Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v41i1.45262

Rapid green synthesis of silver nano particles from *Ziziphus mauritiana* and antibacterial activity against human pathogens

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ABSTRACT. A novel green source *Ziziphus mauritiana* fresh young leaves was opted to synthesize silver nanoparticles and analyze its antibacterial activity. The bioactive compounds present in the plant extracts reduced silver ions to NPs, indicated by change in color from red to dark brown. In this study, we have successfully synthesized nanoparticles using *Z. mauritiana* aqueous leaf extract as a reducing agent and the reaction process of synthesized nanoparticles was monitored by UV-Vis spectroscopy. The UV-Vis absorption peak showed maximum adsorption at 420 nm confirmed the silver nanoparticles synthesis. Further characterization was carried out by FTIR and the results recorded a downward shift of absorption the bands between 400 to 4000 cm^{-1} indicates the formation of silver nanoparticles. Finally, the present research was exploited to study the antibacterial activity of synthesized nanoparticles produced *Z. mauritiana* was studied using different pathogenic bacteria such as *Salmonella* sp., *Proteus sp., Bacillus* sp., *Klebsiella pneumonia* and *E.coli* from the well diffusion results, the synthesized silver nanoparticles displayed the best antibacterial property as compared to the antibiotic has been reported in this paper. To the best of our knowledge, this is the first report that the *Z. mauritiana* aqueous extract facilitate the synthesis of silver nanoparticles and also exhibits antibacterial activity.

Keywords: aqueous extract; silver nanoparticles; anti-bacterial activity; human pathogens.

Received on November 7, 2018.
Accepted on June 26, 2019.

Introduction

Ziziphus mauritiana Lam. syn. *Z. jujuba* Lam., non-Mill, a tropical fruit tree belongs to the family Rhamnaceae and the name *Ziziphus* is associated with Arabic word. Indian *Z. mauritiana* is a spiny, shrub reaches a height of up to 3-4 m tall (Orwa, Mutua, Kindt, Jamnadass, & Anthony, 2009; Ecocrop, 2013). Originally Indian jujube originated from central Asia and spread through Afghanistan, South China, Malaysia and Australia to North Africa and India. The whole plant used as food, fodder, nutrient, medicine, construction material and fuel. In Ayurveda, bark used to treat boils, dysentery and diarrhoea (Nadkarni, 1986). Due to the presence of bitterness and cooling property the root cures coughs, biliousness and headache (Kirtikar & Basu, 1994). The leaves act as antipyretic agent. The fruit cures biliousness, burning sensations, thirst, vomiting (Chopra, Nayar, & Chopra, 1986), tuberculosis and blood diseases while the seeds are used to cure eye diseases and leucorrhoea.

At present an extensive work has been made in the biological synthesis of nanoparticles by using natural products as some researchers tested it for antimicrobial activities. Among the possible biological resources (Bacteria, fungi, yeasts, algae and plant) plant extracts with rich biological active compounds represent excellent scaffolds for NPS green synthesis by serving as both reducing and stabilizing agents (Salam, Rajiv, & Kamaraj, 2012). Very recently many different plants and herbal extracts were utilized for the synthesis of nanoparticles, these includes *Tagetes erecta* flower (Padalia & Chanda, 2014), *Ziziphora tenuior* leaves extract (Sadeghi & Gholamhoseinpoor, 2015), *Tribulus terrestris* leaf extract (Ashokkumar, Ravi, Kathiravan, & Velmurugan, 2014), *Solanum tricobatum* leaf, *Syzygium cumini* leaf, *Centella asiatica* leaf and *Citrus sinensis* peel (Logeswari, Silambarasan, & Abrham, 2013), aqueous beet root (Bindhu & Umadevi, 2015), *Garcinia mangostana* leaf extract (Veerasamy et al., 2011), macro algae *Spirogyra varians* (Zeinab, Firoozeh, Shima, & Ataei, 2016), olive leaf extract (Khalil, Ismail, El-Baghdady, & Mohamed, 2014) and *Acalypha indica* leaf extract (Krishnaraj et al., 2010). As reported (Karnani & Chowdhary, 2013) the synthesis of silver

nanoparticles acts as an alternative to the conventional methods. The mechanism behind the biosynthesis of nanoparticles in plants may be accompanied with the concept of phytoremediation in plants (Salam et al., 2012).

