A green approach to synthesize controllable silver nanostructures from *Limonia acidissima* for inactivation of pathogenic bacteria

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*Cogent Chemistry* (2016), 2: 1144296
PHYSICAL CHEMISTRY | RESEARCH ARTICLE

A green approach to synthesize controllable silver nanostructures from *Limonia acidissima* for inactivation of pathogenic bacteria

E. Chandra Sekhar¹, K.S.V. Krishna Rao²,3*, K. Madhusudana Rao⁴ and S. Pradeep Kumar⁵

Abstract: Controllable silver nanoparticles were developed by a green approach using extracts of both leaves and bark of *Limonia acidissima* tree. Due to the presence of phytochemical compounds in *L. acidissima* leaves and bark; such as saponins, phenolic compounds, phytosterols, and quinines present in extracts act as reductants, hence the silver nanoparticles were easily produced under mild conditions. The formation and kinetic study of silver nanoparticles were verified by UV–vis spectroscopy. Highly stable and uniform size silver nanoparticles were produced using bark extract reduction than leaf extract and confirmed by dynamic light scattering and transmission electron microscopy analysis. Further we applied antibacterial activity on both *Escherichia coli* and *Bacillus subtilis*. The results suggest that the silver nanoparticles suspension exhibits excellent antibacterial activity. The present study is a simple and eco-friendly approach for production of silver nanoparticles in the large scale up and could be easily commercialized, especially biological applications.

Subjects: Materials Chemistry; Nanoscience & Nanotechnology; Physical Chemistry

Keywords: green synthesis; *Limonia acidissima*; silver nanoparticles; antimicrobial activity and biological applications

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PUBLIC INTEREST STATEMENT

There is a growing necessity of nanotechnology for biomedical application. Green chemistry plays an important role for the production of metal nanoparticles from medicinal plants which have significant including DNA interaction, enzyme inhibition, anti-cancer, and anti-bacterial studies. The present study demonstrates bio-reduction of silver (Ag⁺) nanoparticles using *L. acidissima* plant materials. *L. acidissima* aqueous leaf and bark extracts appears to be simple, inexpensive, and environmentally eco-friendly. Aqueous extract of leaf and bark could act not only reducing the silver ion (Ag⁺) but also control the size i.e. average sizes ~25 nm and ~12 nm, respectively. Also silver nanoparticle suspension exhibited potential antibacterial activity against *Escherichia coli* and *Bacillus subtilis*. 

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1. Introduction
Green chemistry is the design of chemical products that reduce the use of hazardous substances, and it applies across the life cycle of a chemical product, including its design, production, use, and decisive disposal (Robert, Morrison Robert, & Boyd, 1992). Green chemistry contributes advantage over physicochemical approaches in terms of its eco-friendly environment and has the ability to reform biomaterials which are more effective and less toxic and could benefit millions of patients throughout the world. Green chemistry has been engaged effectively in the production of functionalized products materials that have a strong correlation to the functionalized nanomaterials projected for a range of forthcoming applications; one would suppose effective application of this method for these emerging materials. Green chemistry is progressively seen as a controlling implement that researchers must use to evaluate the environmental impression of nanotechnology (Zhao & Stevens, 1998). And it refers to investigate on materials that are nanometer in size and pertinent to almost every technology and medicine through medicinal plants.

