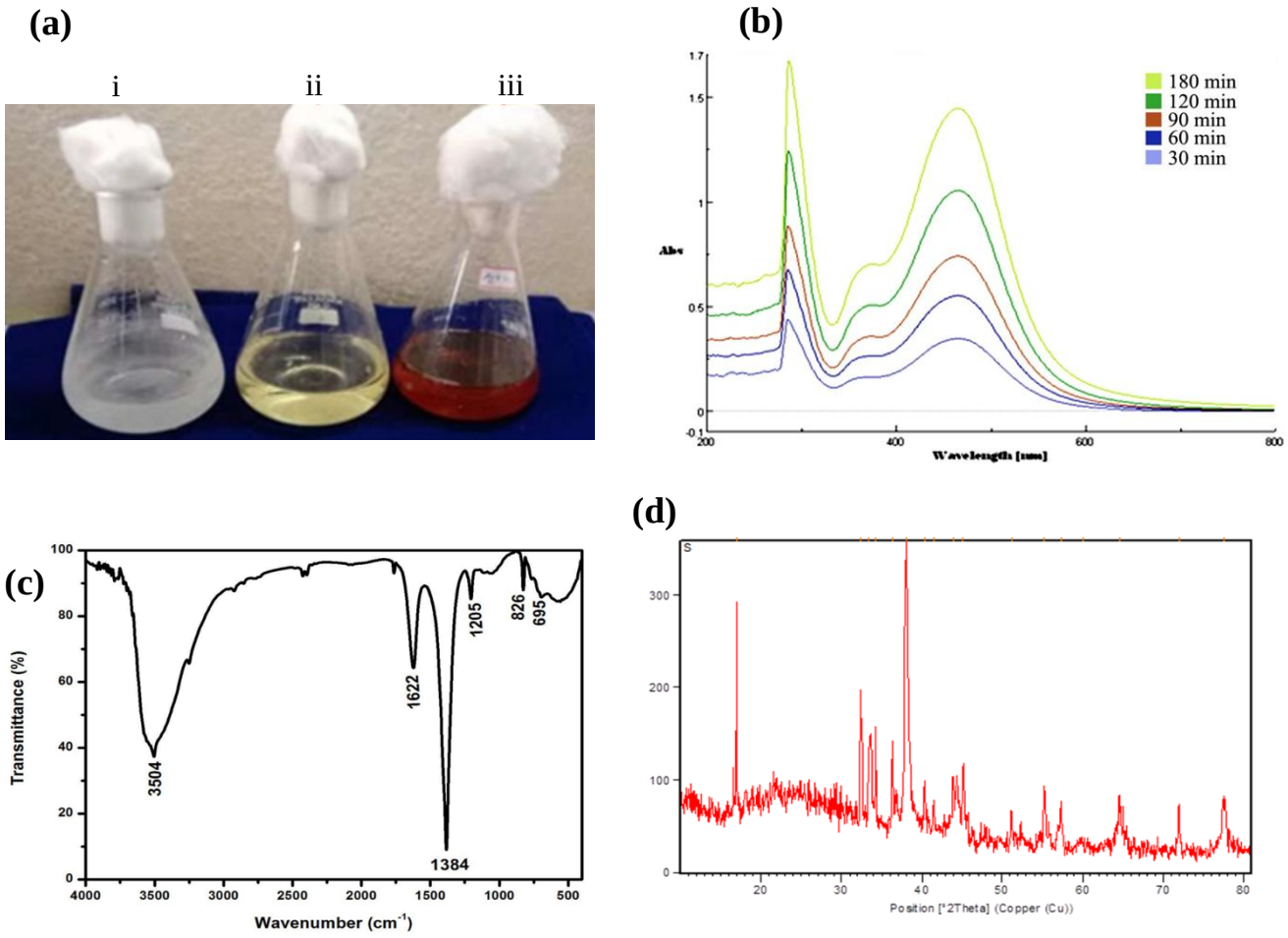

Figure 1 (a) Synthesis of Silver nanoparticles from *W.fruticosa* flower extract (i) Aqueous silver nitrate (ii) *W.fruticosa* flower extract (iii) *Wf*-AgNPs **(b) UV –Vis absorption spectrum of *Wf*-AgNPs, (c) FTIR spectrum of *Wf*- AgNPs (d) XRD analysis of *W. fruticosa* AgNPs**

