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Rapid biological synthesis of silver nanoparticles using *Kalopanax pictus* plant extract and their antimicrobial activity

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Abstract−Silver nanoparticles (AgNPs) have promising potential in biomedicine, energy science, optics, and health care applications. We synthesized AgNPs using plant, *Kalopanax pictus* leaf extract. UV-visible spectrophotometric study showed the characteristic peak for AgNPs at wavelength 430 nm. The optical density at 430 nm increased after addition of plant leaf extract, indicating increase in formation of nanoparticles. Comparative time course analyses for AgNP synthesis carried out at different reaction temperatures (20, 60, and 90 °C) revealed higher reaction rate for *K. pictus* than *Magnolia kobus* plant leaf extract, which showed highest AgNP synthesis rate in the previous report. Electron microscopy analyses confirmed the presence of well dispersed AgNPs, predominantly with spherical shapes. In transmission electron microscopy, the particle size decreased with increase in temperature. Electron dispersive X-ray spectroscopy analyses indicated that Ag content increased with increase in reaction temperature. Fourier transform-infrared spectroscopy studies revealed capping of bioorganics from plant to the synthesized AgNPs. The antimicrobial activity of the synthesized AgNPs against *Escherichia coli* increased with increase in reaction temperature. The observations in this study will prove beneficial in approaching rapid synthesis of AgNPs and their antimicrobial application.

Keywords: Nanoparticles, Silver, *Kalopanax pictus*, Plant Extract, Antimicrobial Activity

INTRODUCTION

Nanoparticles are the basic crucial components of nanotechnology that exhibit remarkable advanced characteristic features due to their size, morphology, and other size dependent properties [1]. The unique features of nanoparticles have promising potential to influence science, economy, and day-to-day life by playing a vital role in biomedicine, energy science, optics, and other health care applications [2-5]. Especially, silver nanoparticles (AgNPs) are receiving increasing attention because of their potential applications in medicine [6], forensic science [7], cosmetics [8], food chemistry [9], agriculture [10], and variety of other fields.

Nanoparticles are commonly synthesized by using physical [11] and chemical processes [12]. However, these methods are not eco-friendly [13] and involve use of toxic chemicals in their synthesis protocols, which may create some hazardous effects in biomedical applications [14]. Therefore, biological synthesis via plant and microbe-mediated processes offers reliable, simple, nontoxic, and eco-friendly alternatives [3,5,6,15-19]. Biosynthesis of nanoparticles by plants is advantageous over other biological methods by reducing the complicated process of maintaining cell culture [20]. Plant-assisted synthesis of nanoparticles has captured considerable interest in the arena of modern nanoscience and technology due to its flexibility, biocompatibility, and eco-friendly nature. Plant materials such as leaves [5,15-18,21,22], seeds [23], fruits [13,24], latex [6,14,25], and barks [26] are reported to be useful for metal reduction process. Many research studies most often concentrate on synthesis and finding size, shape, or application of the synthesized nanoparticles. Along with these studies, there is a need to investigate processes that can synthesize nanoparticles at rapid rate. Besides, production of nanoparticles at technological scale will be possible through investigation of reaction conditions which can synthesize nanoparticles at rapid rate. Song and Kim [15] reported highest rate for *Magnolia kobus* among the investigated plants in their studies and earlier report of Shankar et al. [20] for Neem leaf broth. Investigations of plants and reaction conditions with ability to synthesize nanoparticles at higher rate than *M. kobus* can be useful for developing technology of nanoparticle production.

Kalopanax pictus, a deciduous tree in the family Araliaceae, is distributed mainly in Asian countries [27]. Different parts of the plant (stem, bark, and leaves) possess a variety of biological activities, including anti-diabetic, cytotoxic, anti-lipid peroxidative, anti-fungal, and anti-inflammatory activities [28-32].

In the present study, we compared the time course of AgNP synthesis by leaves extract of *K. pictus* with *M. kobus* at different temperatures. The biosynthesized AgNPs by *K. pictus* were characterized by UV-Vis spectroscopy, electron dispersive X-ray spectroscopy (EDS), Fourier transform-infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The antibacterial effect of the AgNPs synthesized by *K. pictus* was investigated by disc diffusion method.