Nanotechnology is a momentous technology which is widely using in miscellaneous areas like medicine, biology, chemistry, catalysis, photonics, electronics, and bio-labeling (Kim et al., 2007). Outstanding physicochemical properties of nanoparticles, including catalytic activity (Rai, Yadav, & Gade, 2009), optical and electronic properties (Sun, 1988; Valiathan, 1998) as well as cytotoxic and antibacterial properties (Talalay, 2001), researchers showed attainment interest towards the expansion of novel methods for synthesis of nanoparticles. Their antibacterial effect is applicable to stumbling block of respiratory enzyme pathways, interacting with the proteins, and modification of DNA (Robinson & Zhang, 2011; Xavier, Auxilia, & ISelvi, 2013). The combination of traditional and modern knowledge can produce better source of the active components for the treatment of diseases with fewer side effects (Ameenah, 2006). Green synthesis is guiltless and eco-friendly method for originated nanoparticles (Adeleh & Hamed, 2012). The use of nanoparticles as potential drug carriers in the treatment of cancer has also been reported (Anish, Templeton, Munshi, & Ramesh, 2013; Lim, Jang, Lee, Haam, & Huh, 2013; Shankar, 2004; Yang, Mu-Yi, Liu, Huang, & Wei, 2012). Nanoparticles can be produced using different types of chemicals and physical methods to bring under control the problem of toxicity in synthesis, medicinal plants have come to have a major role in the synthesis of nanoparticles. Phytochemical compounds such as saponins, phenolic compounds, phytosterols, and quinines present in plant (Manish, Arun, & Ansari, 2009) have both preservative and reductive activity, and are mainly liable for the reduction of silver ions to nanoparticles using chemical and radiation methods. At present there has been a progressive need to derive an environmentally exonerable nanoparticle synthetic process that does not involve any toxic chemicals. However, metal nanoparticles are synthesized by reducing agents such as hydrazine, 1,2-hexadecanediol, NaBH₄, and ascorbic acid (Kim, Cha, & Gong, 2013; Praveena & Kumar, 2014; Venkateswar Rao & Viswanathan, 2010, 2012) for reduction of silver ions to silver nanoparticles (Ag⁺ to Ag⁰), which are extremely toxic in nature. Green chemistry offers the replacement of anticipatory approach that calls for safe, efficient, and effective materials from the starting to end of a chemical product. Green synthesis facilitates in reduction or elimination of the substances that are hazardous to human health and the environment (Kumar, Vemula, Ajayan, & John, 2008). Nowadays, there is a considerable interest in using green chemistry for fabrication of metal nanoparticles (Arokiyaraj et al., 2014; Christensen, Vivekanandhan, Misra, & Mohanty, 2011; Gandhi, Sirisha, & Sharma, 2014; Geethalakshmi & Sarada, 2010). Sastry et al. (Ahmad, Senapaty, Khan, Kumar, & Sastry, 2003; Shankar, Rai, Ahmad, & Sastry, 2004) synthesized stable Ag, Au, and Ag–Au bimetallic nanoparticles using Azadirachta indica leaf broth. Also, recent literature suggested that green synthesis of Ag and Au nanoparticles from apiin (Kasthuri, Veeropandian, & Rajendiran, 2009), Cinnamomum zeylanicum bark extract (Sathishkumar et al., 2009), mushroom extract (Philip, 2009), Acalypha indica leaf extracts (Krishnaraj et al., 2010), glucose, and starch (Raveendran, Fu, & Wallen, 2006). Considering the above facts, authors chosen a Limonia acidissima plant leaf and bark extract as a reducing agent for green synthesis of silver nanoparticles, which is the easiest, most cost efficient, non-toxic, eco-friendly, efficient method, and rapid method.
**L. acidissima** appertaining to the family **Rutaceae** is a normal-sized deciduous plant (Geeta Vasudevan, Kedlaya, Deepa, & Ballal, 2001). It is given with many medicinal uses in Ayurveda systems of medicine. The fruits are woody, rough, and used as a substitute for “blood alcohol level” in diarrhea and bacterial diarrhea like shigellosis dysentery (Sunitha & Krishna Mohan, 2013). The fruits are used for tumors, asthma, wounds, cardiac debility, and hepatitis. Leaves are aromatic and astringent, oil of leaves useful in relieving itching and when mixed with a pinch of black pepper is used as a carminative; fruits of **L. acidissima** are used to prepare tonic, antiscorbutic, alexipharmic, astringent, stomachic, and stimulant (Absar, Eswar, Omer, & Feronia, 2010). Different parts of **L. acidissima** (stem bark, root bark, and unripe fruit-shells) contain antifungal compounds (Adikaram, Yamuna, Leslie, Gunatilaka, & Bandara, 1989; Parrotta, 2011). Hence, phytochemical constituents of leaves and bark of **L. acidissima** have orientin, psoraline, vitexin, xanthotoxin, amides, feronolide, feronone, limonoids, physcion, quinolones, indole, alkaloids (Kithsiri, Jeratne, Bandara, Leslie, & Gunatilaka, 1992; Parthasarathi, Asok Kumar, & Swapnadip, 1989).

