International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 2, Issue 6, pp: 338-347, 2017
https://doi.org/10.5281/zenodo.1312701
http://www.ijzab.com
Research Article

BIOENGINEERED NANOPARTICLES SYNTHESIZED USING *IPOMOEA PES-TIGRIDIS* FOR IMPROVED ANTIMICROBIAL ACTIVITY AGAINST DRUG RESISTANT MICROBES

A. **Najitha Banu[1]*, A.M. Raut[2] and C. Balasubramanian[3]**

[1]Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara-144411, Punjab, India

[2]Department of Plant Protection, School of Agriculture, Lovely Professional University, Phagwara-144411, Punjab, India

[3]Department of Zoology, Thiagarajar college, Madurai-625 009, Tamil Nadu, India.

Article History: Received 8th November 2017; Accepted 20th November 2017; Published 8th December 2017

ABSTRACT

Green syntheses of silver nanoparticles have fascinated currently and simple method instead of physical and chemical approaches. In the present study, bioengineered nanoparticles were synthesized using fresh leaves of *Ipomoea pes-tigridis*. Synthesized nanoparticles were characterized and confirmed by UV Spectrophotometer, followed by EDX, SEM, XRD and FTIR. The nanoparticle size and morphology was confirmed through scanning electron microscope. The resonant oscillation of conduction of electrons with the sample was observed by UV spectra and obtained the peak 440nm. The energy dispersive X-ray analysis has given a strong signal in the silver region which confirmed the presence of elemental silver in the sample. The functional groups present in *I. pes-tigridis* are responsible for the synthesis of silver nanoparticles were confirmed through FTIR. Further, the synthesized nanoparticles showed high efficiency against drug resistant microbes in the laboratory.

Keywords: *Ipomoea pes-tigridis,* Silver nanoparticles, UV-vis Spectra, FTIR, Antimicrobial activity.

INTRODUCTION

The manifestation of nanotechnology has provided a wide research in recent years by intersecting with diverse other branches of science and creating impact on all forms of life (Baker and Satish, 2012). Nanotechnology and nanoparticles research has find applications in various fields like bio-medical, electronics, optical fibers, agriculture, bio-labeling and in other areas (Salam *et al.*, 2012).

In the recent era, Nanotechnology is playing a vital role in the research of material Sciences due to its specific characteristics such as morphology, size, shape and distribution. Metallic nanoparticles having innovative applications (Andres *et al.*, 2000; Murphy, 2008) and crystalline nanoparticles are used as antibacterial, antifungal and anticancer agents as well as in biomolecular detection, diagnostics and therapeutics (Rai *et al.*, 2009; Elechiguerra *et al.*, 2005; Crook *et al.*, 2001; Gittins *et al.*, 2000). However, there is still a need for economically affordable, stable, viable and environmentally clean synthesis of silver nanoparticles.

From the beginning, only physical and chemical approaches like, reduction in solutions (Goia *et al.*, 1998), chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical and microwave assisted process are used for the nanoparticles synthesis (Taleb *et al.*, 1997; Esumi *et al.*, 1990; Henglein 2001; Rodriguez-Sanchez *et al.*, 2000; Zhu *et al.*, 2000; Pastoriza-Santos *et al.*, 2002). Recently, biological approaches are playing crucial role in the field of nanotechnology, due to the environmentally friendly materials like plant leaves, roots, seed, seed coat, flowers, bacteria, fungi, Actinomycetes, seaweeds etc. (Parashar *et al.*, 2009; Najitha Banu and Balasubramanian 2014a; Najitha Banu and Balasubramanian 2014; Najitha Banu *et al.*, 2014; Najitha Banu and Balasubramanian 2015). Compared to the

*Corresponding Author: Dr. A. Najitha Banu, Assistant Professor, Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara-144411, Punjab, Email: najirila2010@gmail.com, Mobile: +91 7708422990

physical and chemical methods for the synthesis of silver nanoparticles, greener biological method is a very useful eco-friendly method. In the chemical methods some toxic chemicals are used for the synthesis and they may have adverse effect in the medical applications at the same time biological greener approaches are cost effective, easily scaled up for large scale production as well as very easy and no need for high pressure, temperature or toxic chemical (Lok *et al.,* 2007). Silver and Silver nanoparticles are widely used in topical oilments as a wound healing agent (Ip *et al.,* 2006).

In tropical countries, approximately one-half of all death is due to infectious diseases (Venkataswamy *et al.,* 2010). Doss *et al.* (2009) reported that multi drug resistant pathogen is emerged due to many existing antibiotics. New and re-emerging diseases are the misanthrope for the researcher, so there is an immediate need to determine the novel antimicrobial agents with specific mechanism because of re-emerging infectious diseases (Parivuguna *et al.,* 2008). Medicinal plants like natural resources are playing most viable against microbes and all parts of the medicinal plants have specific compounds, these possibly act as an antimicrobial agent. Compare to the different synthetic drugs, greener resources materials have numerous therapeutic value as antimicrobial of plant origin are generally not associated with side effects and have an enormous therapeutic prospective to heal many infectious diseases (Singh and Kumar, 2011).

Most of the medicinal plant parts are used as raw drugs and they possess varied medicinal properties (Mahesh and Satish 2008). Medicinal plants are easily available, less expensive and also have no side effects (Cathrine *et al.,* 2011). Calixto (2005) collected the information from WHO and reported that 65% - 80% of the world's population in developing countries depend on the medicinal plants for their primary health care due to the poverty and lack of access to modern medicine. Medicinal plants represent a rich source of the antimicrobial agent (Mahesh *et al.,* 2008).

Silver nanoparticles were synthesized for the first time in the living system like Alfalfa sprouts by Gardea-Torresdey *et al.* (2003). He has reported that Alfalfa roots have the specific ability to absorb Ag and transferred into the shoots of the plant and Ag atoms are arranged themselves in the shoots. Green syntheses of silver nanoparticles using plants are faster than the microbes quite rapidly. Shankar *et al.* (2003) results showed that synthesis reaction has taken a longer time when compared to Geranium leaves. The 90% synthesis reaction was completed at nine hrs in Geranium leaves and others take 24 to 124 hrs. The nanoparticles synthesis reaction depended on different parameters like type of plants, parts of the plant, temperature, light, agitation, concentration and time (Mittal *et al.,* 2013).