MATERIALS AND METHODS

1. Collection of Plant Materials and Preparation of Plant Extracts

Leaves of plants, *K. pictus* and *M. kobus* were collected and dried for two days at room temperature. The plant leaf extract solutions

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were prepared by taking 5 g of thoroughly washed and finely cut leaves in 300 mL Erlenmeyer flasks with 100 mL of sterile distilled water and then boiling the mixture for 5 min before finally decanting it. The plant leaf extracts were stored at 4 ºC and used within a week.

2. Synthesis and Time Course Studies of AgNPs

Typically, 5 mL of leaf extract was added to 95 mL of 1 mM aqueous precursor $AgNO_3$ solution for reduction of Ag^+ ions taken in an Erlenmeyer flask for a reaction. The reaction was allowed to occur without stirring at different temperatures (20, 60, and 90 ºC). Time course of synthesis of AgNPs by *K. pictus* and *M. kobus* plant extracts was monitored by taking absorption spectrum at 430 nm in UV-Vis spectrophotometer (UV-1601, Shimadzu, Japan).

3. Characterization of AgNPs

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum between 200 to 800 nm wavelengths of the reaction medium using UV-Vis spectrophotometer. The AgNPs synthesised by *K. pictus* at different temperatures were purified by repeated centrifugation at 15,000 rpm for 20 min, followed by redispersion of the pellet in deionized water. The purified dried powder of AgNPs was further analyzed by EDS (Philips XL-30). Morphology of the AgNPs was characterized by SEM (Philip model CM 200). TEM micrographs were obtained using an energy filtering transmission electron microscope (JEM-2100F, Jeol Ltd., Japan) operating at 200 kV. The FT-IR spectra were obtained by mixing the samples in KBr to make pellet and recording in the wave number region of 4,000-400 cm^{-1} using an FT-IR spectrometer (Nicolet Magna-IR 200, Thermo Scientific, USA).

4. Antimicrobial Activity of AgNPs

The antibacterial activity was done by disc-diffusion assay method. In this method, 50 µL of AgNPs synthesized by *K. pictus* leaf extract at different temperatures was applied to pre-sterilized blank filter paper discs (BBL™ Sensi-Disc™ Susceptibility Test Discs, 6 mm in diameter). These discs were air-dried under sterile conditions and were then placed on Luria agar swabbed with *Escherichia coli* at a concentration of 10^6 bacteria/mL. The plates were incubated at 37 ºC for overnight. The zone of inhibition was measured in millimeter after 24 h of incubation and recorded. Each treatment was tested in triplicate, with chloramphenicol discs applied with 30 µg/mL antibiotic concentrations as reference or positive control. The results were also compared with $AgNO_3$ and plant leaf extract.

RESULTS

1. Synthesis and Time Course of AgNPs

The 5% *K. pictus* leaf extract was able to synthesize AgNPs at room temperature. A distinct color change from colorless to dark brown was observed within a few hours of visual observations without stirring in reactions of the 1 mM metal precursor solution ($AgNO_3$) challenged with plant leaf broth for AgNPs. The UV-Vis spectral analysis of the synthesized AgNPs showed a peak at 430 nm (Fig. 1).

Comparison of time course analysis suggested higher synthesis rate by *K. pictus* than *M. kobus* plant extract at different reaction temperatures. The reaction rate for AgNP synthesis increased with increase in temperature, which was indicated by increase in optical density at 430 nm (Fig. 2).

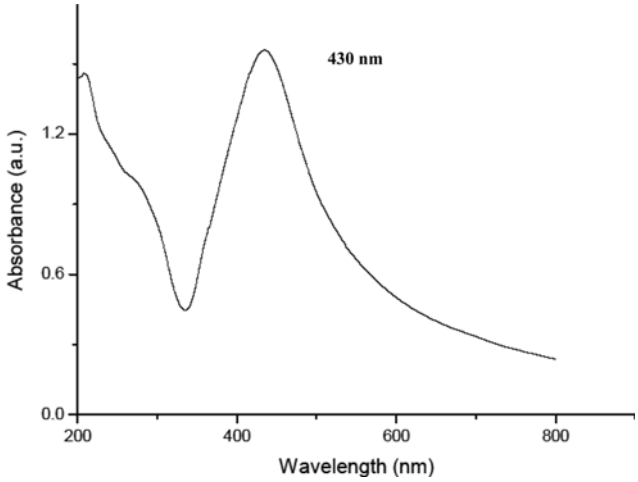
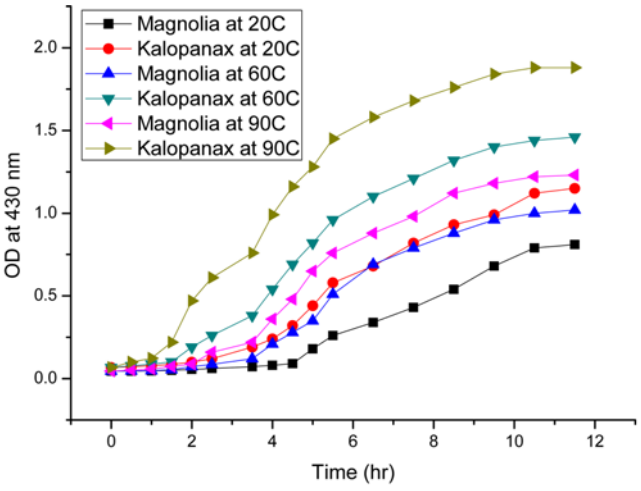