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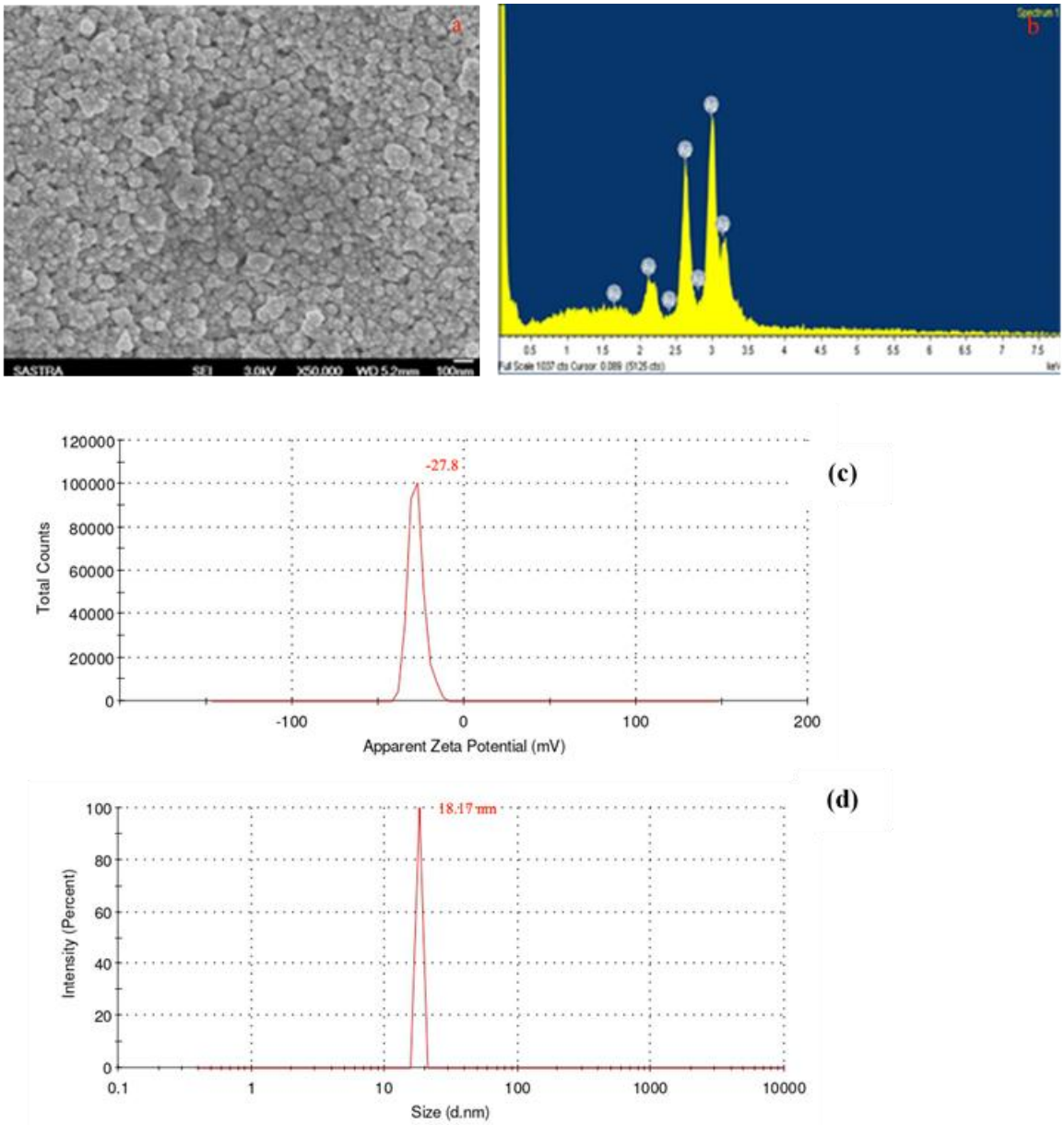