The main reason of using all parts of the plants for the synthesis of nanoparticles are that the plants are having huge medicinal value, easily available and safe to handle and possess a large variety of active agents that can promote the reduction of silver ions as well as plant parts act as a reducing stabilizing agent (Kharissova *et al.,* 2013).

The phytochemical compounds present in the natural source play a major role for the synthesis of nanoparticles. These compounds act as reducing as well as capping agents and possibly form the stable nanoparticles in controlled morphology (Sharma *et al.,* 2009; Kesharwani *et al.,* 2009; Huang *et al.,* 2007; Sathiskumar *et al.,* 2009; Mallikarjuna *et al.,* 2011; Vilchis-Nestor *et al.,* 2008; Kasthuri *et al.,* 2009; Bindhu and Umadevi 2013). The hydroxyl and carboxyl groups present in the plants were able to bind with the metals and the flavonoids and phenols have exclusive power to warp nanoparticles to avoid the agglomeration (Ahmad *et al.,* 2010). Most of the AgNPs synthesized via green approach are investigated for biomedicine and more particularly anti-microbilal agent or for cancer treatment. Therefore, the current study was intended to evaluate *Ipomoea pes-tigridis* synthesized silver nanoparticles against human pathogenic bacteria. Most of the AgNPs synthesized via green approach are investigated for biomedicine and more particularly anti-microbilal agent or for cancer treatment.

MATERIALS AND METHODS

Synthesis of Silver Nanoparticles

Fresh leaves of *I. pes-tigridis* collected from the Keelavalvu, Madurai (District), Tamil Nadu, India. The fresh leaves were washed rapidly with milli-Q water. Five to ten grams of leaf was boiled with 100 mL milli-Q water for 5 to 10 minutes. The obtained extract was filtered through Whatman No 1 filter paper. The filtrate was collected in 250 mL Erlenmeyer flask and stored at 4 ºC for further use. 1mM $AgNO_3$ solution was added in 9:1 ration to *I. pes-tigridis* plant extract and kept in dark condition to avoid photo-oxidation at room temperature. The color change of the plant extract from pale green to dark brown was observed periodically and the aqueous portion was collected for characterization. The formation of brown color indicates the synthesis of silver nanoparticles. The synthesized sample was centrifuged at 5000 rpm for 15 minutes and the pellet was stored for further characterization (Jain *et al.,* 2009).

Characterization of Silver Nanoparticles

The reduction of silver ions was routinely monitored by visual inspection of the solution as well as by measuring the UV-Visible spectra of the solution by periodic sampling of aliquots (2 mL) of the aqueous component. The atomic composition and morphology of bioengineered nanoparticles was confirmed by Energy Dispersive X-ray spectroscopy (EDX), X-ray diffractometer (XRD) and Scanning Electron Microscopy (SEM). The pre-prepared dry powder was collected for the determination of the formation of Ag nanoparticles by an X′ pert pro X-ray diffractometer operated at a voltage of 40 kv and current of 30 mM with Cu Kα radiation in a θ-2θ configuration. The crystallite domain size was calculated from the width of the

XRD peaks, assuming that they are free from non-uniform strains, using the Debye-Scherer formula (Cullity, 1978). Functional groups present in the bioengineered nanoparticles and interactions with protein were analyzed by transform infrared (FTIR) analysis (Najitha Banu and Balasubramanian 2014b).

Antibacterial activity

The standard well diffusion method was followed for the antibacterial activity of the synthesized silver nanoparticles against multi drug resistant clinical isolates such as *Staphylococcus aureus*, *Streptococcus sp.*, *E. coli* and *Pseudomonas sp.* obtained from Entomology Research Institute, Loyola College, Chennai. Approximately 20 to 25 ml of Nutrient agar was poured in sterilized Petri dishes and kept in room temperature for overnight to avoid contamination. For maintaining the virulence, the pathogens were grown in Mueller–Hinton broth at 15 days interval. Further, 24hrs old bacterial pathogens were swabbed in Muller Hinton agar seeded plates and allowed to dry for 10 minutes. A standard cork borer of 6 mm was used to cut six uniform wells on the surface of the agar. The wells were loaded with *I. pes-tigridis* synthesized silver nanoparticles at different concentrations such as 50, 150, 200 µl and control ($AgNO_3$) respectively. The palates were incubated at room temperature for 24 hrs and plates were examined for evidence of zone of growth inhibition after 24hrs. After incubation, the zone of inhibition was measured and mean value for each pathogen was recorded and expressed in millimeter.

RESULTS

The leaf extract of *I. pes-tigridis* was used as the starting material for the synthesis of silver nanoparticles. When the aqueous extracts of *I. pes-tigridis* was added to 1 mM $AgNO_3$ solution, a change in color from colorless to dark brown was observed. The final color deepened with increase in time. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly with in 24 hrs of reaction. Further, there was no change in the color of both positive and negative control (Plant extract & 1mM $AgNO_3$ solution). The color change indicated that reduction reaction occurred in the aqueous solution of *I. pes-tigridis* (Figure 1).