Fig. 1. UV absorption spectra of AgNPs synthesized by leaf broth of *K. pictus*.



(a)

(b)

Fig. 2. (a) Time courses of AgNPs synthesized by leaf broth of *K. pictus* at different temperatures and (b) photograph of the synthesized nanoparticles at different temperatures.

2. Characterization of AgNPs

TEM analysis showed the presence of spherical nanoparticles (Fig. 3). The nanoparticle size decreased with increase in temperature (Fig. 3). SEM analyses revealed presence of spherical nanoparticles (Fig. 4). Silver content in the EDS spectrum of the synthesized nanoparticles increased with increase in temperature (Fig.

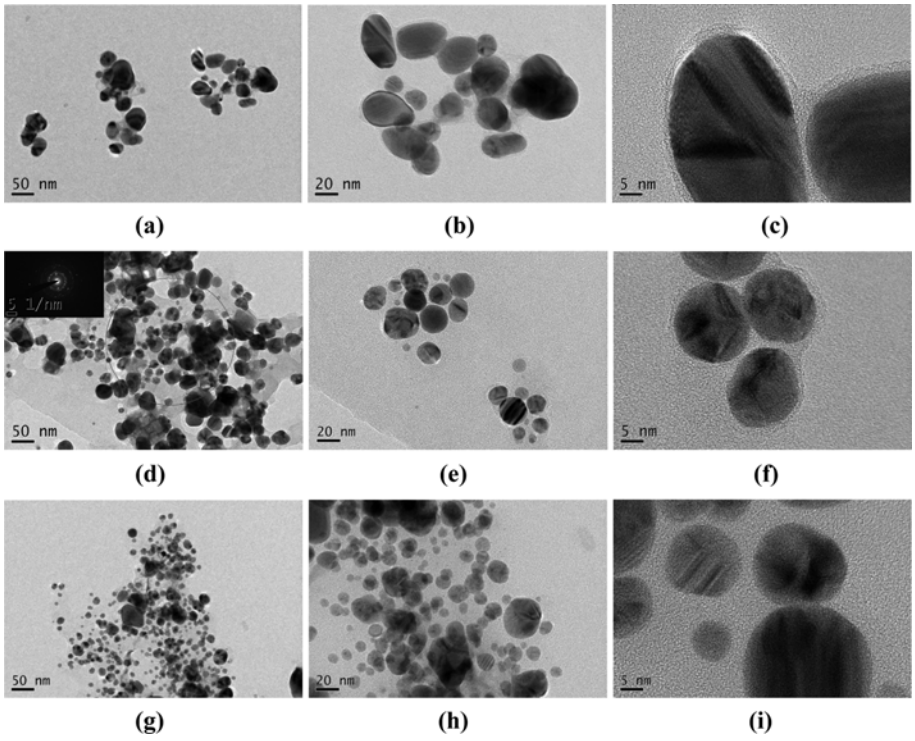


Fig. 3. TEM micrograph of AgNPs synthesized by leaf broth of *K. pictus* at different temperatures, (a) to (c) 20 °C, (d) to (f) 60 °C, and (g) to (i) 90 °C; inset in (d) shows a representative SAED pattern of AgNPs formed at 60 °C.