To the best of the authors’ knowledge there is no report on synthesis of silver nanoparticles utilizing an aqueous leaf and bark extracts of **L. acidissima**. In this work, **L. acidissima** extract in a concentrated aqueous solution of silver nitrate (AgNO₃) resulted in reduction of silver ions (Ag⁺) and formation of silver nanoparticles.

2. **Materials and methods**

2.1. **Materials**

**L. Acidissima** plant materials (leaves and bark) were collected in the month of 19 October 2014 in Yogi Vemana University Andhra Pradesh; India. Silver nitrate (Analytical grade) was received from MERCK Specialties Pvt. Ltd, INDIA. Throughout the experiment double-distilled water was used and all reagents were used without further purification.

2.2. **Preparation of Extracts of L. Acidissima Leaves and bark**

The **L. acidissima** plant derivatives (leaves and bark) were collected and immersed in a distilled water bath to remove the surfaces adhered to dust particles; the derivatives were removed from the bath and allowed for drying in a dust-free environment at room temperature for 48 h. These plant derivatives were cut into small pieces for further study. 10 g of leaves/10 g bark were added to 100 mL of distilled water in 500 mL Erlenmeyer flask for 60 min at 70°C. The extract was filtered with Whatmann grade No. 1 filter paper. Collected extract was preserved at 4°C for further experiment.

2.3. **The synthesis of silver nanoparticles**

Aqueous AgNO₃ solution and the aqueous **L. acidissima** plant (leaf and bark) extracts were used for fabrication of Ag nanoparticles through bio-reduction process. Briefly, to synthesize silver nanoparticles, 0.01 M AgNO₃ 300 μL for leaf extract (5 mL) and 100 μL for bark extract (5 mL) were added in a bottle. After a few minutes, silver nanoparticles were formed and monitored by brown color of colloidal suspension. Different feed compositions for synthesis of silver nanoparticles are presented in Table 1. The schematic representation of fabrication of silver nanoparticles is shown in Figure 1. Further, formation of silver nanoparticles was verified by UV–vis spectrophotometer at different time intervals, and effect of concentration of extract and concentration of silver ion solution.

2.4. **Characterization**

UV–vis absorbance spectroscopy: bio-reduction of the nanoparticles by silver nitrate was monitored using a Series 3000 double beam ultraviolet-visible spectrophotometer (UV–vis Spectrophotometer, LAB INDIA, UV-3092). Samples were loaded into a 1 cm path length quartz cuvette for analysis. The UV–vis spectrophotometric readings were recorded at a scanning speed of 0.5 nm intervals and were scanned from 200 to 800 nm. Fourier transforms infrared analysis (PerkinElmer Spectrum Two, UK) spectrophotometer was measured to **L. acidissima** silver nanoparticles. Samples of **L. acidissima** leaf extract silver nanoparticles and **L. acidissima** bark extract silver nanoparticles were dried at 40°C for 2 days, and mixed with KBr in a ratio of 10:200 (w/w) and pressed under vacuum to form pellets.
Fourier transforms infrared analysis of the samples was recorded in transmittance mode. Dynamic light scattering study: The zeta-sized nanosequence performs size measurements using advancement called dynamic light scattering spectroscopy measures Brownian motion and relates this to the size of the particles. Mean diameter and size circulation of the nanoparticles were determined by dynamic light scattering method using a Brookhaven BI-9000 AT instrument (Brookhaven Instruments Corporation, USA). Transmission electron microscopy: Samples of bio-synthesized silver nanoparticles (5 mL of leaf extract vs. 300 μL of AgNO₃ for leaf extract silver nanoparticles and 5 mL of bark extract vs. 100 μL of AgNO₃ for bark extract silver nanoparticles) were prepared by placing a drop of the colloidal solution on carbon-coated copper grids, allowing the films on the transmission electron microscopy grids to stand for two minutes, removing the excess solution with blotting paper, and letting the grid dry prior to measurement.

