Journal of Nanoscience and Technology 2(1) (2016) 32–36

ISSN: 2455-0191

Contents List available at JACS Directory

Journal of Nanoscience and Technology

journal homepage: www.jacsdirectory.com/jnst

Lovoa trichilioïdes Root Back Mediated Green Synthesis of Silver Nanoparticles and Rating of Its Antioxidant and Antibacterial Activity against Clinical Pathogens

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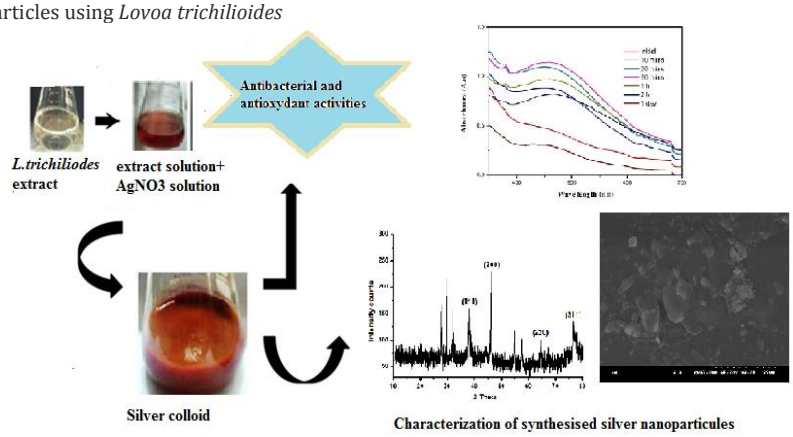
GRAPHICAL ABSTRACT

Green synthesis of silver nanoparticles using *Lovoa trichilioides*



ARTICLE DETAILS

Article history:
Received 25 October 2015
Accepted 02 November 2015
Available online 04 November 2015

Keywords:
Silver Nanoparticles
Antibacterial Activity
Antioxidant Activity
L. trichilioïdes

ABSTRACT

Biosynthesis of silver nanoparticles (AgNPs) was achieved by a novel, simple green chemistry procedure. The effect of time on the synthesis of silver nanoparticles was carried out at room temperature, at 10 min, 30 min, 1 hr and 2 hrs. The successful formation of silver nanoparticles has been confirmed by UV–vis, FTIR, XRD, and SEM analysis. *In vitro* antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella planticola* (Gram-negative), and *Staphylococcus pneumonia, Bacillus subtillus, Entrococcus caccae, lactobacillus acidophilus* (Gram-positive) has been studied by agar diffusion method and compared between three standard antibiotic ampicillin (AMP), chloramphenicol (CHL) and tetracycline (TET). The maximum zone of inhibition was observed in the Au NPs against *E. coli*, (31.00±0.00 mm), *K. planticola* (20.00±0.00 mm), *B subtillus* (19.00±0.00 mm) and *E caccae* (18.00±0.00 mm). *In vitro* antioxidant activity was carried out using DPPH method with 3.125 to 100 µg/mL concentration of silver nanoparticles. The *in vitro* antioxidant activity was found to show biphasic response and inhibitory activity was found between 100 to 25 µg/mL followed by stimulatory activity from 12.5 to 3.25 µg/mL which was not significant. It can be concluded silver nanoparticles synthesized by green synthesis using possess antioxidant activity and act as an effective antibacterial agent.

1. Introduction

Nanoparticles are often referred to as particles with a maximum size of 100 nm. Nanoparticles exhibit unique properties, which are quite different than those of larger particles. New properties of nanoparticles related to variation in specific characteristics like size, shape and distribution have been demonstrated [1]. Among the noble metals (e.g. Ag, Pt, Au and Pd), silver (Ag) is the metal of choice for potential applications in the field of biological systems, living organisms and medicine [2]. Due to their exclusive properties, silver nanoparticles (AgNPs) may have several applications, such as catalysts in chemical reactions [3], electrical batteries and in spectrally selective coatings for absorption of solar energy [4, 5], as optical elements [6], pharmaceutical components and in chemical sensing and biosensing [7, 8]. Nanoparticles formation has been reported using chemical and physical methods. There are various methods for Ag-NPs formation such as sol-gel process, chemical precipitation, reverse micelle method, hydrothermal method, microwave, chemical vapor deposition and biological methods, etc., [9–11]. Recently, biosynthetic methods have been investigated as a new way for the production of AgNPs. Biological methods are currently gaining importance because they are eco-friendly,