The production of nanoparticles by using plant extracts is at present under development as few researchers worked on it and examination of potential towards antimicrobial property. For the last two eras a widespread work has been done to develop new drugs from natural products because of the resistance of micro-organisms to the existing drugs. Nature has been an important source of a products currently being used in medical practice.

Material and methods

Materials

Silver nitrate ($AgNO_3$), nutrient agar and nutrient broth were obtained from Merck. Double distilled water and ampicillin used throughout the experimental procedure was procured from a local chemist shop.

Selection of plant

The fresh *Ziziphus mauritiana* leaves were collected from Vellore District between December and January. The taxonomic identity of the plant was confirmed with known identity and a voucher specimen (11003) was deposited in the herbarium of D. K. M. College for Women.

Preparation of leaf extract

Thoroughly the leaves were washed several times with normal tap water followed by distilled water in order to remove the impurities. The 10 g of fresh leaves were cut into small pieces and boiled in 100 mL of double distilled water. After 10 min., the prepared solution was cooled to room temperature and initially filtered through a normal filter paper mesh so that the leafy materials could be filtered out. The filtrate was again filtered through Whatman filter paper n. 1. The filtered extract was stored in the refrigerator at 4°C.

Synthesis of silver nanoparticles

Thirty mL of 2 mM aqueous solution of silver nitrate was taken. Then 70 mL of *Z. mauritiana* aqueous leaf extract was added to it at room temperature. The mixture was warmed at 80°C for 10 min. The silver nanoparticle formation is indicated by a change in colour. After period of 24 hour, the solution was centrifuged in an ultracentrifuge at 10,000 rpm for 30 min. to obtain a dark brown precipitate. The precipitate was washed thrice with MiliQ water and air dried to yield silver nanoparticles.

Characterization of silver nanoparticles

The formation of silver nanoparticles was confirmed by UV-Visible spectrum recorded between the ranges from 300 to 600 nm using UV-VIS spectrophotometer instrument. To determine the bio-functional moieties present in the leaf extract and SNP, FTIR analysis were carried out with the spatial resolution of 4 cm^{-1} in the transmission mode, between 400-4000 cm^{-1} respectively.

Antimicrobial susceptibility testing

The antibacterial activities of the synthesized silver nano- particles were assessed against both Gram positive (*Bacillus sp*. and *Proteus sp*.) and Gram negative (*Escherichia coli*, *Pseudomonas sp*. and *Klebsiella sp*.) bacteria through standard well diffusion method (Khyade & Vaikos, 2009). For comparison, antibacterial activities of *Z. mauritiana* extract and ampicillin solution were also studied. The growth of the bacterial culture was carried out by growing the organism overnight in Muller-Hinton nutrient agar medium at 37°C and the inoculum of each bacterium was subcultured in Muller-Hinton agar at 37°C overnight. By using a sterile swab bacterial cultures were swabbed on a Mueller-Hinton agar plate. The surface of the agar plate was punched with a sterilized cork borer to make holes (well). Then the prepared wells were filled with different concentration of SNPs (20 and 10 µL), plant extract (10 µL) and standard drug (ampicillin 10 µL) separately. After a period of incubation, the inoculated plates were incubated at appropriate temperature for 24 hour. The anti- bacterial activity was expressed as the zone of inhibition (ZI) and was measured with a Vernier caliper.

Results and discussion

UV-Vis spectral studies

As the *Ziziphus mauritiana* aqueous leaf extract mixed with aqueous solution of the silver nitrate, the color starts to change from red to dark brown due to the silver ion reduction (Figure 1). By using UV-visible spectrophotometer a spectrum was obtained in the visible range of 300-600 nm to confirm the presence of nanoparticles. From this UV-Vis spectral analysis, absorbance peak at around 420 nm, indicated the presence of Ag nanoparticles. The results of different concentration of a leaf extract on the size of AgNPs were studied by Ashokkumar, Ravi, Kathiravan, and Velmurugan (2015). The results we obtained were very similar to what they found. As the concentration of leaf extract gets increased, a more number of biomolecules will be available to accommodate the metal reduction process.