2.5. Antimicrobial activity

Antimicrobial activity of leaf extract silver nanoparticles/bark extract silver nanoparticles was performed by disc method of Bauer-Kirby (Kirby, Yoshihara, Sundsted, & Warren, 1957). Mueller Hinton Agar (M173) plates were prepared for rapid growth of *Bacillus subtilis* (G⁺ve) and *Escherichia coli* (G⁻ve) bacteria for this study. 10 μL of leaf extract silver nanoparticles/bark extract silver nanoparticles colloidal solution and pure extract was impregnated onto filter paper disks of 5 mm diameter, under aseptic conditions with different concentrations, then situated onto a cultured Mueller Hinton agar plates using a mechanical dispenser or sterile forceps. The plates were incubated for 18–20 h, at 37°C in the incubation chamber. Antimicrobial activity was estimated in identical by computing the zone of inhibition for the test organisms. The inhibition zone diameter that is produced will specify the susceptibility or resistance of a bacterium to the extract. Antibacterial activity can be resolute by

| Table 1. Different feed compositions for synthesis of silver nanoparticles |
|---------------------------------------------------------------|
| S. No. | Volume of extract (mL) | Volume of AgNO₃ (μL) |
|--------|------------------------|---------------------|
| **Variation of plant leaf extract at constant AgNO₃, (Figure 1(c))** |
| 1      | 2                      | 300                 |
| 2      | 3                      | 300                 |
| 3      | 4                      | 300                 |
| 4      | 5                      | 300                 |
| 5      | 6                      | 300                 |
| **Variation of AgNO₃ at constant plant leaf extract (Figure 1(d))** |
| 1      | 5                      | 200                 |
| 2      | 5                      | 250                 |
| 3      | 5                      | 300                 |
| 4      | 5                      | 350                 |
| 5      | 5                      | 400                 |
| **Variation of plant leaf extract at constant AgNO₃, (Figure 2(c))** |
| 1      | 3                      | 100                 |
| 2      | 4                      | 100                 |
| 3      | 5                      | 100                 |
| 4      | 6                      | 100                 |
| **Variation of AgNO₃ at constant plant bark extract (Figure 2(d))** |
| 1      | 5                      | 50                  |
| 2      | 5                      | 100                 |
| 3      | 5                      | 150                 |
| 4      | 5                      | 200                 |

Fourier transforms infrared analysis of the samples was recorded in transmittance mode. Dynamic light scattering study: The zeta-sized nanosequence performs size measurements using advancement called dynamic light scattering spectroscopy measures Brownian motion and relates this to the size of the particles. Mean diameter and size circulation of the nanoparticles were determined by dynamic light scattering method using a Brookhaven BI-9000 AT instrument (Brookhaven Instruments Corporation, USA). Transmission electron microscopy: Samples of bio-synthesized silver nanoparticles (5 mL of leaf extract vs. 300 μL of AgNO₃ for leaf extract silver nanoparticles and 5 mL of bark extract vs. 100 μL of AgNO₃ for bark extract silver nanoparticles) were prepared by placing a drop of the colloidal solution on carbon-coated copper grids, allowing the films on the transmission electron microscopy grids to stand for two minutes, removing the excess solution with blotting paper, and letting the grid dry prior to measurement.