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Cite this Article as: L.S. Sidjui, M. Ponnanikajamideen, M. Malini, L.N. Famen, R. Sindhu, J. Uthaya Chandirika, G. Annadurai, G.N. Folefoc, *Lovoa trichilioïdes* root back mediated green synthesis of silver nanoparticles and rating of its antioxidant and antibacterial activity against clinical pathogens, J. Nanosci. Tech. 2(1) (2016) 32–36.

cost effective, and don't involve the use of any toxic chemicals for the synthesis of nanoparticles [12–14]. The biosynthesis of inorganic nonmaterial has been performed using eukaryotic organisms such as fungi to produce nanoparticles of silver [15, 16]. Synthesis of nano silver particles using ascorbic acid and citrate as reducing agents has recently been reported [17]. An earlier study showed that Shewanella algae were found able to reduce silver ions, forming 10–20 nm gold nanoparticles [18]. During recent times several groups have achieved success in the synthesis of Ag, Au, and Pd nanoparticles using extracts obtained from unicellular organisms like bacteria [19-22] and fungi [23-25] as well as extracts from plant parts, such as *Basella alba*, *Helianthus annus*, *Saccharum officinarum*, *Oryza sativa*, *Sorghum bicolor, Zea mays* [26], *Aloe vera* [27], *Medicago sativa* (Alfalfa) [28], *Capsicum annuum* [29], *Magnolia kobus* [30], *Cinnamomum camphora* leaf [31], and *Geranium sp*. [32] for pharmaceutical and biological applications. A green synthesis of nano silver particles using a methanolic extract of *Eucalyptus hybrida* leaves was reported [33], another study related to the synthesis of nanoparticles from *vitex negundo* L. leaf extract in water solution with heat treatment [34]. Recently, some studies have shown that specially formulated AgNPs have good antibacterial activity [35]. The bacteria usually are incapable of developing resistance against AgNPs, because these nonmaterial can at the same time attack a broad range of targets in microorganisms such asproteins with thiol groups, cell walls and cell membranes. A recent study on *E. coli* has shown that AgNPs react with cell walls and cytoplasmic membranes, resulting in pits in the cell wall of bacteria, and finally killing them [36]. It has been reported that greenly synthesized titania (TiO_2) and silver nanocomposites (TANCs) can easily damage the cell walls of *E. coli* [37]. The antimicrobial and antiviral activity of silver ion, silver compounds and AgNPs have been thoroughly investigated in previous studies [38–40]. There are several reports on the use of natural materials sources like plants, bacteria, fungi, yeast and honey for synthesizing silver nanoparticles. In continuation of the efforts for synthesizing silver nanoparticles by green route, here we present are port on the facile, rapid and single-pot aqueous biosynthesis of these nanoparticles using the root backs extract of *L. trichilioïdes* (meliaceae). The family meliaceae has 50 genera and about 140 species ranging from trees to shrubs. Plants of the family meliaceae are rich sources of limonoids and use in traditional medicine as anticancer, antimalarial, antimicrobial, antitumor, antiviral, anti-inflammatory, anti-insecticidal and anti-ulcer [41-44].

In this paper, we report on the biosynthesis of pure metallic nanoparticles of silver by the reduction of aqueous Ag^+ ions with the methanolic root backs extract of *L. trichilioïdes* and its determination of in *vitro* antibacterial and antioxidant activity.

2. Experimental Methods

2.1 Plant Materiel

The roots back of *L. trichilioïdes* were collected in November 2014 from the Mont Kala locality in the Central region of the Republic of Cameroon. Plant material was identified by Mr. Victor NANA, a plant taxonomist at the National Herbarium of Cameroon. A voucher specimen was deposited (36420/HNC) at the National Herbarium of Cameroon.

The air-dried powdered stem barks (500 g) of *L. trichilioïdes* was exhaustively macerated successively with n-hexane (n-hex, 2 L), Methyline chloride (DCM 2 L), ethyl acetate (AcOEt, 2 L) and Methanol (MeOH, 2 L) respectively at room temperature for 72 h. The macerate was filtered and evaporated under reduced pressure to obtain crude extracts labeled LM1 (4 g), LM2 (6 g), LM3 (6 g), LM4 (5 g) respectively.