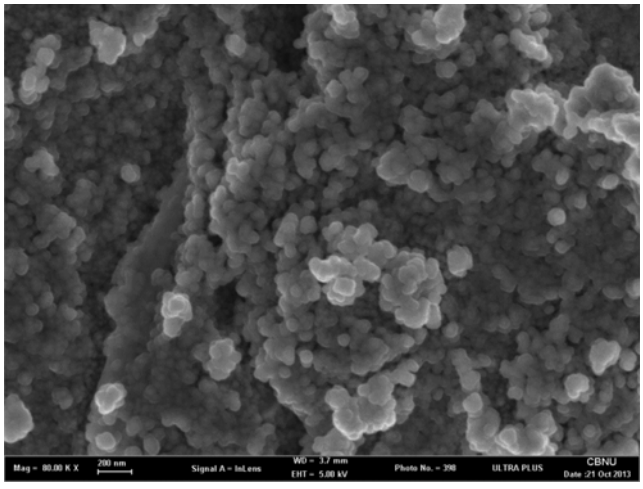


Fig. 4. SEM micrograph of AgNPs synthesized by leaf broth of *K. pictus* at 60 °C.

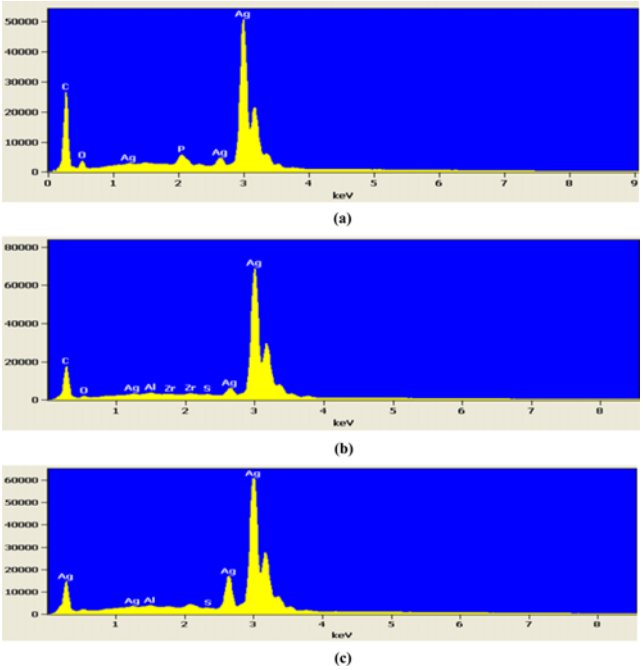


Fig. 5. EDS spectra of AgNPs synthesized by leaf broth of *K. pictus* at different temperatures, (a) 20 °C, (b) 60 °C, and (c) 90 °C.

5, Table 1). The FT-IR spectrum pattern for the obtained AgNPs at different temperatures differed slightly from each other (Fig. 6).

3. Antimicrobial Activity Studies

E. coli was sensitive to treatment of AgNPs and chloramphenicol at concentration of 30 μg/mL on the discs (Fig. 7). The controls of 1 mM $AgNO_3$ also showed antimicrobial potential but less than AgNPs. Plant leaf broth did not show antimicrobial effect (Fig. 7).

DISCUSSION

AgNPs are known to exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in AgNPs [20]. The UV-Vis spectral analysis of the synthesized AgNPs showed the maximum absorbance at ca. 430 nm, which steadily increased in intensity as a function of reaction time. Similar observations about UV-Vis spectral analysis of AgNPs were reported in previous stud-

Table 1. Element content of AgNPs in EDS analyses

Element type	Content (wt%)		
	AgNPs synthesized at 20 °C	AgNPs synthesized at 60 °C	AgNPs synthesized at 90 °C
Ag	87.0	93.7	99.3
C	7.03	3.46	-
O	4.52	1.10	-
P	1.49	-	
Al	-	0.31	0.29
S	-	0.36	0.40
Zr	-	1.04	-