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comparing the zone diameter obtained with the known zone diameter size for susceptibility (Tetracycline). The antimicrobial activity of L. acidissima extracts was evaluated for both B. subtilis (ATCC 6633) and E. coli (ATCC 25922) (Sripairoj, Suwanborirux, & Tanasupawat, 2013).

3. Results and discussion

3.1. Formation of silver nanoparticles by leaf extract
The production of silver nanoparticles was confirmed by witnessing UV–vis analysis. Figure 2 shows the UV–vis spectral bands of silver nanoparticles manufactured by mixing leaf extract into Ag⁺ ion solution at Lab. Conditions. The UV–vis spectral bands of formation of silver nanoparticles (5 mL of leaf extract with 300 μL of AgNO₃) for various intervals of time are predicted in Figure 2(a). Within 30 s the reduction was started but no clear SPR band appeared. The color of solution turned into reddish brown color, which designates the evidence of formation of silver nanoparticles. After 5 min a strong SPR band identified at 425 nm and the intensity of absorption band grows with increasing the reaction time and subsequent color fluctuations were observed, without shifting the wavelength.
during the reaction. Mostly, the silver nanoparticles formation was started at 5 min and it might be completed within 4 h. For instance, Krishna raj et al. reported the synthesis of silver nanoparticles using the leaf extract of Acalypha indica (Krishnaraj et al., 2010). The reduction of Ag⁺ to silver nanoparticles during disclosure to the plant leaf extracts could be followed by color changes (Ankamwar, Chaudhary, & Sastry, 2005; Chandran & Chaudhary, 2006; Shankar, 2004). A graph plotted between absorbance vs. time, which indicates a constant absorbance value obtained after 40 min.

3.2. Effects of leaf extract concentration
To study the effect of the extract of L. acidissima, volume of the extract was varied from 2 to 6 mL with constant volume (Table 1) of 0.01 M AgNO₃ solution (Figure 2(c)). With the increase in the volume of extract, the SPR band was shifted to longer wavelength that proposed the development of enlarged size of silver nanoparticles. After addition of 5 mL extract increase in the number and size of the silver nanoparticles came to an end, may be due to the decline of the Ag⁺ in the aqueous leaf extract of L. acidissima. The optimization of exact volume of aqueous AgNO₃ solution required to reduce the 5 mL of leaf extract is examined by varying both leaf and metal solutions (Table 1). 300 μL of Ag⁺ was reduced by 5 mL of the extract as shown in the Figure 2(d).

3.3. Formation of silver nanoparticles by bark extract

3.3.1. Effect of contact time
Reduction of Ag⁺ existing in the aqueous solution of Ag complex during the reaction with the phytochemical constituents present in the bark extract of L. acidissima detected by the UV–vis spectroscopy disclosed that nanoparticles in the solution may be correlated with the UV–vis analysis. A strong band noticed around 425 nm was predicted as SPR band and recognized the excitation of free electrons in the nanoparticles. The color changes were started from pale green to brown color at 5 min and its resolution increased up to 1 h reaches reddish brown color indicates the production of silver nanoparticles. Silver nanoparticles formation started between 420 nm and 590 nm whereas the strong peak was detected at 425 nm at the reaction time of 50 min due to excitation of exterior vibrations occurred in the surface of the silver nanoparticles. The intensity of SPR band primarily rises exponentially with time after that it tends to attain a persistent absorption value (Figure 3(a)) signifying the completion of the reaction. SPR band at 425 nm designates the formation of silver nanoparticles which was further confirmed by TEM analysis.