Fig 2 (a) FE-SEM images of *Wf*-AgNPs (b) EDX pattern of AgNPs (c) DLS analysis of AgNPs. (d) Zeta potential distribution of AgNPs

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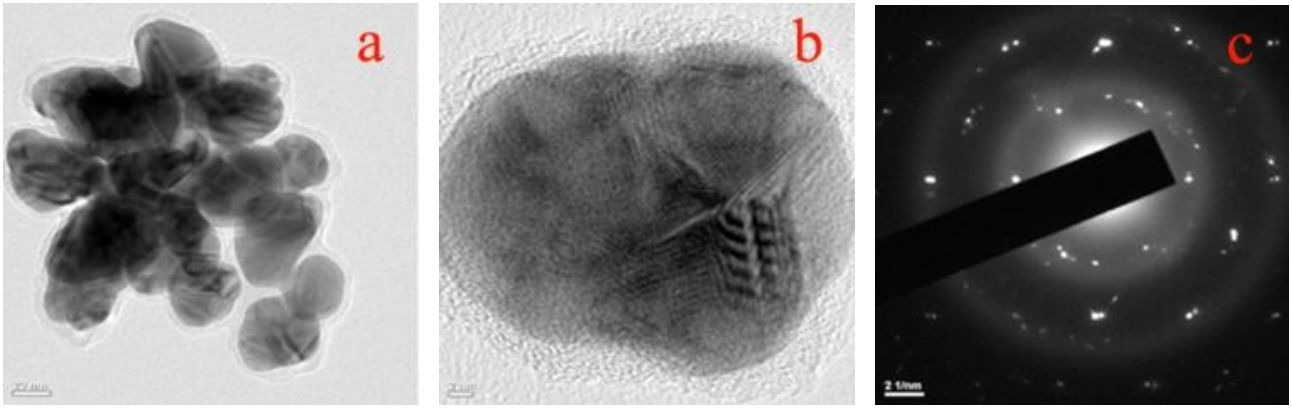

Fig 3. TEM images of *Wf*-AgNPs (a) 20X resolution (b) High resolution image of nanoparticles with lattice fingers 2X (c) SAED pattern