2.2 Green synthesis of Silver Nanoparticles

Typical synthesis process of silver nanoparticles 10 mL of pure root extract solution mixed with aqueous solution of 90 mL of 1mM silver nitrate ($AgNO_3$) solution and kept in room temperature for constant stirring at 120 rpm. A color change of the solution noted by visual inspection and the absorbance calculated by UV-vis spectroscopy at different time and wavelength confirming by synthesis of silver nanoparticles.

2.3 Purification and Characterization of Silver Nanoparticles

The bioremediation of silver ions in aqueous solution using root extract was observed by double beam UV-vis spectrophotometer at different wave length from 300 to 700 nm (Perkin Elmer Singapore). The synthesized silver nanoparticles are purified by distilled water by repeated centrifugation at 8000 rpm 15 min crystalline nature of silver nanoparticles was analyzed by XRD (Panalytical 'X' Part Pro X-ray Diffractometer) and particles morphologic structure was characterized by Scanning Electron Microscope (SEM). The functional carboxyl groups present in the plant extract responsible for silver nanoparticles formation were characterized by FT-IR (Perkin Elmer Singapore). The dried silver nanoparticles were measured at the wavelength range from 100 to 4000 cm^{-1}.

2.4 Characterization

Preliminary characterization of the silver nanoparticles was carried out using UV–visible spectroscopy. The reduction of silver ions to the nanoparticle form was monitored by measuring the UV–Visible spectra of the solutions after diluting the sample with deionized water. The UV–visible spectra were recorded on a Shimadzu UV-1601 spectrophotometer with samples in quartz cuvette operated at a resolution of 1 nm from 400 to 700 nm. Deionized water was used as blank. The spectra recorded were then re-plotted using Origin 6.0 version.

High Resolution Scanning Electron Microscopic (HRSEM) image and Energy Dispersive X-ray (EDAX) analysis samples were prepared on carbon coated copper grids, which was dried and measured on a FEI Quanta FEG 200 HRSEM equipped with EDAX.

Silver colloids were centrifuged (14,000 rpm, 25 °C) for 10 min, washed several times with distilled water, and were then freeze dried and subjected to XRD analysis. The XRD studies was performed with XPERTTVPVR-7130 (Philips), Cu Kα radiation (k = 1.54 nm) in the 2 hrs range of 30–80 operate data voltage of 40 kV and a current of 30 mA.

FTIR measurement, the solution of silver nanoparticles was centrifuged at 10,000 rpm for 15 min. The pellet was dried and mixed with KBr pellet and analyzed on a FT-IR instrument (Perkin Elmer Spectrum1).

2.5 Antibacterial Assay of Silver Nanoparticles

The silver nanoparticles synthesized by using root extract was examined for antimicrobial activity by agar well diffusion method against pathogenic microbes for *Escherichia coli, Klebsiella planticola, Pseudomonas sp., Streptococcus pneumonia, Lactobacillus acidophilus, Bacillus subtillus, Entrecoccus caccae*. The pure cultures of bacteria were sub cultured on nutrient broth. Each strain was swabbed homogenously on the individual plates using sterile cotton swabs. Wells of 10 mm diameter were through on Muller Hinton agar using gel puncture different concentration of silver nanoparticles 100 µg, 200 µg, and 300 µg was poured on each well. After 24 hrs incubation the various level of zone of inhibition was measured. Three replicates of experiments were carried out.

2.6 In vitro Antioxidant Activity by DPPH Method

In vitro antioxidant activity/Free radicals scavenging potential of silver nanoparticles were tested against a Methanol solution of 1,1–Diphenyl–2 –picryl hydrazyl (DPPH) [45]. Antioxidants reacts with DPPH and convert it to 1,1–Diphenyl–2–picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the extract. The change in the absorbance produced at 517 nm has been used as measure of Antioxidant activity. Different concentration of silver nanoparticles (3.125 to 100 µg/mL) were mixed with DPPH methanol solution in 3 mL of total reaction mixture and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 517 nm and converted to percent antioxidant activity. For a comparative study, the ascorbic acid was used as the standard. The percentage inhibition activity was calculated by using a formula.

