INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

BIOSYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES OF *EUPHORBIA MILII* DES MOUL. LEAF EXTRACT

A.Ch. Pradyutha [1], V. Umamaheswara Rao [2]* and YRKV Tirupati Rao [2]
[1]Department of Microbiology, R.B.V.R.R Women's College, Hyderabad, Telangana, India
[2]Dept. of Botany & Microbiology, Acharya Nagarjuna University, Nagarjunanagar, Guntur Dt., Andhra Pradesh, India
*Corresponding Author Email: umrvanga@yahoo.co.in

Article Received on: 01/06/18 Approved for publication: 20/06/18

DOI: 10.7897/2230-8407.096108

ABSTRACT

Synthesis of nanomaterials may involve various routes including physical, chemical and biological approaches. The biological green synthesis of nano particles is gaining attention due to its cost effective, ecofriendly and possibility for easy large scale production. In this present study, leaf extract of *Euphorbia milii* was used to synthesize silver nano particles and aimed to study the antibacterial activity of the synthesized silver nanoparticles. The silver nano particles synthesized were confirmed by their change of colour to dark brown due to the phenomenon of surface plasmon resonance. The rapid reduction of silver (Ag+) ions was monitored by using UV-visible spectrophotometer. Transmission electron microscopy (TEM) and Scanning electron Microscopy (SEM) showed that the synthesized nano particles are varied from 20-50 nm with spherical shape. FTIR analysis showed the functional groups involved in the AgNps formation. Biological evaluation of silver nanoparticles was also done against Gram positive and Gram negative bacteria for their future applications in biomedicine. The synthesized silver nanoparticles exhibited good antibacterial activity against both Gram-negative and Gram-positive bacteria tested. This work proved the potential antibacterial activity of silver nano particles synthesized from leaves of *E.milii*.

Key words: Nanoparticles, *Euphorbia milii*, SEM, TEM, FTIR, UV-Visible Spectrophotometer.

INTRODUCTION

Nanoparticles are special and interesting as their chemical and physical properties are different from their macro counterparts. The synthesis of nanoparticles and applications are gaining intense importance in biomedicine for therapeutic purpose in different dosage forms and dosing routes due to the smaller size of nanoparticles (1–100 nm), high surface area and reactivity[1]. An important area of research in nanotechnology is the synthesis of nano silver particles using biosources. Silver has long been recognized as having an inhibitory effect towards many bacterial strains and microorganisms[2]. Biological methods of nanoparticle synthesis using microorganisms[3], enzymes[4], fungus[5] and plants or plant extracts[6, 7] have been suggested as possible ecofriendly alternatives to chemical and physical methods. In recent times, plant-mediated synthesis of nanoparticles has garnered wide interest owing to its inherent features such as rapidity, simplicity, eco-friendliness and cheaper cost[8].

Euphorbia is a large genus of smooth and spiny shrubs and cactus-like succulents from 4" to 20 feet in the spurge family (Euphorbiaceae). Of the more than 1,600 species, crown of thorns, *Euphorbia milii* is a small tropical species from Madagascar that has long been grown as a houseplant or ornamental in warm climates. *E.milii* is widely used in folk medicine for the treatment of warts (South Brazil), cancer and hepatitis (China). It has been reported that *Euphorbia milii* possesses antifungal and antinociceptive property, acts as natural molluscicide, can curb the spread of schistosomiasis[9]. Therefore the present study has been planned to synthesize and evaluate the antibacterial activity of silver nano particles from the leaves of *E.milii*.

MATERIALS AND METHODS

Collection and Authentication of plant

The *E.milii* plants were collected from different places of Hyderabad and authenticated by a plant taxonomist from the Department of Botany, Osmania University.

Extraction of *E.milii* leaves

The leaves of *E.milii* plants were washed, air dried in shade and powdered in a mechanical grinder. Fifteen grams of powered leaf material was placed in 100 ml of deionized water and boiled for 15 minutes at 60°C. After cooling to room temperature, the extract was filtered using Whatman No.1 filter paper to get clear solution and stored at 4°C for further analysis.

Synthesis of AgNPs

The silver nitrate of AR grade used in this study was obtained from Hi-Media. To 20 ml of leaf extract, 80 ml of 1 mM AgNO3 solution was added and further heated upto 60°C for 15 minutes. The colour change of the solution from yellow to brown indicates the formation of AgNPs from the leaf. This was used for the characterization and antimicrobial activity.

Characterization of the Synthesized Silver Nanoparticles

The bioreduction of silver ions to silver nanoparticles was monitored by measuring the UV-Vis spectrum of the reaction solution. The technique outlined above has proven to be very useful for the analysis of nanoparticles[10]. Furthermore, the

solution was centrifuged at 20000 rpm for 30 minutes to remove free biomass residue that is not the capping ligand of the nanoparticles. After centrifugation, separated nanoparticles settled at the bottom were collected and washed thrice with double distilled water, then dried in an oven at 60°C for 2 hrs. The stabilized powder form of the nanoparticles was stored for further characterization.