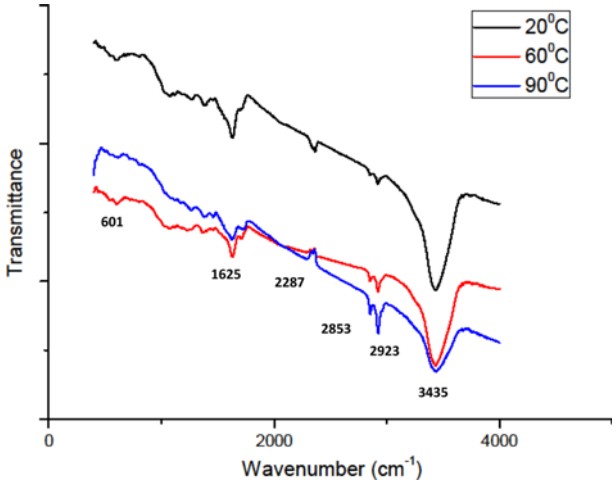
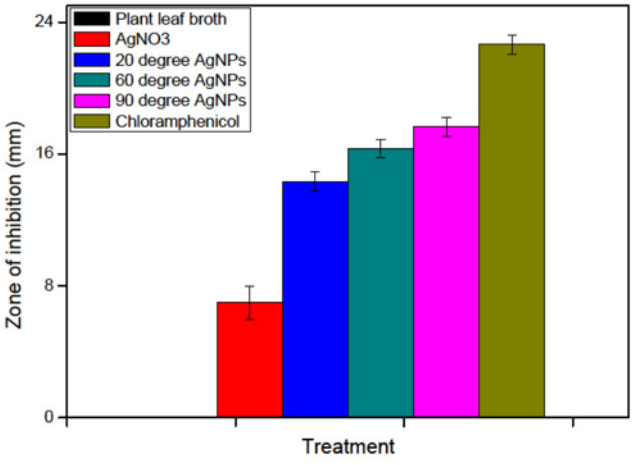
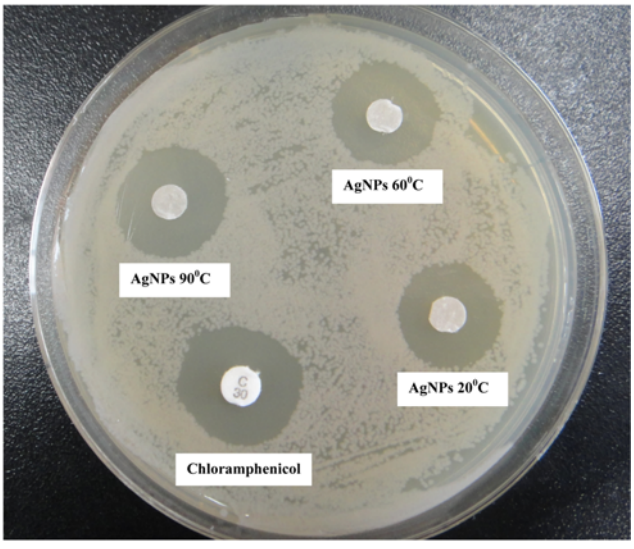


Fig. 6. FT-IR spectra of AgNPs synthesized by leaf broth of *K. pictus* at different temperatures, (a) 20 °C, (b) 60 °C, and (c) 90 °C.



(a)



(b)

Fig. 7. Antimicrobial activity of AgNPs synthesized by leaf broth of *K. pictus* at different temperatures against *E. coli*, (a) zone of inhibition and (b) a representative image.

ies [15,19,20]. The time course of AgNP formation can be quantitatively monitored by measuring the absorbance at 430 nm [15]. In the present study, the rates for the production of AgNPs were higher for *K. pictus* than *M. kobus* leaf broth at all reaction temperatures tested. As the reaction temperature increased, AgNP synthesis rate increased using *K. pictus* leaf broth. The final absorption intensities at 430 nm were more than 1.5 a.u. and increased up to 2.0 a.u. using *K. pictus* plant extract. These values are higher than that reported for *M. kobus* leaf broth [15] and Neem leaf broth [20], suggesting that the conversion to AgNPs may be higher using the plant leaf extracts in this study. The observations about the increase of reduction rate with increasing the reaction temperature were made in earlier studies for silver, gold, platinum, and copper nanoparticles using *M. kobus* and *Diopyros kaki* leaf extracts [5,15-17] and gold nanotriangles using lemongrass extract [33]. Synthesis of AgNPs using culture supernatants of *Enterobacteria* was fast, but the synthesized AgNPs were unstable after 5 min [34]. The AgNP synthesis by plant extracts in this study remained stable, which suggests that use of plants is more advantageous than bacteria.

TEM analysis showed the presence of well-dispersed spherical nanoparticles without aggregations. The particle size decreased with increase in temperature. The particle size decreased from around 30 nm at 20 °C to less than 10 nm at 90 °C. The particle shape changed from irregular at lower temperature to spheres at higher temperatures. Regarding effect of temperature on AgNP size, Song and Kim [15] reported that the reaction rate increases with increase in the reaction temperature, and most silver ions are consumed in the formation of nuclei, stopping the secondary growth process on the surface of the preformed nuclei. Rai et al. [33] also reported similar observations for gold nanotriangles synthesized using lemongrass extract.