Percent Inhibition = 100 x (Absorbance of Blank- Absorbance of Sample) / Absorbance of Blank

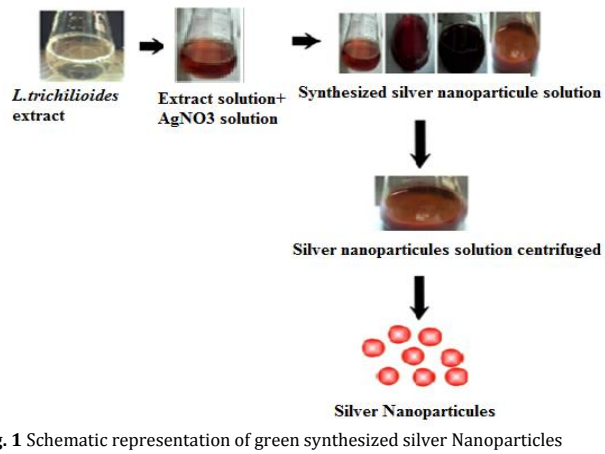

Fig. 1 Schematic representation of green synthesized silver Nanoparticles

3. Results and Discussion

3.1 Visual Identification

Diminution of silver ions to silver nanoparticles was visually distinguished by color change from yellow to brown in the aqueous solution of reaction mixture at 30 min incubation time (Fig. 1). Brown color formation took place due to the oscillation free electrons in the reaction mixture the color change depended on the incubation time. The deep brown color for silver nanoparticles was happened upon at 24 hrs pointing that the increasing of color intensity is directly proportion to the time of incubation [46]. Furthermore, the nanoparticles formation by the root extract confirmed by UV-vis spectroscopy at different wave length.

3.2 UV-Vis Spectroscopy Analysis

UV-vis spectroscopy analysis depends on the arising of color in the reaction due to the excitation of surface plasmon resonance band in reaction mixture and was recorded as different functional time such as 10 min, 20 min, 30 min, 1 hr, 2 hrs and 24 hrs. The peaks that are observed 460 nm indicates the presence of silver nanoparticles that are synthesized by *L. trichilioïdes* root extracts. The UV spectrum *L. trichilioïdes* root extracts signifies that the absorbance of silver nanoparticles is slowly increased from 10 min to 2 hrs. Fig. 2 shows that, there is no peak were formed at the initial stage it indicates that there is no silver nanoparticles were noted. After 30 min of incubation time the surface plasmon resonance band for silver nanoparticles was positioned at around 460 nm and the synthesis was steadily decreasing with the increasing in the time of reaction with slight change in the peak position. The nanoparticles synthesis was completed at 2 hrs time of incubation.

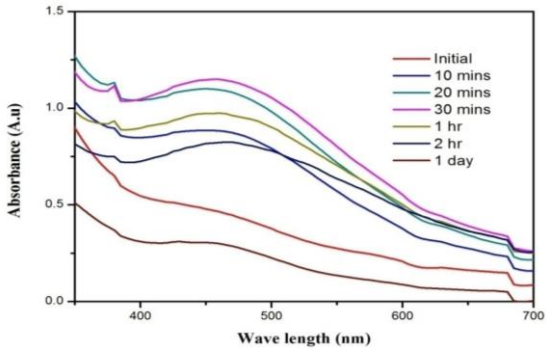

Fig. 2 UV- visible Spectrum of silver nanoparticles from stem backs methanolic extract of *L. trichilioïdes*

3.3 X-Ray Diffraction (XRD) Analysis

The XRD spectra are used to confirm the crystalline nature of the silver nanoparticles synthesized by using *L. trichilioïdes* root extracts and the figure is presented in Fig. 3. The four distinct diffraction peaks of the 2θ values of 38.04°, 46.22°, 57.46°, 76.75° should be assigned the plane of (111), (200), (220), (311), respectively indicating that the silver nanoparticles are fcc (face centered cubic) and crystalline in nature. The synthesized silver nanoparticles are compared with standard silver nitrate and pure silver particles which are published by Joint Committee on Powder Diffraction Standards (File nos. 04-0783). The XRD spectrum clearly shows that the synthesized silver nanoparticles using the above mentioned extracts are crystalline in nature.