3.3.2. Effects of Ag ion concentration
The volume of the silver nitrate solution was varied (i.e. 50, 100, 150, and 200 μL) with constant volume i.e. 5 mL of L. acidissima bark extract for 50 min. In the UV–vis spectroscopy it was observed that the highest intense peak at 445 nm at the concentration of 100 μL of AgNO₃ solution which may be due to higher reduction of Ag⁺ into silver nanoparticles (Figure 3(d)) and denoted that formation of silver nanoparticles was found to be at lower concentration of Ag⁺ solution. In aqueous extract of L. acidissima bark there is a better reduction at concentration of 100 μL of AgNO₃ for 5 mL of bark extract (Figure 3(c)), which clearly states that very low quantity of AgNO₃ was required to convert Ag⁺ ions to silver nanoparticles whereas in other plants higher volume of plant extract required the formation of silver nanoparticles (Dipankar & Murugan, 2012; Kotakadi et al., 2013; Philip, Philip, 2009; Raju et al., 2012; Rathi Sre, Reka, Poovazhagi, Arul Kumar, & Murugesan, 2015).

3.3.3. Effect of bark extracts concentration
Optimized quantity of the aqueous AgNO₃ (100 μL) was added to the various amounts of L. acidissima bark extract (3, 4, 5, and 6 mL) and their consistent SPR bands detected between 200 and 800 nm. Figure 3(c) suggests that different volumes of extract (Table 1) were monitored to synthesize the silver nanoparticles; and the production increased with increasing volume of the extract without a significant change in the size of the silver nanoparticles. Interestingly, the SPR bands noticed that the absorbance peak was substantially increased without any shift in the wavelength and sharp acute peak was observed at 446 nm with 5 mL of bark extract due to the higher reduction with 100 μL of silver nitrate (Figure 3(d)). This may be due to the presence of numerous acid and
carboxylic groups in the bark extract of the *L. acidissima*. UV–vis spectroscopy is well known to investigate shape and size controlled of nanoparticles, frequency and width of the absorption peak depend on the size and shape of the metal nanoparticles (Srinivasan, 2011).

### 3.4. Kinetic studies of formation of silver nanoparticles from leaf and bark extracts

Kinetic studies of the reduction process exposed that the synthesis of nanoparticles starts within 30 s and reaches the flat terrain after 60 min showed in Figure 2(a), and 40 min for bark extract in Figure 3(a), inset. In the first 10 min there is an exponential increase in absorbance followed by slow and linear increase in the absorbance values which finally flattens out after 60 min thus, suggesting very fast reaction kinetics of silver nanoparticles synthesis. Similar control solution kept in dark for 14 days did not show much color enhancement, suggesting that light has vital role in the synthesis of silver nanoparticles. In the current study, the addition of aqueous AgNO$_3$ to leaf and bark aqueous extract, Ag$^+$ ions were attracted by the –O$^-$ groups of phytochemicals like phenols, flavonoids, and lysine. The exposure of the reaction medium to sunlight would have enabled the quick transfer of electrons from O$^-$ to Ag$^+$ ion resulting in the complete reduction of Ag$^+$ ions. The pseudo-first-order rate constant $k_{obs}$ was obtained from the slope of the plot of log($A_t - A_\infty$)/($A_0 - A_\infty$) vs. time $t$ (Figures 2(b) and 3(b)), conferring to the equation $A_t = A_\infty + (A_0 - A_\infty) \exp(-k_{obs}t)$, where $A_0$ and $A_\infty$ are the initial
and final absorbance, respectively (Philip, 2009). The $k_{obs}$ was found to be $4.79 \times 10^{-2}$ min$^{-1}$ with $R = 0.975$ for bark extract silver nanoparticles (Figure 3(b)) and $3.91 \times 10^{-2}$ min$^{-1}$ with $R = 0.970$ for leaf extract silver nanoparticles (Figure 2(b)), respectively.

$$A_t = A_{\infty} + (A_0 - A_{\infty})e^{-k_{obs}t}$$ (1)