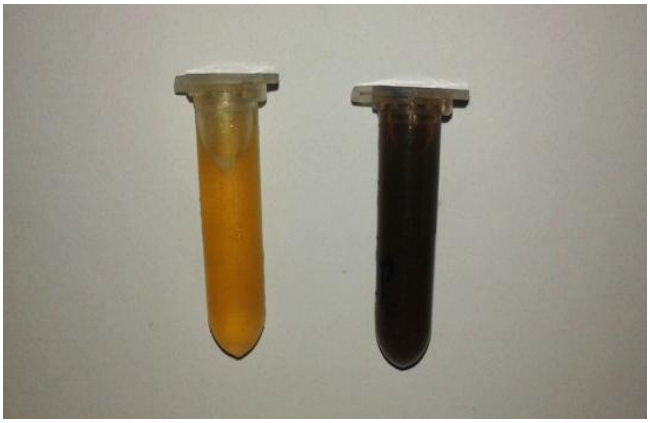

Figure 1. Colour observed in aqueous leaf extract and synthesised silver nanoparticle.

Fourier infrared spectroscopy

FTIR spectroscopy characterizes and identifies the chemical composition on the surface of the silver nanoparticles. FTIR analysis recognizes the functional groups of the biomolecules that are responsible for reduction of Ag+ ions to Ag nanoparticles and its sub-sequent stabilization present in *Z. mauritiana* leaf extract performed can be seen in Figure 2 and 3. The peak at 1141.86 cm^{-1} revealed that bands were due to C-N stretching, absorption frequency at 1516.05 and 1633.715 cm^{-1}, thereby confirming that the stretching vibration of C = C group, N-H band at 3352.28 and 3728.40 cm^{-1} corresponded to hydroxyl absorption. FTIR analysis showed the presence of 5 bands at about 3360.00, 2362.80, 1635.64, 1132.21 and 3360.50 cm^{-1} which is characteristic of the OH stretching of phenolic group. The absorption band at 2362.80 cm^{-1} could be due to the stretching of the O-H group. The band at 1635.64 cm^{-1} corresponds to C = O group present in silver nanoparticles. The band at 1132.21 and 1049.28 cm^{-1} indicated CO group of the ester. The spectrum obtained so reveals that the phytoconstituents particularly tannin protects the silver nanoparticles from the aggregation. A similar FTIR pattern of silver nanoparticles synthesized by *Hibiscus rosa sinensis* was demonstrated by Dairy Philip, which coincides with results of *Z. mauritiana* leaf extract mediated synthesis of silver nanoparticles.

Phytochemical analysis

The qualitative analysis of plant extract was performed to determine the presence of secondary metabolites. The carbohydrates, flavonoids, alkaloids, steroids, glycosides, saponins, terpenoids, aminoacids, protein, tannins and phenols are tested with the aqueous extract of *Z. mauritiana*.

In *Z. mauritiana* plant aqueous extract alkaloids, steroids, saponins, tannins and phenols compounds are present. Flavonoids, glycosides are absent. While AgNp's possess tannin, saponin, steroids and phenol. Flavonoids, alkaloids and glycosides are absent.

Phenolic compounds possess hydroxyl and carboxyl groups, which are able to bind to metals. The leaf aqueous extract contains high levels of phenolic compounds which may inactivate ions by chelating. Presence of the chelating ability of phenolic compounds is directly related to the high nucleophilic character of the aromatic rings (Table 1).