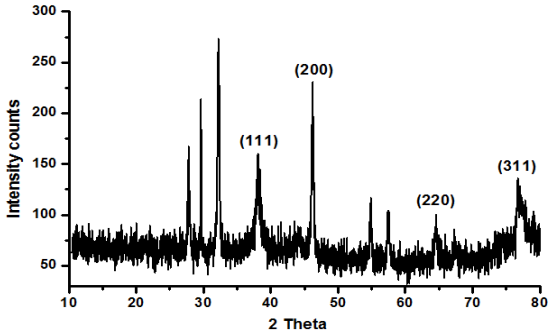

Fig. 3 XRD pattern of Silver nanoparticles from stem backs methanolic extract of *L. trichilioïdes*

3.4 Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscope is one of the potent tools to identify the morphology of the nanoparticles. The silver nanoparticles synthesized by the *L. trichilioïdes* root extract are shows (Fig 4) that the sizes at around 20 nm with many shapes which are triangle, rod, spherical are clearly observed. This SEM image also showed the aggregation of the silver nanoparticles. Similar results were showed in the silver nanoparticles using stem extracts of *C. quadrangularis* with the size ranging from 37 to 43 nm [47]. High density of silver nanoparticles is shown in the SEM image which is uniformly distributed on the surface of the root extract.

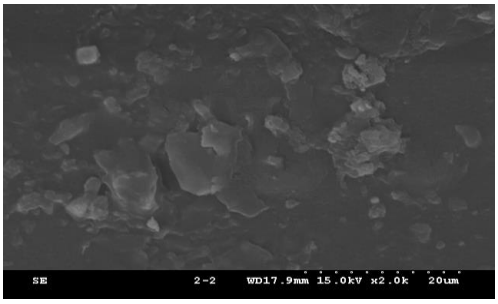

Fig. 4 SEM image of silver nanoparticles from stem backs methanolic extract of *L. trichilioïdes*

3.5 Fourier Transformed Infrared (FTIR) Spectroscopy Analysis

The potential functional groups of phytochemicals in plant extracts involved in nanoparticles synthesis are identified by FT-IR analysis. The silver nanoparticles synthesized by root extract of *L. trichilioïdes* (Fig. 5 (a)), expose acute absorption peaks at 3271 cm^{-1} representing to N-H stretching of primary amine. The weak band observed at 2923 cm^{-1} indicates the H-C-H asymmetric and symmetric stretching of alkanes. The absorption band at 1017 cm^{-1} corresponding the C-O stretching of ethers group of root extracts. The originating of these functional groups in FT-IR spectrum designates the affiliation of silver nanoparticles with the phytochemicals. In root-derived crude extract (Fig 5(b)), the broad spectrum at 3251 cm^{-1} comprises the presence of hydrogen-bonded O-H stretch phenols and alcohols. The band at 2923 cm^{-1} and 2853 cm^{-1} denotes the H-C-H asymmetric and symmetric stretching of alkanes. The strong band observed at 1020 cm^{-1} C-O stretching of ethers. FT-IR exposed that soluble organic compounds or proteins in the extracts may bind with the silver ions to nanoparticles.

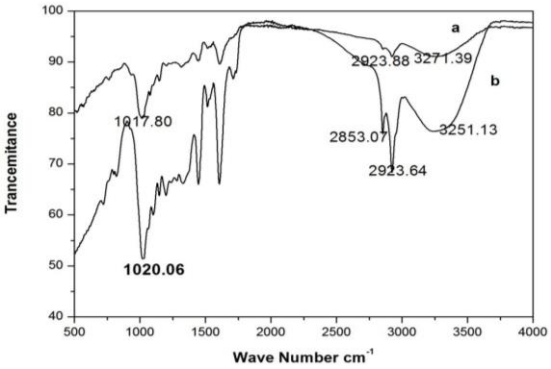

Fig. 5 FTIR image of stem backs extract of *L. trichilioïdes* (5a) and Synthesized silver nanoparticles (5b)

3.6 Antibacterial Study

Antibacterial properties of triangular and spherical shaped Ag nanoparticles against gram negative strain *S. pneumonia*, *L. acidophilus, B. subtillus*, *E. caccae*, *S. entomphila*, *Pl. sp.* and gram positive strain *E. coli*, *K. planticola*, *P. sp.* was performed and is given Table 1. It is well know that *E. coli* is the most characterized bacterium and cause gastroenteritis, urinary, tract infection and neonatal infection, *S. pneumonia* can cause a range of illnesses from minor skin infection, to life-threading diseases such as pneumonia, chest pain, and bacteremia. From the table, it was found that inhibition diameter was concentration dependent then, increase with concentration. Moreover, at 100 µg/disc the bacteria are less sensible to nanoparticles compare concentration to 300 µg/disc. *Pseudomonas sp.* and *Serratia entomphila* were the more resistant microorganisms at 100 µg/disc.