### 3.5. Fourier transforms infrared analysis

*L. acidissima* leaves and bark extract contains mainly saponins, phenolic compounds, phytosterols, and quinines beta carotene, vitamin B, vitamin C, thiamin, flavanone, and riboflavin (Grotewold, Grotewold, & A. S., 2006; Malarkodi et al., 2014). To determine whether during silver nanoparticles synthesis some biomolecules particularly those with free carboxylic (−COOH) or amino (−NH$_2$) groups present in the leaf and bark extracts have been bound to surface of the Ag in the silver ion solution and formed silver nanoparticles. Fourier transforms infrared analysis spectrum of leaf extract, silver nanoparticles leaf extract and bark extract silver nanoparticles showed distinctive bands for several functional groups. Fundamental absorption peaks for phenolic (−OH), aromatic amines, aliphatic amines, carbonyl (>C=O), C−H, and C=C (benzene) functional groups were observed at 3,436, 3,401, 1,601, 1,384, 1,261, 1,040, 1,123, 1,630, 1,390, 1,178, 2,848, and 1,604 cm$^{-1}$, respectively in (Figure 4).

The functional group analysis performed to identify the phytochemicals responsible for the reduction of Ag$^+$ ions to Ag$^0$ and its stabilization. Silver nanoparticles showed distinctive bands similar to those of the *L. acidissima* leaf and bark extracts (Figure 4) indicating that silver nanoparticles were coated with the biomolecules from both extracts. Peak at 1,633 cm$^{-1}$ may be assigned to stretching...
vibrations of C=C. There was a major shift from 3,436 to 3,401 cm⁻¹ showed that the corresponding functional groups such as stretching vibration of O–H of alcohol or N–H of amines may be liable for the reduction of Ag⁺ to stable silver nanoparticles. Very sharp intense peak was observed 1,348 cm⁻¹ in both leaf extract silver nanoparticles and bark extract silver nanoparticles indicating that the phenolic carboxyl groups were affected by Ag⁺ and to form stable Ag o. Broad absorption band located at around 3,391 cm⁻¹ may be attributed to bending vibrations of –O–H stretching and these conclusions were evaluated and the profile of phytochemicals present in leaf and bark extracts of L. acidissima (Banerjee, Someshwar, Subrata, & Chandra, 2011).

3.6. Transmission electron microscopic analysis and dynamic light scattering studies
Transmission electron microscopic analysis of silver nanoparticles supported to get the precise size and shape information. Figure 5 shows picture of leaf extract silver nanoparticles and bark extract silver nanoparticles, particles exhibited average mean sizes and further conformed by dynamic light scattering in Figure 6. All produced (5 mL of leaf extract vs. 300 μL of AgNO₃ for leaf extract silver nanoparticles and 5 mL of bark extract vs. 100 μL of AgNO₃ for bark extract silver nanoparticles) nanoparticles seem to be spherical in their morphology. Figure 5 shows that the particles are almost uniform within the volume of the particle, signifying the presence of silver nanoparticles supporting the probnation of a single SPR band. Figure 5 shows that the silver nanoparticles obtained under normal conditions demonstrate that both leaf extract silver nanoparticles and bark extract silver nanoparticles with average sizes 25 ± 2.9 nm and 12 ± 3.7 nm, respectively.