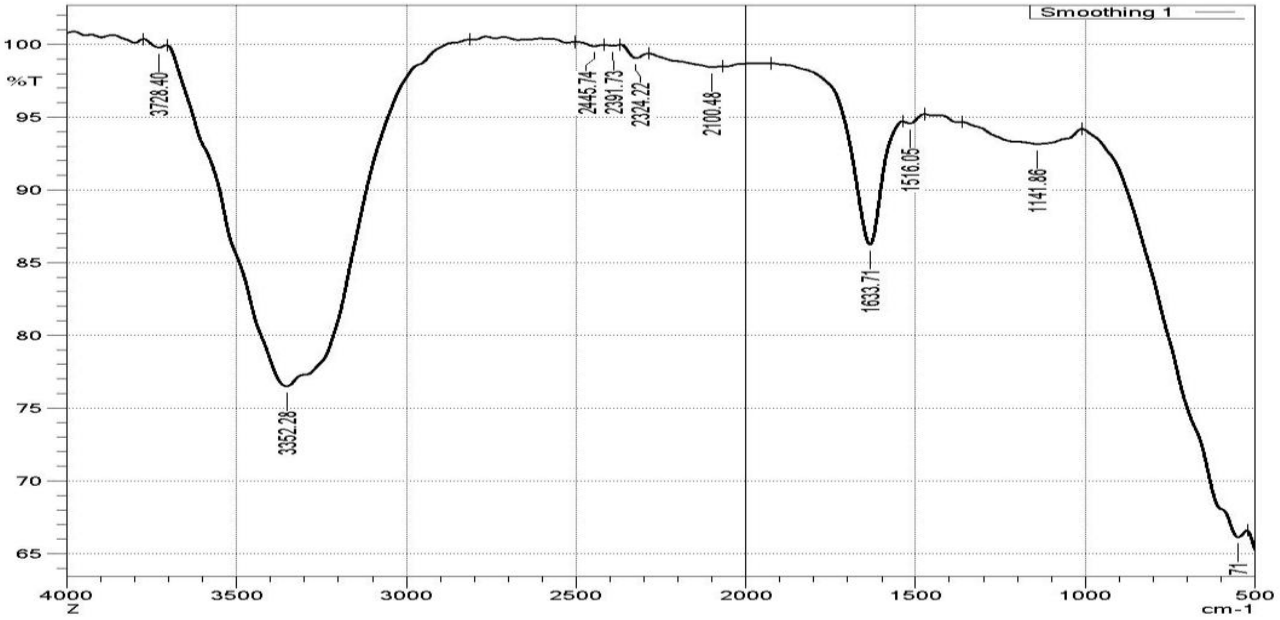

Figure 2. FTIR spectra of *Ziziphus mauritiana* aqueous leaf extract.