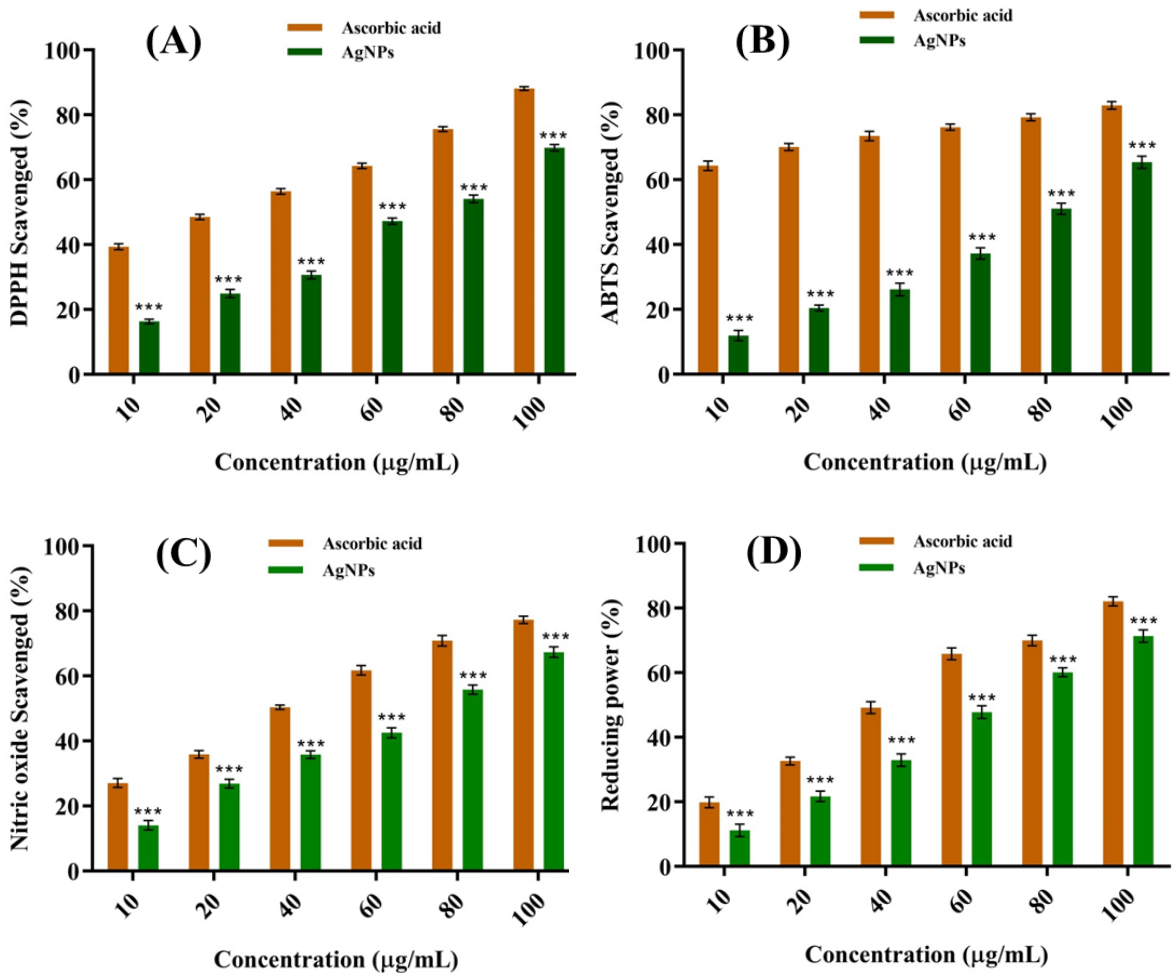

Figure 4. Antioxidant activity of *W. fruticosa* AgNPs (A) DPPH Scavenging (B) ABTs radical Scavenging (C) Nitric oxide Scavenging (D) Reducing power capacity