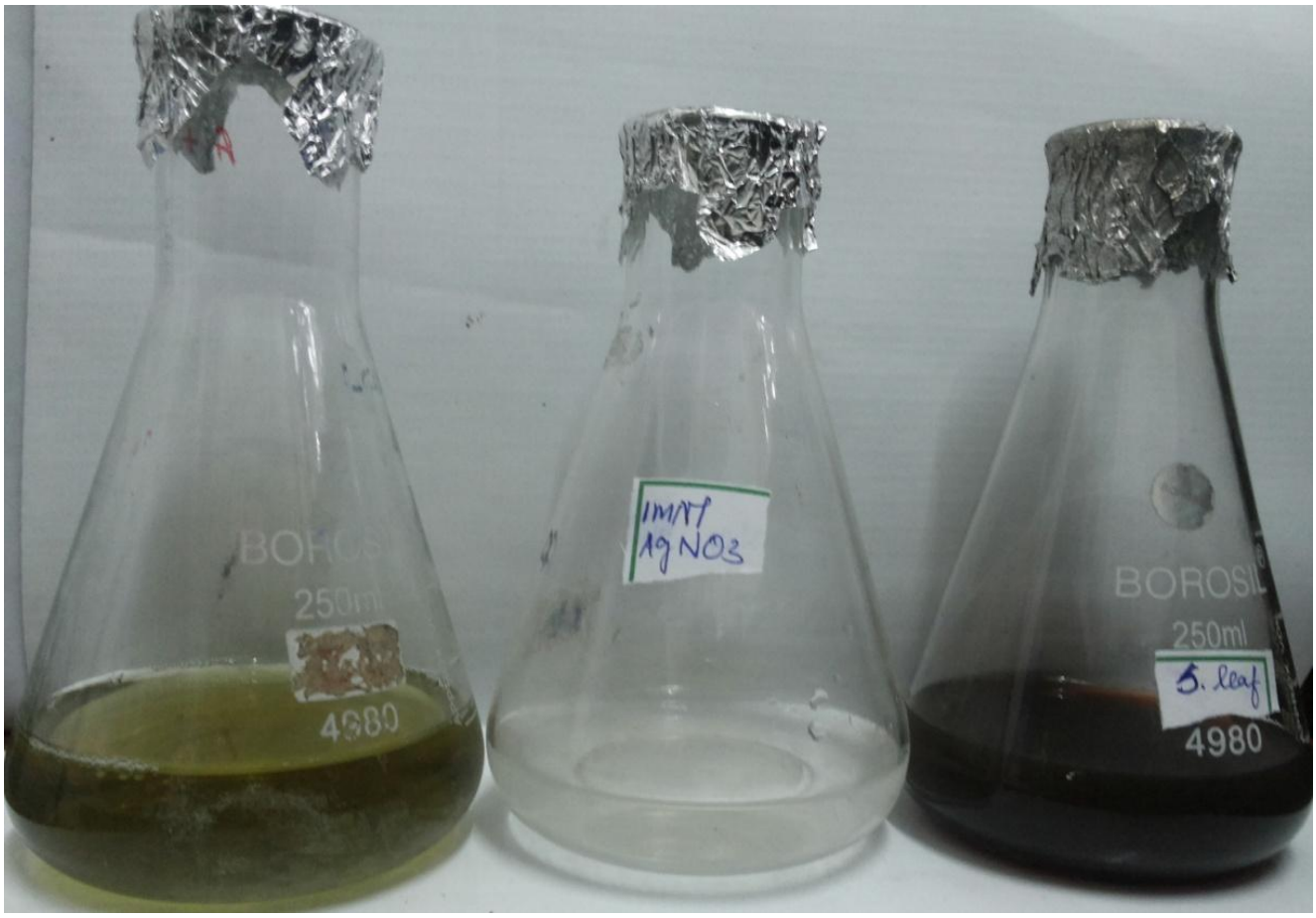


Figure 1: *Ipomoea pes tigridis* crude extract, 1mM $AgNO_3$, *Ipomoea pes-tigiridis*+$AgNO_3$.

UV-Vis Spectroscopy Analysis

The free electrons present in the AgNps gave rise to a surface Plasmon resonance absorption band. The formation of peak was due to the combined vibration of electrons of the metal nanoparticles in resonance with the light wave. The 440 nm surface Plasmon resonance peak was obtained through UV-Vis spectra (Figure 2).

Topographical and elemental analysis

The bioengineered nanoparticle solution was centrifuged at10,000 rpm for 5-10 mts and the pellet was redispersed in Milli Q water. The purified pellets were characterized by EDX, SEM, XRD and FTIR analysis. The EDX result shows the reduction of Ag+ to Ag0 in the sample through the very sharp peak of Ag at ranges from 2.8 to 3.8 keV (Figure 3).

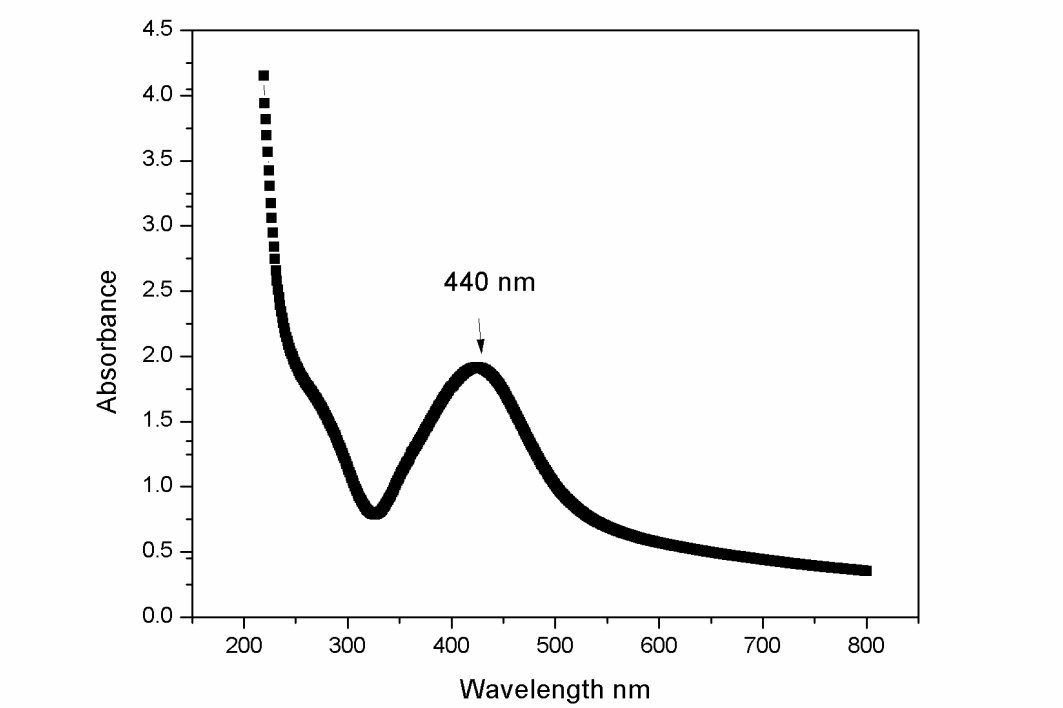


Figure 2. UV-vis spectra of *Ipomoea pes-tigridis* synthesized silver nanoparticles.