UV-Vis Spectroscopy

The synthesized AgNPs were characterized and confirmed by UV-Vis spectroscopy by measuring the wavelength of reaction mixture (from 200 to 800 nm) in the UV-Vis spectrophotometer of the Perkin Elmer.

TEM & SEM Analyses

Transmission electron microscopy (Model-Philips CM 200) and Scanning electron microscopy (Model-SEM Hitachi - S520, Japan) were used to study the morphology of AgNPs.

Fourier Transform Infra-red Spectroscopy (FTIR)

The pellet of AgNPs obtained after oven drying was mixed with KBr and the resultant KBr-AgNPs mixture was subjected to FTIR (Model-Thermo Nicolet Nexus 670) analysis to ensure the formation of AgNPs with encapsulation of biomolecules of *E.milii* leaves.

Screening of Antibacterial activity of synthesized AgNPs

The antibacterial activity of AgNPs synthesized from leaves of *E.milii* was tested against some Gram positive and Gram negative bacteria viz., *Micrococcus luteus* MTCC 106, *Arthrobacter protophormiae* MTCC 2682, *Rhodococcus rhodochrous* MTCC 265, *Staphylococcus aureus* MTCC 737, *Proteus mirabilis* MTCC 425, *Pseudomonas aeruginosa* MTCC 1688, and *Salmonella enterica* MTCC 3858 by agar well diffusion method[11]. Into each agar well, 100 µl of sample prepared by dissolving 100 µg of nanoparticle material in 1 ml of dimethyl sulfoxide (DMSO) was placed. In a separate well, DMSO was also dispensed to maintain the control. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the zone of inhibition was measured. For each sample and bacterial species, triplicates were maintained.

RESULTS AND DISCUSSION

Visual observation

Silver nanoparticles formation was primarily and visually identified by the colour change of *E.milii* leaf extract that was treated with silver nitrate aqueous solution from yellowish brown to dark brown (Figure-1A & B). The colour change was a clear indication for the formation of silver nanoparticles[12]. Silver nanoparticles arise due to the surface plasmon vibrations in the aqueous solution.

UV-Vis spectral analysis

The UV-Vis absorption spectrum of AgNPs obtained showed the absorption maxima ranged from 425 to 475 nm which gives the confirmation for the AgNPs synthesis[13]. UV-Vis absorption spectrum of silver nanoparticles formed in the reaction mixture has displayed a strong and broad absorbance peak at 444.38 nm wavelength (Figure-2), which gives the confirmation of the silver nano particles synthesized from the leaves of *E.milii*. Broadening of peak indicates the polydispersed nature of the silver nanoparticles[14]. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within a hour of reaction, making it one of the fastest bio-reducing methods to produce Ag nano structures that have been reported till the date[15].

TEM and SEM Analyses

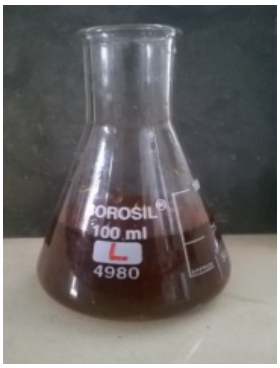
Morphology and size of silver nanoparticles synthesized were characterized by TEM (Figure-3) which revealed that the nanoparticles were evenly distributed without agglomeration. Synthesized silver nanoparticles were found mostly spherical and their dimension ranged from 20 to 50 nm. Thus, TEM characterization studies confirmed the nanometer size range of the synthesized silver nanoparticles. The SEM image (Figure-4) showed the high density AgNPs synthesized by using the leaf extract of *E.milii* leaf and also further confirmed the development of silver nanostructures.

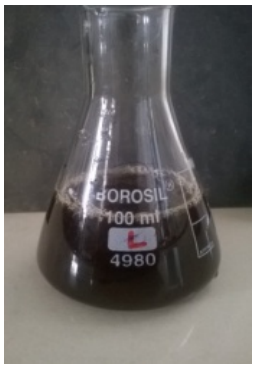
FTIR Analysis

The dual role of the plant extract as a reducing and capping agent and presence of some functional groups was confirmed by FTIR analysis of silver nanoparticles (Figure-5). A broad band at 3287 cm^{-1} is due to the N–H stretching vibration of group NH_2 and OH, the overlapping of the stretching vibration is attributed for water and *E.milii* leaf extract molecules. The band at 1634 cm^{-1} corresponds to amide C=O stretching and a peak at 2656 cm^{-1} can be assigned to alkyne group present in phyto-constituents of extract. The observed peaks at 1223 cm^{-1} denote –C–OC- linkages, or –C–O- bonds. The observed peaks are mainly attributed to flavonoids and terpenoids excessively present in plants extract[16, 17]. From FTIR results, it can be concluded that some of the bioorganic compounds from *E.milii* leaf extract formed a strong coating/capping on the nanoparticles.