3.7. Antibacterial activity
Antimicrobial activity of the leaf extract silver nanoparticles and bark extract silver nanoparticles was examined opposed to B. subtilis (ATCC 6633) and E. coli (ATCC 25922) using standard zone of inhibition microbiology assay. The aqueous extract effect of AgNO₃ has antibacterial activity against E. coli and B. subtilis. Previous studies reported that silver nanoparticles have microbial effect on micro-organisms (Mallikarjuna & John Sushma, 2014). Silver nanoparticles showed inhibition zone towards all tested bacteria (Figure 7) and maximum zone of inhibition was found to be leaf extract silver nanoparticles 1 = 14.94 ± 0.71 mm for B. subtilis and leaf extract silver nanoparticles 3 = 15.1 ± 0.82 mm for E. coli with respect to leaf extract silver nanoparticles (Figure 7) at different volumes (leaf extract = 10 μL, leaf extract silver nanoparticles 1 = 10 μL, leaf extract silver nanoparticles 2 = 11 μL, leaf extract silver nanoparticles = 12 μL, and leaf extract silver nanoparticles 4 = 13 μL of each tested bacteria) of silver nanoparticles colloidal solutions. Figure 7 suggested that bark extract silver nanoparticles (bark extract = 10 μL, bark extract silver nanoparticles 1 = 10 μL, bark extract silver nanoparticles 2 = 11 μL, bark extract silver nanoparticles 3 = 12 μL, and bark extract silver nanoparticles 4 = 13 μL of each tested bacteria) were low zone of inhibition when compared to leaf extract silver nanoparticles and reported that bark extract silver nanoparticles 2 = 13.86 ± 0.51 mm for B. subtilis and leaf extract silver nanoparticles 2 = 11.92 ± 1.09 mm for E. coli at various volumes of tested leaf extract silver nanoparticles. However, both leaf extract silver nanoparticles and bark extract silver nanoparticles showed better activity compared to plain leaf and bark extracts. Fascinatingly noticed that E. coli with thin cell wall is more susceptible to cell wall damage compared to B. subtilis with thick cell wall by leaf extract silver nanoparticles (Prasad &
Venkateswarlu, 2014). The intensified antimicrobial effects of silver nanoparticles are analyzed and also specified that once inside the cell, silver nanoparticles would interface with the pathogenic growth indication pathway by regulating polyphenols for cell viability and the nanoparticles were not in direct contact even within the aggregates, indicating stabilization of nanoparticles by a capping agent (Duran, Marcato, Alves, De souza, & Esposito, 2005; Rahman et al., 2014).

4. Conclusion

The present study demonstrates bio-reduction synthesis of nanosized silver nanoparticles using L. acidissima plant materials. L. acidissima aqueous leaf and bark extracts appear to be environmentally eco-friendly, so that this procedure could be used for rapid production of silver nanoparticles. Silver nanoparticles synthesized by green approach in this study using L. acidissima leaf and bark extracts. Aqueous extract of leaf and bark extract could act not only reducing Ag⁺ but also control the size i.e. average sizes 25 ± 26 nm for leaf extract silver nanoparticles and 12 ± 14 nm for bark...
extract silver nanoparticles. Further the silver nanoparticles formations conformed by UV–vis and Fourier transforms infrared analysis and size of both leaf extract silver nanoparticles and bark extract silver nanoparticles studied by dynamic light scattering and transmission electron microscopic analysis. In the future, selection of such plants may create a new platform for realizing the potential of herbal medicines in nanoscience for drug delivery and biomedical applications. The antibacterial activity of silver nanoparticles suspension showed enhancement in the activity towards E. coli and B. subtilis. By using UV–vis spectrometric analysis the rate constant \( k_{\text{abs}} \) was also calculated in this study. In future we will also study the anticancer and anti-HIV activity of silver nanoparticles colloidal suspension. In addition, this report provides a theoretical and experimental foundation for investigation of the biosynthesis of other nanoparticles.

**Funding**

Author Dr K.S.V. Krishna Rao thanks to University Grants Commission (UGC), New Delhi, India, for financial support under UGC-RAMAN Postdoctoral Fellowship program (No. F 5-8/4- 2014 (IC)).

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**Citation information**

Cite this article as: A green approach to synthesize controllable silver nanostructures from Limonia acidissima for inactivation of pathogenic bacteria. E. Chandra Sekhar, K.S.V. Krishna Rao, K. Madhusudana Rao & S. Pradeep Kumar, Cogent Chemistry (2016), 2: 1144296.

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