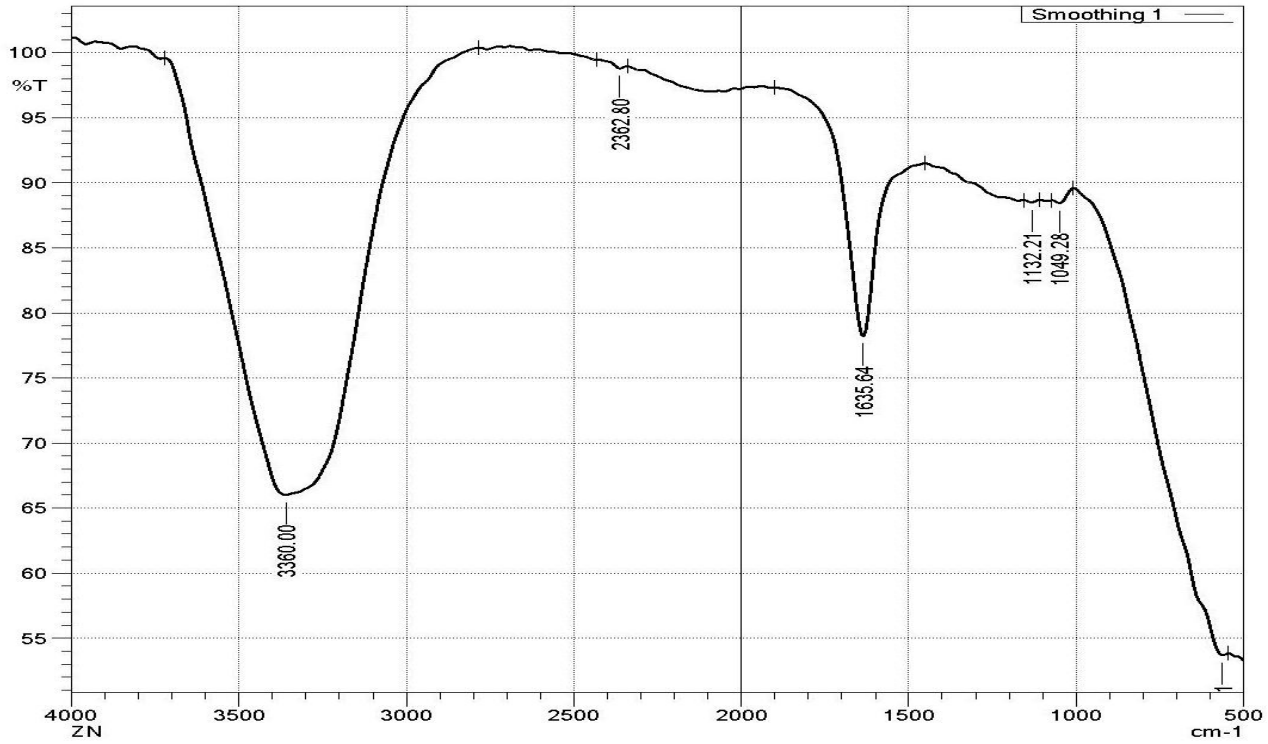

Figure 3. FTIR spectra of *Ziziphus mauritiana* synthesized silver nanoparticles.

Table 1. Phytochemical analysis of aqueous leaf extract and synthesized silver nanoparticles.

s. n.	Phytochemical test	Plant extract (*Ziziphus mauritiana*)	AgNP'S
1.	Test for Tannin (Lead acetate Test)	+ ve	+ ve
2.	Test for Saponins (Foam Froth)	+ ve	+ ve
3.	Test for flavonoids (Sodium hydroxide)	- ve	- ve
4.	Test for Steroids (Salkowski)	+ ve	+ ve
5.	Test for Alkaloids (Wagner & Hager)	+ ve	- ve
6.	Test for Phenol (Lead acetate)	+ ve	+ ve
7.	Test for Glycoside (Bontrager's)	- ve	- ve

- ve represents not found, + ve represents found.

Antibacterial activity

The antibacterial activity of synthesised Ag nanoparticles produced by 30 mL of *Z. mauritiana* aqueous leaf extracts was studied against both bacteria (gram positive and gram negative). The typical antibacterial activity of synthesised Ag nanoparticles against the selected bacterial strains varied between 10 to 14 mm (the zone of inhibition). Ag nanoparticles had enlightened a maximum activity for *Salmonella* and *Proteus sp*., with 14 mm zone of inhibition. While, the diameters of the inhibition zones against *Bacillus* and *Klebsiella pneumonia* were found to be 12 mm, respectively. The representative zones of inhibition are shown in Table 2, Figure 4. *Z. mauritiana* aqueous leaf extract of about 10 µL did not indicate any distinct microbial inhibition zone, whereas AgNPs synthesized by *Z. mauritiana* fresh leaf extract (20 µL) presented distinguishing microbial inhibition zones against all four pathogenic bacteria in the well diffusion method with a zone of inhibition of 13 (*Salmonella*, *Proteus*), 12 (*Bacillus*) and 10 mm (*Klebsiella pneumonia*). Many reports have conveyed that AgNPs can be an effective bactericidal agent (Sondi & Salopek-Sondi, 2004; Lara, Ayala- Núñez, Turrent, & Padilla, 2010; Rai, Deshmukh, Ingle, & Gade, 2012). An analogous result has been obtained with standard antibiotic ampicillin (10 µL) 35mm for *salmonella*, 24 mm for *K. pneumonia*, 19 mm for *E.coli*, 14 mm for *Bacillus* and *Proteus*. It was seen that no antimicrobial activity against *E. coli* as reported by AgNPs.