Antibacterial activity

The synthesized silver nanoparticles of *E. milii* leaves exhibited good antibacterial activity with some variation among the Gram-positive and Gram-negative bacteria tested (Figure-6). Based on the zone of inhibition produced, synthesized silver nanoparticles exhibited relatively more antibacterial activity against *Micrococcus luteus*, *Arthrobacter protophormiae*, *Staphylococcus aureus* and *Salmonella enterica* than *Rhodococcus rhodochrous*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. However, *Arthrobacter protophormiae*, *Rhodococcus rhodochrous*, *Staphylococcus aureus* and *Salmonella enterica* were found to be more susceptible to synthesized nanoparticles when compared to that of standard antibiotic, streptomycin. Though, *Micrococcus luteus*, *Proteus mirabilis* and *Pseudomonas aeruginosa* found sensitive to silver nanoparticles of *E. milii* leaves, but not high than to that of streptomycin. The results indicating the more effective inhibitory activity of synthesized *E.milii* leaf silver nanoparticles against Gram positive bacteria than Gram negative bacteria tested. This may be due to the action of silver nanoparticles on microbes in causing the microbicidal effect. Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequent penetration into it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and ultimately death of the cell[18].





(A) **(B)**

Fig. 1: A) *E. milii* leaf extract with $AgNO_3$ solution before reaction, B) *E. milii* leaf extract with $AgNO_3$ solution after reaction