Cite this Article as: L.S. Sidjui, M. Ponnanikajamideen, M. Malini, L.N. Famen, R. Sindhu, J. Uthaya Chandirika, G. Annadurai, G.N. Folefoc, *Lovoa trichilioïdes* root back mediated green synthesis of silver nanoparticles and rating of its antioxidant and antibacterial activity against clinical pathogens, J. Nanosci. Tech. 2(1) (2016) 32–36.

Table 1 Bacteria inhibition zone diameters of AgNPs nanoparticles

Microorganisms tested	Inhibition zone diameter ± S.D (mm)					
	AgNPs: Conc (µg/disc)			References		
	100	200	300	AMP	CHL	TET
E.coli	11.0±0.0	18.0±0.0	31.0±0.0	0.0±0.0	17.0±0.0	12.0±0.0
K.planticola,	12.0±0.0	16.0±0.0	20.0±0.0	0.0±0.0	16.0±0.0	16.0±0.0
P. sp.	0.0±0.0	11.0±0.0	14.0±0.0	6.0±0.0	0.0±0.0	37.0±0.0
S.entomphila	0.0±0.0	13.0±0.0	14.0±0.0	15.0±0.0	38.4±0.2	31.0±0.0
S.pneumonia	10.0±0.0	12.0±0.0	13.0±0.0	0.0±0.0	28.0±0.0	29.0±0.0
L.acidophilus	15.0±0.0	15.0±0.0	16.0±0.0	0.0±0.0	25.0±0.0	27.0±0.0
B. subtillus	14.0±0.0	17.0±0.0	19.0±0.0	0.0±0.0	12.0±0.0	27.0±0.0
E. caccae	15.0±0.0	17.0±0.0	18.0±0.0	13.0±0.0	40.0±0.0	6.0±0.0
Pl. sp.	11.0±0.0	15.0±0.0	16.0±0.0	17.0±0.0	35.3±0.2	13.0±0.0

Each experiment was performed three times, and the data were averaged (n = 3). Values are means of three replication ± SD. Microorganisms: (Gram+) E. coli: Escherichia coli, K. planticola: Klebsiella planticola, P. sp.: Pseudomonas sp., (Gram-) S. pneumonia: Streptococcus pneumonia, La: Lactobasillus acidophilus, B. subtillus: Bacillus subtillus. E. caccae: Entrecoccus caccae, S. entomphila: Serratia entomphila, Pl. sp.: Planomicrobium sp. Reference (Positif control): AMP: Amplicillin, CHL: Chloramphenicol, TET: Tetracycline.

Table 2 *In vitro* antioxidant activity of silver NPs by DPPH assay

Concentration [µg/mL]	% Inhibition
100	40.4 ± 0.033
50	14.14 ± 0.003
25	5.05 ± 0.040
12.5	2.02 ± 0.028
6.25	5.05 ± 0.029
3.125	-3.03 ± 0.075

3.7 Antioxidant Activity

Table 2 shows the *in vitro* antioxidant activity of silver nanoparticle. The *in vitro* antioxidant activity was found to show biphasic response and inhibitory activity was found between 100 to 25 µg/mL followed by stimulatory activity from 12.5 to 3.25 µg/mL which was not significant with both 1 mM silver nanoparticles synthesized using methanol and water at room temperature. The *in vitro* activity of nanoparticle synthesized at 75 °C did not show good activity.

4. Conclusion

Our results demonstrating synthesis of disease specific silver nanoparticles by using plant phytochemical will provide unprecedented opportunities towards and development of nanomedicine. The synthesized nanoparticles displayed efficient antibacterial and antioxidant activity. These methods for synthesis for silver nanoparticles only through biosynthesis; did not used any chemicals and thus has the potential for exploit in biomedical application.

Acknowledgements

L.S.S. acknowledges the Centre for International Co-operation in Science (CICS) for a research grant INSA JRD-TATA Fellowship (DO/CICS/4785/2014) at the Manonmaniam Sudaranar University, Tirunelveli, India.

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Cite this Article as: L.S. Sidjui, M. Ponnanikajamideen, M. Malini, L.N. Famen, R. Sindhu, J. Uthaya Chandirika, G. Annadurai, G.N. Folefoc, *Lovoa trichilioïdes* root back mediated green synthesis of silver nanoparticles and rating of its antioxidant and antibacterial activity against clinical pathogens, J. Nanosci. Tech. 2(1) (2016) 32–36.