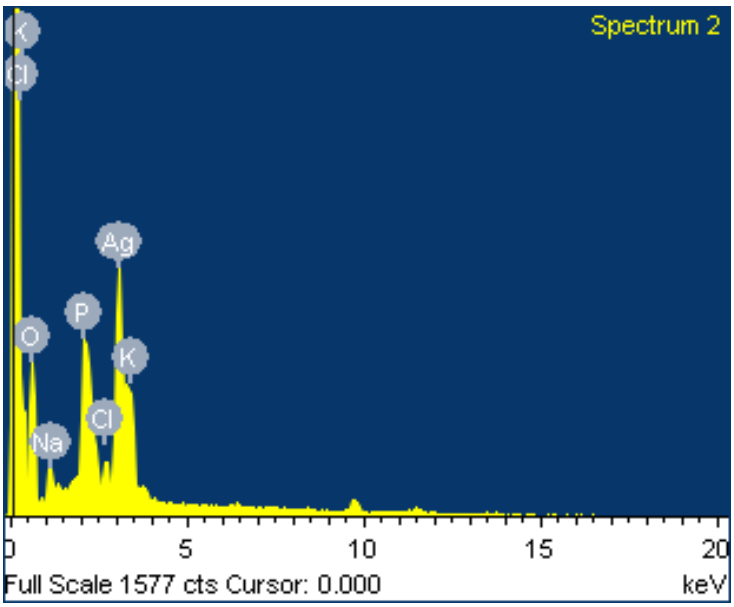


Figure 3. EDX- spectra of *Ipomoea pes-tigridis* synthesized silver nanoparticles was showed reducing Ag+ to Ag0.

The topography of bioengineered nanoparticles was confirmed through SEM image and the particles were irregular in shape and the diameter of particles was below 100 nm i.e., in the range of 40-82.73 nm (Figure 4).

The crystalline nature of the silver nanoparticles was confirmed through XRD analyses. The diffraction peaks were recorded from 10º to 90º at 2θ angles.

XRD analysis (Figure 5) showed strong intense peaks at 2θ values of 31.8836 assigned to the 122 planes of a faced centre cubic structure of silver nanoparticles. The target was Cu Kα with a wavelength of 1.54060 Aº. The ontrol thin films of the leaf extract as well as $AgNO_3$ did not show the characteristic peaks that indicated high purity of the silver nanoparticles. The observed peak broadening and noise were probably related to the effect of nanosized particles and the presence of various crystalline biological macromolecules in the plant extract. The obtained results showed that Ag^+ had definitely been reduced by *I. pes-tigridis* plant extract under reaction conditions (Table 1).

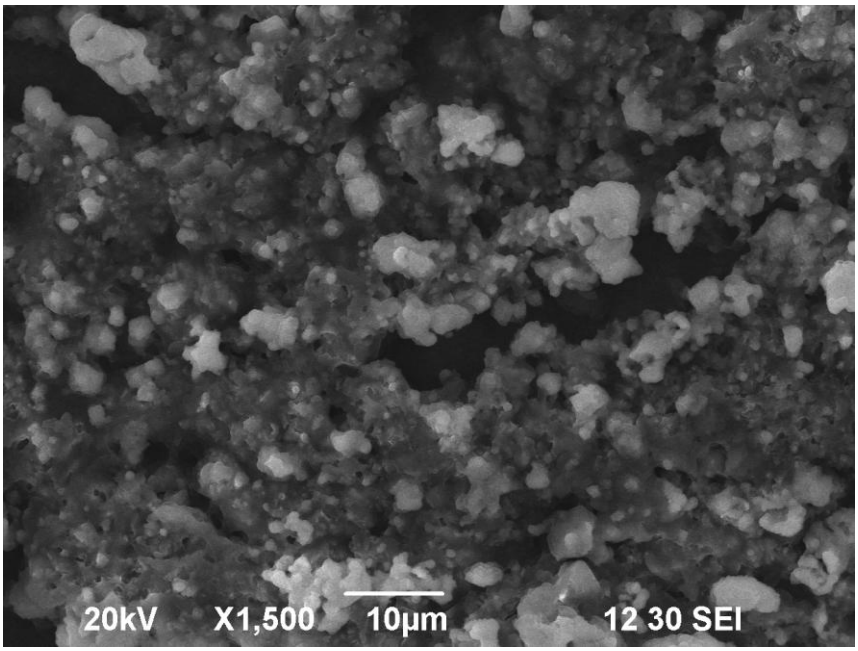


Figure 4. SEM- image of *Ipomoea pes-tigridis* synthesized silver nanoparticles was illustrated the particles in below 100 nm.

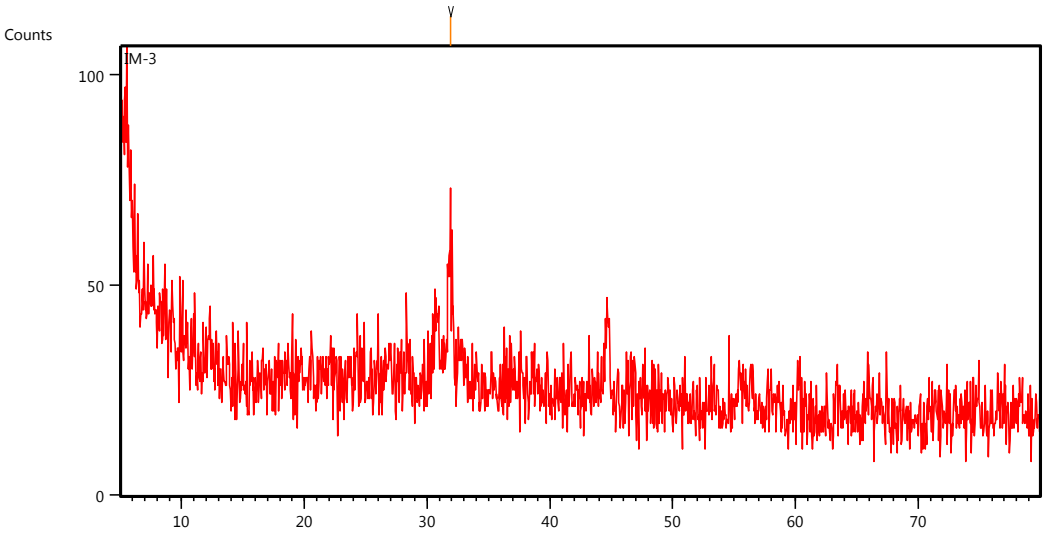


Figure 5. The crystalline structure of *Ipomoea pes-tigridis* synthesized silver nanoparticles characterized through XRD.