Table 2. Antibacterial activity of *Ziziphus mauritiana* silver nanoparticles against gram positive and gram negative bacteria.

s. n.	Agent	Conc (µL)	Zone of inhibition (mm)				
			Salmonella	*Bacillus*	*E. coli*	*Klebsiella Pneumonia*	*Proteus*
1.	AgNP'S	20	13	12	-	10	13
		30	14	12	-	12	14
2.	Antibiotic	10	35	14	19	24	14
3.	Plant extract	10	-	-	-	-	-

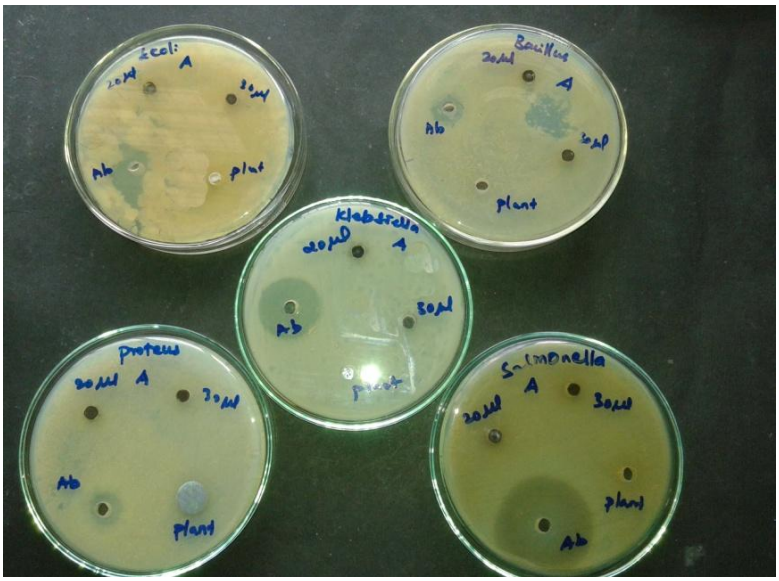

Figure 4. Antibacterial activity of *Ziziphus mauritiana* extract and its silver nanoparticles against gram positive and Gram negative bacteria.

Also, the antimicrobial activities of biologically synthesized AgNPs are suggested to be more sensitive against gram negative bacteria than the Gram positive bacteria. The higher sensitivity of Gram negative bacteria may be due to their difference in the structure of their cell wall. In Gram negative bacteria, the cell wall is made up of a thin layer of peptidoglycan, comprising of linear polysaccharide chains resulting in the formation of thinner structures, thus, causes an easy penetration of silver nanoparticles when compared to that of Gram positive bacteria, where the cell wall is composed of a thick layer of peptidoglycan. The high bactericidal activity is certainly due to the release of silver cations from synthesised silver nanoparticles that in turn act as reservoirs for the Ag+ ions, a bactericidal agent. Even though the precise mechanism exists behind the antibacterial activity of the SNP is still indefinite, some researchers assumed that the high bactericidal activity might be due to strong interaction between the silver cations released from silver nanoparticles and ionic charges that exist on the surface of cell membrane. Thus, a synergistic action exists

when SNP adhered on the surface of bacterial cell and thereby kills the bacterial cell by apoptosis/lysis of bacteria (Rawani, Ghosh, & Chandra, 2013). Possibly, the inhibition of bacterial growth may be due to the following possible mechanisms underlying SNP's antibacterial activity(a) disruption during cell wall synthesis; (b) interference during transcription process and protein bio-synthesis and (c) disruption of primary metabolic pathways involving deactivation of enzymes (Kumar et al., 2004; Tenover, 2006; Shrivastava et al., 2007; Bindhu & Umadevi, 2015).

From the result it was observed that SNP synthesized using 0.002M showed good antibacterial activity as compared with standard drug and plant extract. These observations were found to be similar to other researchers who calculated the MIC for SNPs produced by the plant extracts. Minimum inhibitory concentration exhibited by silver nanoparticle synthesised using rice paper plant stem (Zeng et al., 2007) and bamboo leaves (Sohail, Liu, & Yao, 2013) were found to be 14.1 and 20 mg L^{-1}, respectively. Similarly, *Terminalia bellirica* fruit extract (Anand & Mandal, 2015) and *Withania somnifera* leaf extract (Raut, Mendhulkar, & Kashid, 2014) synthesized SNPs displayed MIC of 20 mg mL^{-1} against both *E.coli* and *S. aureus* and 1.8 ± 0.20 and 1.2 ± 0.14 mg L^{-1} respectively, while *Paederia foetida* mediated leaf extracts (Kumar, Singh, Halder, & Mitra, 2014) showed 5.394 and 4.454 mg L^{-1} MIC for *E.coli* and *S. aureus*, respectively. Overall, the results of this report, pointed out that the silver nanoparticle produced by *Z. mauritiana* showed excellent antibacterial activity compared to the plant extract.