The inset of Fig. 3(d) shows the selected area electron diffraction (SAED) pattern recorded from the AgNPs. The ring-like diffraction pattern indicates that the particles are crystalline. The diffraction rings could be indexed on the basis of the fcc structure of silver. Four rings arise due to reflections from (111), (200), (220), and (311) lattice planes of silver, respectively. Similar SAED pattern was obtained with AgNPs synthesized using *Aloe vera* extract [35] and *M. kobus* plant extract [15].

SEM provided further insight into the morphology and size details of the AgNPs. The nanoparticles observed were spherical. The peaks around 3.40 keV in EDS spectrum correspond to the binding ener-

gies of Ag. The EDS profile shows strong silver signal along with a weak oxygen and carbon peak, which may originate from the biomolecules that are bound to the surface of the AgNPs. Silver signal increased to 99.3% at 90 °C from 87.0% at 20 °C, suggesting increase in purity of nanoparticles with increase in temperature.

The FT-IR spectrum of AgNPs synthesised at 20 °C showed absorption peaks at 3,434, 2,364, and 1,631 cm^{-1}. The peaks found at 60 °C were 3,433, 2,922, 1,629, and 602 cm^{-1}. The peaks found at 90 °C were 3,435, 2,923, 2,854, 2,287, and 1,626 cm^{-1}. The intense broad line at 3,433-3,435 cm^{-1} is characteristic of the hydroxyl functional group in alcohols and phenolic compounds. The bands at 2,922 cm^{-1} could be due to alkane C-H stretch, which is associated with lipid molecules in the leaf broth. The IR band at 1,631 cm^{-1} is due to amide II bond from proteins. The bands at 2,287 and 602 cm^{-1} can be assigned to C=N and phenyl (C-H) stretch, respectively. These observations are indicative of the binding of protein with Ag nanoparticles through free carboxylate group. Currently, the mechanism of biological synthesis of nanoparticles is not fully understood. With Neem leaf broth, it was reported that terpenoids are believed to be the surface active molecules stabilizing the nanoparticles, and reaction of the metal ions is possibly facilitated by reducing sugars and/or terpenoids present in the Neem leaf broth [20]. Results with *Capsicum annuum* L. extract indicated that the proteins which have amine groups played a reducing and controlling role during the formation of AgNPs in the solutions, and that the secondary structure of the proteins changed after reaction with silver ions [36]. FT-IR results suggest that the bioorganics from broth of *K. pictus* formed a strong coating/capping on the nanoparticles. The compounds like hederagenin glycosides such as kalopanaxsaponin A-K, phenolic glycosides of liriodendrin, syringing, and coniferyl aldehyde 4-O-glucoside have been reported in this plant [37-39]. More elaborate studies are required to elucidate the mechanism of biological synthesis of nanoparticles.

The variability of the susceptibility of *E. coli* can be regarded to size of AgNPs. Size-dependent variability in antimicrobial activity was investigated earlier by different researchers. The AgNPs synthesized at higher temperature show presence of smaller nanoparticles. These nanoparticles may be responsible for the increase in antimicrobial ability.

The AgNPs of small size easily enter in the bacterial cell and affect the intracellular processes such as DNA, RNA, and protein synthesis. The binding of AgNPs with bacteria depends on the surface area for the interaction. Smaller particles affect the larger surface area of the bacteria; thus they have more bactericidal activity than the larger nanoparticles [18,40]. The mechanism of inhibitory action of AgNPs on microorganisms is partially known. AgNPs have positive charge and can attach to the negatively charged microorganisms by electrostatic attraction in the cell wall membrane [41]. AgNPs are associated with thiol groups of cell wall and result in the generation of reactive oxygen species, thereby disrupting the cell [42]. AgNPs closely associate with cell wall of bacteria by forming 'pits' and affect the permeability resulting in cell death [43].

CONCLUSIONS

We successfully synthesised AgNPs by using an environmentally friendly method with leaf broth of plant *K. pictus*. The rate of AgNP synthesis using *K. pictus* was faster than using *M. Kobus*, which was known to be fastest in the previous report. The characterization studies by spectroscopic and microscopy techniques confirmed synthesis of spherical AgNPs of small size. The average particle size decreased from around 30 to less than 10 nm by increasing the reaction temperature. The synthesised AgNPs were able to inhibit growth of *E. coli*. We believe that our observations will prove beneficial in approaching rapid synthesis of nanoparticles and using them for controlling microorganisms associated with human and environmental health.

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