Table 1. XRD characteristics analysis spectra values of silver nanoparticles synthesized by *Ipomoea pes-tigridis* leaf extracts.

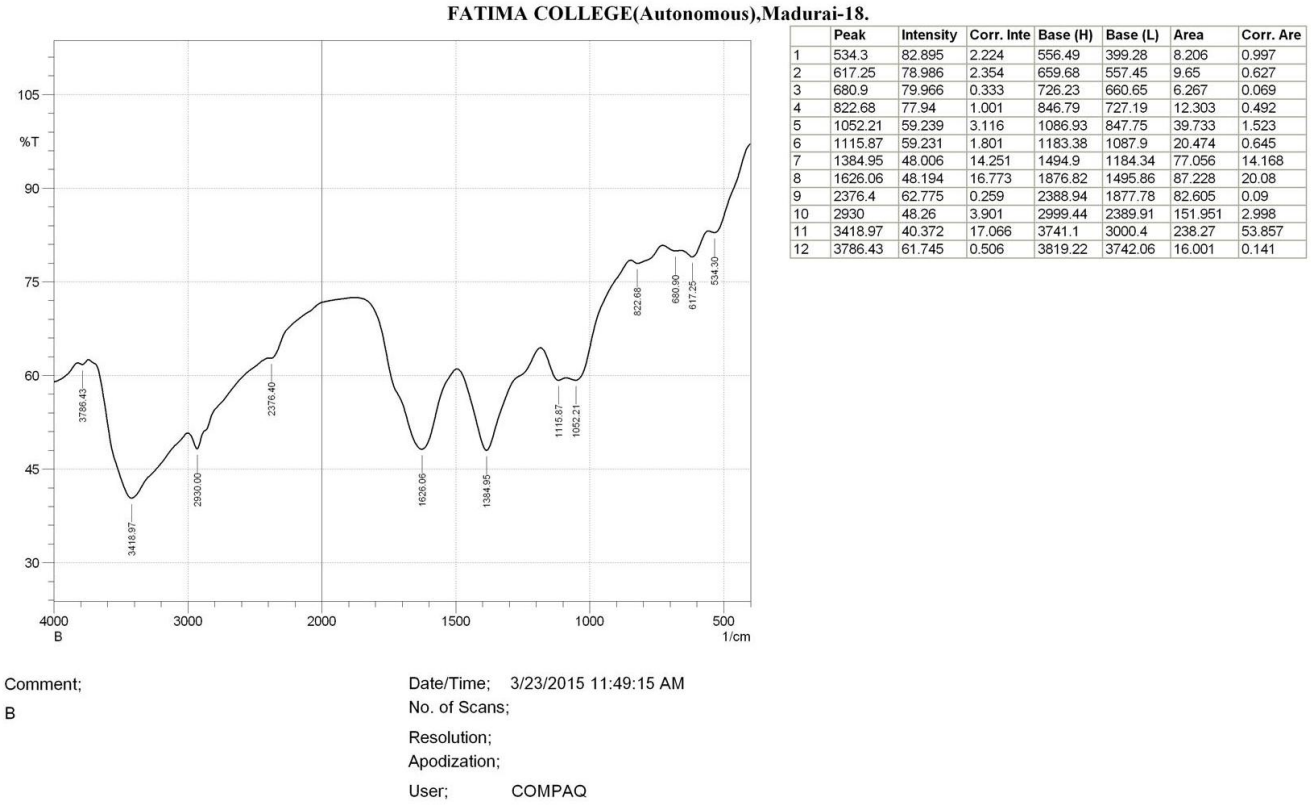
Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
31.8836	29.66	0.4800	2.80455	100.00

Values are 2TH =2θ , FWHM-Full with half maxima.

FT-IR spectroscopic studies were carried out to investigate the reducing and capping agent present in the *I. pes-tigridis* responsible for the synthesis of silver nanoparticles. The peaks were located at about 3786.43, 3418.97, 2930.00, 2376.40, 1626.06, 1384.95, 1115.87 and 1052.21cm^{-1} in the region 4000-400 cm^{-1} (Figure 3). The FTIR spectral analysis revealed the presence of =C-H stretch and bend strong sharp peak, C-H bend sharp peak, C-H stretch weak very strong nitro and carboxyl beak and hydroxyl beak mechanism behind the formation of these silver nanoparticles and offer information regarding the functional groups (Table 2 and Figure 6).

Table 2. FTIR Characteristics peaks values and functional groups of *Ipomoea pes-tigridis* leaf extracts synthesized silver nanoparticles.

Vibration Assignment/Functional Groups	Observed wave Number (cm^{-1})	Visible Intensity
OH stretch	2917.69	Broad
C-H stretch	1732.53	Strong Sharp
C-H stretch	1643.99	Sharp
NO_2	1356.64	Strong Sharp
C=O stretch	1209.43	Sharp
C-O stretch	1025.83	Very Strong and Sharp
OH stretch	904.17	Sharp



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	534.3	82.895	2.224	556.49	399.28	8.206	0.997
2	617.25	78.986	2.354	659.68	557.45	9.65	0.627
3	680.9	79.966	0.333	726.23	660.65	6.267	0.069
4	822.68	77.94	1.001	846.79	727.19	12.303	0.492
5	1052.21	59.239	3.116	1086.93	847.75	39.733	1.523
6	1115.87	59.231	1.801	1183.38	1087.9	20.474	0.645
7	1384.95	48.006	14.251	1494.9	1184.34	77.056	14.168
8	1626.06	48.194	16.773	1876.82	1495.86	87.228	20.08
9	2376.4	62.775	0.259	2388.94	1877.78	82.605	0.09
10	2930	48.26	3.901	2999.44	2389.91	151.951	2.998
11	3418.97	40.372	17.066	3741.1	3000.4	238.27	53.857
12	3786.43	61.745	0.506	3819.22	3742.06	16.001	0.141

Figure 6. FTIR spectra of *Ipomoea pes-tigridis* synthesized silver nanoparticles.