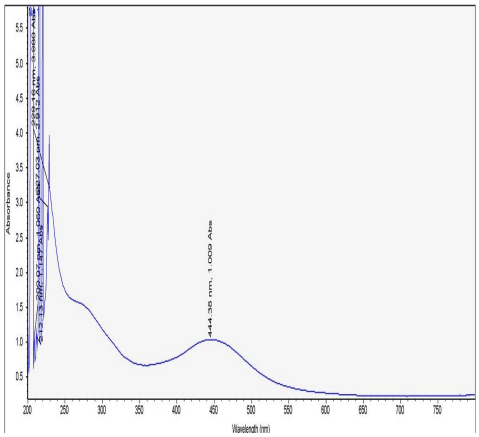


Fig. 2: UV-Vis absorption spectrum of AgNPs of *E.milii* leaves

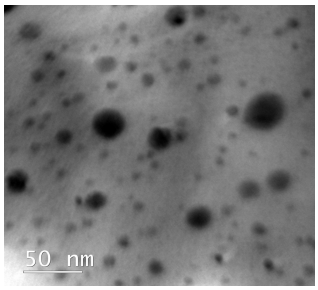


Fig-3: Transmission electron microscopy image of AgNPs of *E.milii* leaves

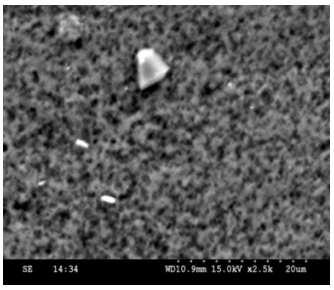


Fig-4: Scanning electron microscopy image of AgNPs of *E.milii* leaves

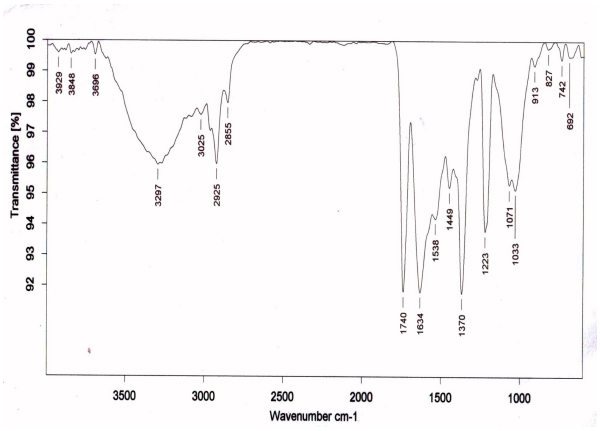


Fig 5: FTIR spectrum of AgNPs of *E.milii* leaf extract

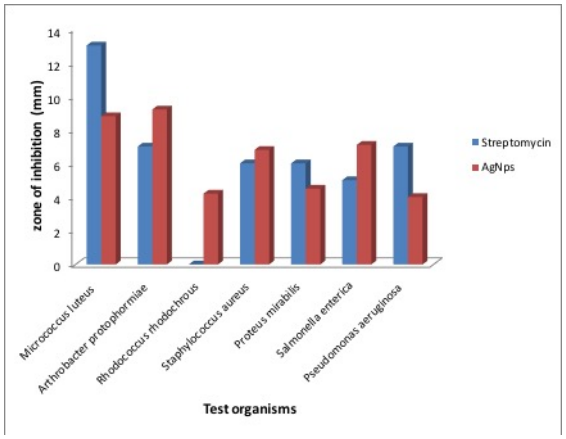


Fig. 6: Antibacterial activity of *E.milii* leaf extract Silver nanoparticles

CONCLUSION

A simple and biosynthetic method of colloidal AgNPs synthesis directly by using leaf extract of *E.milii* is successfully achieved. The primary colour change of reaction mixture, spectral analyses of UV-Vis absorption and FTIR spectroscopy confirmed the formation of silver nanoparticles as well. The morphology and shape of synthesized silver nanoparticles of *E. milii* leaf extract were identified by TEM and SEM images. The green synthesized silver nanoparticles of *E. milii* leaves showed very good and effective antibacterial activity against the tested bacteria. The present study concludes that the leaves of *E.milii* plant can be used as an excellent source for synthesizing the silver nanoparticles having potential antibacterial activity which can be introduced into future biomedical applications to combat various bacterial diseases.

REFERENCES

1. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res. Pharm. Sci. 2014; 9 (6): 385–406 .
2. Jiang H, Manolache S, Wong ACL, Denes FS. Plasma-enhanced deposition of silver nanoparticles on to polymer and metal surfaces for the generation of antimicrobial characteristics. Journal of Applied Polymer Science 2004; 93 (3): 1411–1422.
3. Nair B, Pradeep T. Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains. Cryst Growth Des. 2002; 2: 293–298.
4. Willner I, Baron R, Willner B. Growing metal nanoparticles by enzymes. J Adv Mater. 2006; 18:1109–1120.
5. Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paraliker KM, Balasubramanya RH. Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Mater Lett. 2007; 61: 1413–1418.
6. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol Prog. 2006; 22: 577–583.
7. Jae YS, Beom SK. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng. 2009; 32: 79–84.
8. Dubey M, Bhadauria S, Kushwah BS. Green synthesis of nanosilver particles from extract of *Eucalyptus hybrida* (Safeda) leaf. Dig J Nanomat Biostruct. 2009; 4(3): 537–543.
9. Rauf A, Khan A, Uddin N, Akram N, Arfan M, Uddin G, Quasir M. Preliminary Phytochemical screening, antimicrobial and antioxidant activities of *Euphorbia milii*. Pak J Pharm Sci. 2014; 27(4): 947-51.
10. Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, Kumar R, *et al*. Enzyme mediated extracellular synthesis of CdS nanoparticles by the fungus, *Fusarium oxysporum*. J Am Chem Soc. 2002; 124 (41): 12108-12109.
11. Olurinola PF. A laboratory manual of pharmaceutical microbiology. Idu, Abuja, Nigeria: National Institute for Pharmaceutical Research and Development 1996: 69-105.
12. Arunachalam R, Dhanasingh S, Kalimuthu B, Uthirappan M, Rose C, Mandal AB. Phytosynthesis of silver nanoparticles using *Coccinia grandis* leaf extract and its application in the photocatalytic degradation. Colloids and Surfaces B: Biointerfaces. 2012; 94 : 226–230.
13. Nagababu P, Umamaheswara Rao V. Pharmacological Assessment, Green synthesis and Characterization of Silver Nanoparticles of *Sonneratia apetala* Buch.-Ham. Leaves. Journal of Applied Pharmaceutical Science 2017; 7 (8): 175-182.
14. Geethalakshmi R, Sarada DVL. Synthesis of plant-mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their anti microbial activities. Inter J. Engineering Sci. Technol. 2010; 2 (5): 970- 975.
15. Shiv Shankar S, Ahmad A, Pasricha R, Sastry M. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. J. Materials. Chem. 2003; 13: 1822-1826.
16. Banerjee P, Mantosh S, Mukhopahayay A, Das P. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing 2014; 1(3): 1–10.
17. Prathna TC, Chandrasekaran N, Raichur AM, Mukherjee A. Biomimetic synthesis of silver nanoparticles by *Citrus limon* (lemon) aqueous extract and theoretical prediction of particle size. Colloids and Surf B Biointerfaces 2011; 82 : 152–159.
18. Bindhu MR, Umadevi M. Silver and gold nanoparticles for sensor and antibacterial applications. Spectrochimica Acta A 2014; 128: 37-45.

Cite this article as:

A.Ch. Pradyutha *et al*. Biosynthesis, characterization and antibacterial activity of silver nanoparticles of *Euphorbia milii* Des Moul. leaf extract. Int. Res. J. Pharm. 2018;9(6):154-157 http://dx.doi.org/10.7897/2230-8407.096108

Source of support: Nil, Conflict of interest: None Declared