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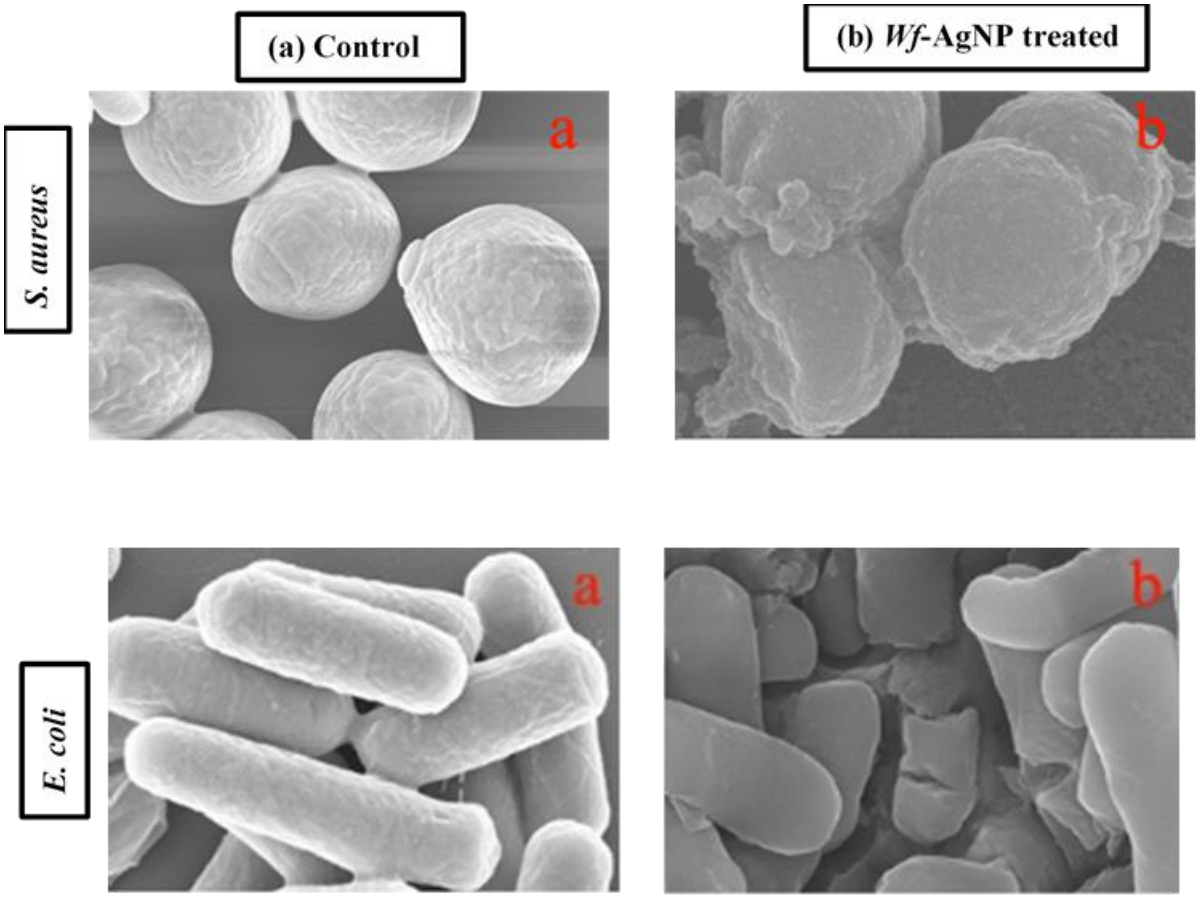

Figure 5. FE-SEM images of AgNPs treated *E. coli* and *Staphylococcus aureus*

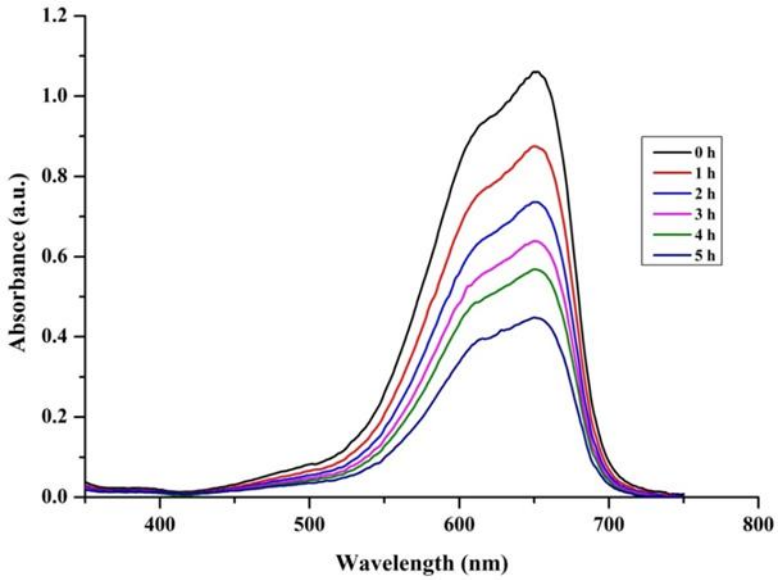

Figure 6. Photocatalytic degradation of Methylene blue dye by *Wf*- AgNPs