Conclusion

To conclude, *Z. mauritiana* a conjugated Ag nanoparticles were synthesized using their leaves extract through bio-reduction of aqueous Ag+ ions. The synthesized AgNPs were analyzed using UV–vis spectrophotometer and FTIRrevealed the formation of Ag nanoparticles. Antibacterial study dis-played that the biosynthesized silver nanoparticles from the fresh leaves of *Z. mauritiana* were attributed to have excellent antibacterial performance against Gram negative bacteria, than Gram positive bacteria in a concentration dependent manner using *Z. mauritiana* leaves extract. While, fresh leaves extract does not exhibit any activity towards the growth of tested bacteria's. Therefore, AgNPs producing *Z. mauritiana* may be potentially utilized for the economical production of AgNPs form any pharmaceutical applications.

References

Anand, K. K. H., & Mandal, B. K. (2015). Activity study of biogenic spherical silver nanoparticles towards microbes and oxidants. *Spectrochimica Acta A, Molecular and Biomolecular Spectroscopy, 135*, 639-645. doi: 10.1016/j.saa.2014.07.013

Ashokkumar, S., Ravi, S., Kathiravan, V., & Velmurugan, S. (2014). Synthesis, characterization and catalytic activity of silver nanoparticles using *Tribulus terrestris* leaf extract. *Spectrochimica Acta A, Molecular and Biomolecular Spectroscopy, 121*, 88-93. doi: 10.1016/j.saa.2013.10.073

Ashokkumar, S., Ravi, S., Kathiravan, V., & Velmurugan, S. (2015). Synthesis of silver nanoparticles using *A. indicum* leaf extract and their antibacterial activity. *Spectrochimica Acta A, Molecular and Biomolecular Spectroscopy, 134*, 34-39. doi: 10.1016/j.saa.2014.05.076

Bindhu, M. R., & Umadevi, M. (2015). Antibacterial and catalytic activities of green synthesized silver nanoparticles. *Spectrochimica Acta A, Molecular and Biomolecular Spectroscopy, 135*, 373-378. doi: 10.1016/j.saa.2014.07.045

Chopra, R. N., Nayar, S. C., & Chopra, I. C. (1986). *Glossary of indian medicinal plants*. New Delhi, IN: Council of Industrial and Scientific Research.

Ecocrop. (2013). *Ecocrop database*. Rome, IT: FAO.

Karnani, R., & Chowdhary, A. (2013). Biosynthesis of silver nanoparticle by eco-friendly method. *Indian Journal of Nanoscience, 1*(1), 25-31.

Khalil, M. M. H., Ismail, E. H., El-Baghdady, K. Z., & Mohamed, D. (2014). Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. *Arabian Journal of Chemistry, 7*(6), 1131-1139. doi: 10.1016/j.arabjc.2013.04.007

Khyade, M. S., & Vaikos, N. P. (2009). Phytochemical and antibacterial properties of leaves of *Alstonia scholaris* R Br. *African Journal of Biotechnology, 8*(22), 6434-6436. doi: 10.5897/AJB2009.000-9489

Kirtikar, K. R., & Basu, B. D. (1994). *Indian medicinal plants* (vol. II, 2nd ed.). Dehradun, IN: Bishen Sigh Mahendrapal Singh.

Krishnaraj, C., Jagan, E. G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P. T., & Mohan, N. (2010). Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and Surfaces. B, Biointerfaces, 76*(1), 50-56. doi: 10.1016/j.colsurfb.2009.10.008

Kumar, S., Singh, M., Halder, D., & Mitra, A. (2014). Mechanistic study of antibacterial activity of biologically synthesized silver nanocolloids. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 449*, 82-86. doi: 10.1016/j.colsurfa.2014.02.027