Antimicrobial activity of I. pes-tigridis synthesized silver nanoparticles

The antimicrobial efficacy of the *I. pes-tigridis* synthesized AgNPs was examined against four multi drug resistant bacterium like *S. aureus, Streptococci sp., E. coli* and *Pseudomonas sp.* Primary screening disclosed that the green synthesized AgNPs showed important activity, as compared to the plant extract. The synthesized AgNPs displayed significant activity against *Pseudomonas* sp. (18 ±0.0 mm), *S. aureus* (14.3±0.6 mm), and *Streptococci* sp. (14±0.0 mm) and moderate activity against *E. coli* (12.8±0.00 mm) (Table 3). The activity was dose dependent and increased proportionately with the concentration.

Table 3. Antibacterial activity of *Ipomoea pes tigiridis* AgNPs against selected human pathogenic bacteria.

Organisms	Zone of inhibition in mm (Mean±SD)			
	Control	50 µl	150 µl	200 µl
Staphylococcus aureus	0.00±0.00	9.0±0.5	10.0±1.0	14.3±0.6
Streptococcus sp.	0.00±0.00	10.0±0.0	10.5±0.5	14±0.00
E. coli	0.00±0.00	9.8±0.0	10.6±0.4	12.8±0.00
Pseudomonas sp.	0.00±0.00	10.2±0.4	11.8±0.2	18±0.00

Values are ±SD Standard deviation.

DISCUSSION

Ipomoea pes-tigridis is a flowering plant of the Convolvulaceae family. This family comprises of plants with high industrial, pharmaceutical, scientific, and cultural significance. It is distributed across continents and has its presence in Senagal Nigeria, tropical Africa and into Asia, Mascarene Island, Malaysia and Australasia. Typically, it is used in folk medication for the treatment of hemorrhoids, diabetes, bronchitis and arthritis (Shubhangi and Patil, 2004).

Arokiyaraj *et al.* (2014) reported that the phytochemical components present in the aqueous extract of *Chrysanthemum indicum* was perhaps responsible for the formation of AgNPs and acted as reducing and capping agent that prevented agglomeration and provided stability to the medium (Jha *et al.*, 2009). Flavonoids, tannins, proteins, and reducing sugars that are present in plants have been reported to act as bioreductants and capping agents (Li *et al.*, 2007). Similar results were observed in the present investigation also that *I. pes-tigridis* plant extract acted as both reducing and capping agent for synthesis silver nanoparticles and phytochemical components are responsible for reduction of Ag+ to A^0.

In the present study, initially the reduction reaction was confirmed through color change of the aqueous solution from colorless to yellowish brown to dark brown at 24 hrs**.** Bandi and Vasundhara (2012) reported this characteristic difference in color is due to the excitation of the surface plasmon resonance in the metal nanoparticles.

Vilchis Nestor *et al.* (2008) and Chandran *et al.*, (2006) reported reduction of silver ion and formation of stable nanoparticles with 4 hrs of reaction in *Camellia sinensis* extract and 24 hrs in *Aloe vera*. The UV–Vis spectrum is one of the imperative techniques used to find low-level concentrations of metal nanoparticles in solution. In this study, the absorption spectra were noted at 440 nm, and a broadening of the peak indicated that the particles were polydispersed. A similar trend was observed by Loo *et al.* (2012) and Arokiyaraj *et al.* (2014).

Mandal *et al.* (2006) reported that, enzymes, like reductase present in the proteins of various microbial systems responsible for the reduction of metal ions into atom. The present study, has confirmed through EDX analysis the reduction of Ag+ into Ag0. Reducing agents present in the *I. pes-tigridis is* responsible for the synthesis of silver nanoparticles. Similarly, Gole *et al.* (2001) reported that proteins can bind to nanoparticles either through free amine groups or cysteine residues or through the electrostatic attraction of negatively charged carboxylate groups in enzymes present in the cell wall of mycelia, and therefore stabilization of the silver nanoparticles by protein occurs. Topography and morphology of the nanoparticles was confirmed through SEM analysis. The obtained particles were below 100 nm, the present result was correlated with Prakash *et al.* (2013).

Prakash *et al.* (2013) characterized the *Mimusops elengi* synthesized silver nanoparticles through FTIR spectral analysis and they obtained the characteristic peak at 3415, 2928, 118, 1445 and 1041cm^{-1}. Similar results were observed in the present study silver nanoparticles synthesized by *I. pes tigridis* and the FTIR spectral analysis revealed the presence of =C-H stretch and bend strong sharp peak, C-H bend sharp beak, C-H stretch weak very strong nitro and carboxyl beak and hydroxyl beak. The band at 3415cm^{-1} was responsible for OH stretching (Prathna *et al.*, 2011 and Prakash *et al.* (2013). The crystalline nature of silver nanoparticles was confirmed through XRD analysis and the diffraction of 100% intensity peak was observed in the range of 31.8836.

Antibacterial activity of *I. pes-tigridis* synthesized silver nanoparticles showed maximum zone of inhibition in *Pseudomonas sp.* (18 ±0.0 mm) followed by *S. aureus* (14.3±0.6 mm), *Streptococcus* sp. (14±0.0 mm) and they

exhibited moderate activity against *E. coli* (12.8±0.00 mm) respectively. These results corroborate with Prakash *et al.* (2013) and Arokiyaraj *et al.* (2014).

CONCLUSION

The present study demonstrated that the bioengineered nanoparticles using plant resources like *I. pes-tigridis* is a better alternative to physical and chemical synthesis, since this green synthesis is pollutant free and eco-friendly. The results suggested that *I. pes-tigridis* plays an important role in the attenuation and stabilization of silver nitrate to silver nanoparticles. The particles illustrate high antibacterial activity against the multi drug resistant clinical pathogenic bacteria. Further study needs to carry out that the bioengineered nanoparticles for the drug delivery instead of modern medicine.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the management, Thiagarajar College and Lovely Professional University for the all laboratories facilities.