Kumar, V. S., Nagaraja, B. M., Shashikala, V., Padmasri, A. H., Madhavendra, S. S., & Raju, B. D. (2004). Highly efficient Ag/C catalyst prepared by electro-chemical deposition method in controlling microorganisms in water. *Journal of Molecular Catalysis A: Chemical, 223*(1-2), 313-319. doi: 10.1016/j.molcata.2003.09.047

Lara, H. H., Ayala-Núñez, D. C. L., Turrent, C. I. L., & Padilla, C. R. (2010). Bactericidal effect of silver nanoparticles against multi drug resistant bacteria. *World Journal of Microbiology and Biotechnology, 26*(4), 615-621. doi: 10.1007/s11274-009-0211-3

Logeswari, P., Silambarasan, J., & Abrham, J. (2013). Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties. *Scientia Iranica, 20*(3), 1049-1054. doi: 10.1016/j.scient.2013.05.016

Nadkarni, K. M. (1986). *Indian materia medica* (vol. 1). Bombay, IN: Popular Prakashan.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Anthony, S. (2009). *Agroforestree Database: a tree reference and selection guide version 4.0*. Nairóbi, KE: World Agroforestry Centre.

Padalia, H., & Chanda, S. (2015). Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. *Arabian Journal of Chemistry, 8*(5), 732-741. doi: 10.1016/j.arabjc.2014.11.015

Rai, M., Deshmukh, S., Ingle, A., & Gade, A. (2012). Silver nanoparticles: the powerful nano weapon against multidrug-resistant bacteria. *Journal of Applied Microbiology, 112*(5), 841-852. doi: 10.1111/j.1365-2672.2012.05253.x

Raut, R. W., Mendhulkar, V. D., & Kashid, S. B. (2014). Photosensitized synthesis of silver nanoparticles using *Withania somnifera* leaf powder and silver nitrate. *Journal of Photochemistry and Photobiology B: Biology, 132*, 45-55. doi: 10.1016/j.jphotobiol.2014.02.001

Rawani, A., Ghosh, A., & Chandra, G. (2013). Mosquito larvicidal and antimicrobial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of *Solanum nigrum* L. (Solanaceae: Solanales). *Acta Tropica, 128*(3), 613-622. doi: 10.1016/j.actatropica.2013.09.007

Sadeghi, B., & Gholamhoseinpoor, F. (2015). A study on the stability and green synthesis of silver nanoparticles using *Ziziphora tenuior* (Zt) extract at room temperature. *Spectrochimica Acta. Part A: Molecular Biomolecular Spectroscopy, 134*(5), 310-315. doi: 10.1016/j.saa.2014.06.046

Salam, H., Rajiv, P., & Kamaraj, M. (2012). Plants: green route for nanoparticle synthesis. *International Research Journal of Biological Sciences, 1*(5), 85-90.

Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P., & Dash, D. (2007). Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology, 18*(22), 225103-225111. doi: 10.1088/0957-4484/18/22/225103

Sohail, Y., Liu, L., & Yao, J. (2013). Biosynthesis of silver nanoparticles by bamboo leaves extract and their antimicrobial activity. *Journal of Fiber Bioengineering and Informatics, 6*(1), 77-84. doi: 10.3993/jfbi03201307

Sondi, I., & Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science, 275*(1), 177-182. doi: 10.1016/j.jcis.2004.02.012

Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *American Journal of Infection Control, 34*(5 Suppl. 1), S3-S10. doi: 10.1016/j.ajic.2006.05.219

Veerasamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N., & Dhanaraj, S. A. (2011). Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *Journal of Saudi Chemical Society, 15*(2), 113-120. doi: 10.1016/j.jscs.2010.06.004

Zeinab, S., Firoozeh, D., Shima, D., & Ataei, S. A. (2016). Sustainable synthesis of silver nanoparticles using macroalgae *Spirogyra varians* and analysis of their antibacterial activity. *Journal of Saudi Chemical Society, 20*(4), 459-464. doi: 10.1016/j.jscs.2014.10.004

Zeng, F., Hou, C., Wu, S., Liu, X., Tong, Z., & Yu, S. (2007). Silver nanoparticles directly formed on natural macroporous matrix and their antimicrobial activities. *Nanotechnology, 18*(5), 055605-055613. doi: 10.1088/0957-4484/18/5/055605