REFERENCES

Ahmad, N., Sharma, S., Alam, M.K., 2010. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. *Colloids Surf B: Biointer.*, 81(1), 81-86.

Andres, R.P., Datta, S., Janes, D.B., Kubiak, C.P., Reifemberger, R. and Naiwa, H.S., 2000. Handbook of Nanostructured Material and Nanotechnology, Vol. 4. HS Nalwa ed., p.179-231.

Arokiyaraj, S., Arasu, M.V., Vincent, S., Udaya Prakash, N., Choi, S.H., Kyoon, Y., Choi, K.C., Kim, K.H., 2014. Rapid green synthesis of silver nanoparticles from *Chrysanthemum indicum* L. and its antibacterial and cytotoxic effects: an in vitro study. *Int. J. Nanomed.*, 9, 379-388.

Baker, S. and Satish, S., 2012. Endophytes: toward a vision in synthesis of nanoparticle for future therapeutic agents. *Int. J. Bio-Inorg. Hybd. Nanomat*., 1(2), 67-77.

Bandi, S. and Vasundhara, K., 2012. Green synthesis of silver nanoparticles using Adhatoda vasica methanolic extract and its biological activities. *J. Atoms. Mol.*, 2(4), 282.

Bindhu, M.R. and Umadevi, M., 2013. Synthesis of monodispersed silver nanoparticles using Hibiscus cannabinus leaf extract and its antimicrobial activity. Spectrochimica *Acta—Part A: Mol. Biomole. Spectros.* 101, 184-190.

Calixto, J.B., 2005. Twenty-five years of research on medicinal plants in Latin America: a personal view. *J. Ethnopharm.*, 100, 131-134.

Cathrine, L. and Prabavathi nagarajan, N., 2011. Preliminary phytochemical analysis and antibacterial activity of leaf extracts of *Vitex leucoxylon* L.F. *Int. J. Cur. Pharm. Res.*, 3(2), 71-73.

Chandran, S. P., Chaudhary, M., Pasricha, R., Ahmad, A., and Sastry, M., 2006. Synthesis of gold nanotriangles and silver nanoparticles using Aloevera plant extract. *Biotechnol. Prog.*, 22, 577-583.

Crooks, R.M., Lemon III, B.I., Sun, L., Yeung, L.K. and Zhao, M., 2001. Dendrimer-encapsulated metals and semiconductors: synthesis, characterization, and applications. In: Dendrimers III. Springer Berlin Heidelberg. p. 81-135.

Cullity, B.D., 1978. Elements of X-ray diffraction, 2nd Edition, Addison-Wesley Publishing Company Inc., Phillippines, p.102

Doss, A., Mubarack, H.M. and Dhanabalan, R., 2009. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. *Indian J. Sci. Technol.*, 2(2), 41-43.

Elechiguerra, J.L., Burt, J.L., Morones, J.R., Camacho-Bragado, A., Gao, X., Lara, H.H. and Yacaman, M.J., 2005. Interaction of silver nanoparticles with HIV-1. *J. nanobiotechnol.*, 3(1), 6.

Esumi, K., Tano, T., Torigoe, K. and Meguro, K., 1990. Preparation and characterization of bimetallic palladium-copper colloids by thermal decomposition of their acetate compounds in organic solvents. *Chem. Mater.*, 2(5), 564-567.

Gardea-Torresdey, J.L., Gomez, E., Peralta-Videa, J.R., Parsons, J.G., Troiani, H. and Jose-Yacaman, M., 2003. Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. *Langmuir*, 19(4), 1357-1361.

Gittins, D.I., Bethell, D., Schiffrin, D.J. and Nichols, R.J., 2000. A nanometre-scale electronic switch consisting of a metal cluster and redox-addressable groups. *Nature*, 408(6808), 67.

Goia, D.V. and Matijevic, E., 1998. Preparation of monodispersed metal particles. *New J. Chem.* 22(11), 1203-1215.

Gole, A., Dash, C., Ramakrishnan, V., Sainkar, S.R., Mandale, A.B., Rao, M. and Sastry, M., 2001. Pepsin− gold colloid conjugates: preparation, characterization, and enzymatic activity. *Langmuir*, 17(5), 1674-1679.

Henglein, A., 2001. Reduction of Ag (CN) 2-on silver and platinum colloidal nanoparticles. *Langmuir*, 17(8), 2329-2333.

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H., Wang, Y., Shao, W., He, N. and Hong, J., 2007. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. *Nanotechnology*,18(10),105104.

Ip, M., Lui, S.L., Poon, V.K., Lung, I. and Burd, A., 2006. Antimicrobial activities of silver dressings: an in vitro comparison. *J. Med. Microbial.*, 55(1), 59-63.

Jain, D., Daima, H.K., Kachhwaha, S. and Kothari, S.L., 2009. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. *Dig. J. Nanomater. Biostruct.*, 4(3), 557-563.

Jha, A.K., Prasad, K., Prasad, K., Kulkarni, A.R., 2009. Plant system: nature's nanofactory. *Colloids Surf. B. Biointerfaces*, 73(2), 219-223.

Kasthuri, J., Kathiravan, K., Rajendiran, N., 2009. Phyllanthin-assisted biosynthesis of silver and gold nanoparticles: a novel biological approach. *J. Nanopart. Res.*, 11(5), 1075-1085.

Kesharwani, J., Yoon, K.Y., Hwang, J., Rai, M., 2009. Phytofabrication of silver nanoparticles by leaf extract of Datura metel: hypothetical mechanism involved in synthesis. *J. Bionanosci.*, 3(1), 39-44.

Kharissova, O.V., Dias, H.R., Kharisov, B.I., Pérez, B.O., Pérez, V.M.J., 2013. The greener synthesis of nanoparticles. *Trends Biotechnol.*, 31(4), 240-248.

Li, S., Shen, Y., Xie, A., Yu, X., Qiu, L., Zhang, L., Zhang, Q., 2007. Green synthesis of silver nanoparticles using Capsicum annuum L. *Extract. Green Chem*., 9(8), 852-858.

Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun, H., Tam, P.K.H., Chiu, J.F., Che, C.M., 2007. Silver nanoparticles: partial oxidation and antibacterial activities. *J. Biol. Inorg. Chem.*, 12(4), 527-534.

Loo, Y.Y., Chieng, B.W., Nishibuchi, M., Radu, S., 2012. Synthesis of silver nanoparticles by using tea leaf extract from Camellia sinensis. *Int. J. Nanomed*. 7, 4263.

Mahesh, B. and Satish, S., 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J. Agr. Sci.*, 4(5), 839-843.

Mallikarjuna, K., Narasimha, G., Dillip, G.R., Praveen, B., Shreedhar, B., Lakshmi, C.S., Reddy, B.V.S. and Raju, B.D.P., 2011. Green synthesis of silver nanoparticles using *Ocimum* leaf extract and their characterization. Digest. *J. Nanomater. Biostruc.*, 6(1), 181-186.

Mandal, D., Bolander, M.E., Mukhopadhyay, D., Sarkar, G. and Mukherjee, P., 2006. The use of microorganisms for the formation of metal nanoparticles and their application. *Appl. Micro. Biotechnol.*, 69(5), 485-492.

Mittal, A.K., Chisti, Y. and Banerjee, U.C., 2013. Synthesis of metallic nanoparticles using plant extracts. *Biotechnol. Adv.*, 31(2), 346-356.

Murphy, C.J., 2008. Sustainability as an emerging design criterion in nanoparticle synthesis and applications. *J. Mater. Chem.*, 18(19), 2173-2176.

Najitha Banu, A., Balasubramanian, C., 2014a. Myco-synthesis of silver nanoparticles using Beauveria bassiana against dengue vector, Aedes aegypti (Diptera: Culicidae). *Parasitol. Res.*, 113(8), 2869-2877.

Najitha Banu, A. and Balasubramanian, C., 2015. Extracellular synthesis of silver nanoparticles using Bacillus megaterium against malarial and dengue vector (Diptera: Culicidae). *Parasitol. Res.*,114, 4069–4079.

Najitha Banu, A., Balasubramanian., C., Vinayaga Moorthi, P., 2014. Biosynthesis of silver nanoparticles using Bacillus thuringiensis against dengue vector, Aedes aegypti (Diptera: Culicidae). *Parasitol. Res.*, 113, 311-316.

Najitha Banu., A., Balasubramanian, C., 2014b. Optimization and synthesis of silver nanoparticles using Isaria fumosorosea against human vector mosquitoes. *Parasitol. Res.*, 113, 3843-3851.

Parashar, V., Parashar, R., Sharma, B., Pandey, A.C., 2009 Parthenium leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. *Digest J. Nanomater. Biostruct*., 4, 45-50.

Parivuguna, V., Gnanaprabhal, R., Dhanabalan, R., Doss, A., 2008. Antimicrobial Properties and Phytochemical Constituents of Rheo discolor Hance. *Ethno. Botanical. Leaflets*,. 12, 841-845

Pastoriza-Santos, I., Liz-Marzán, L.M., 2002. Formation of PVP-protected metal nanoparticles in DMF. *Langmuir*, 18(7), 2888-2894.

Prakash, P., Gnanaprakasam, P., Emmanuel, R., Arokiyaraj, S. and Saravanan, M., 2013. Green synthesis of silver nanoparticles from leaf extract of Mimusops elengi, Linn. for enhanced antibacterial activity against multi drug resistant clinical isolates. *Colloids and Surf. B: Biointer.*, 108, 255-259.

Prathna, T.C., Chandrasekaran, N., Raichur, A.M. and Mukherjee, A., 2011. Biomimetic synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical prediction of particle size. *Colloids Surf. B: Biointer*., 82(1), 152-159.

Rai, M., Yadav, A. and Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.*, 27(1),76-83.

Rodriguez-Sanchez, L., Blanco, M.C. and Lopez-Quintela, M.A., 2000. Electrochemical synthesis of silver nanoparticles. *J. Phy. Chem. B*, 104(41), 9683-9688.

Salam, H.A., Rajiv, P., Kamaraj, M., Jagadeeswaran, P., Gunalan, S. and Sivaraj, R., 2012. Plants: green route

for nanoparticle synthesis. *Int. Res. J. Biol. Sci.*, 1(5), 85-90.

Sathishkumar, M., Sneha, K., Won, S.W., Cho, C.W., Kim, S. and Yun, Y.S., 2009. Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. *Colloids Surf. B: Biointer.*, 73(2), 332-338.

Shankar, S.S., Ahmad, A. and Sastry, M., 2003. Geranium leaf assisted biosynthesis of silver nanoparticles. *Biotechnol. Prog.*,19(6), 1627-1631.

Sharma, V.K., Yngard, R.A. and Lin, Y., 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. *Adv. Colloid Inter. Sci.*, 145(1), 83-96.

Singh, G., Kumar, P., 2011. Extraction and antimicrobial activity of alkaloids of *Euphorbia Hirta*. *J. Pharm. Res.*, 4(4), 1228-1230.

Taleb, A., Petit, C. and Pileni, M.P., 1997. Synthesis of highly monodisperse silver nanoparticles from AOT reverse micelles: a way to 2D and 3D self-organization. *Chem. Mater.*, 9(4), 950-959.

Venkataswamy, R., Doss, A., Mubarack, H.M. and Sukumar, M., 2010. Phytochemical, HPTLC finger printing and antibacterial activity of *Acacia nilotica* (L.) Delile. *Hygeia J.D. Med.*, 2(2), 38-42.

Vilchis-Nestor, A.R., Sanchez-Mendieta, V., Camacho-Lopez, M.A., Gomez-Espinosa, R.M., Camacho-López, M.A. and Arenas-Alatorre, J.A., 2008. Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract. *Mater. Lett.*, 62(17), 3103-3105.

Zhu, J., Liu, S., Palchik, O., Koltypin, Y. and Gedanken, A., 2000. Shape-controlled synthesis of silver nanoparticles by pulse sonoelectrochemical methods. *Langmuir*, 16(16), 6396-6399.