Int J Pharm Bio Sci 2014 Jan; 5(1): (B) 457 - 467

Research Article Nanotechnology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

A FACILE GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *PSORALEA CORYLIFOLIA* L. SEED EXTRACT AND THEIR *IN-VITRO* ANTIMICROBIAL ACTIVITIES

SUNITA D. DANAI-TAMBHALE*[1] AND PARAG V. ADHYAPAK[2]

[1]*Department of Botany, Annasaheb Magar Mahavidyalaya, Pune-411028, India.*
[2]*Centre for Materials for Electronics Technology, Panchwati, off Pashan Road, Pune-411008, India.*

ABSTRACT

In this study, the biosynthesis of silver nanoparticles was carried out by using *Psoralea corylifolia* L. (family Leguminosae) as reducing agent. As per our knowledge, this is the first report where *Psoralea corylifolia* seed extract was found to be suitable for the green synthesis of silver nanoparticles. Synthesis of silver nanoparticles was confirmed on the basis of UV-Vis Spectrometer which showed a peak between 400 nm to 440 nm. The synthesized silver nanoparticles were characterized by X-ray diffraction analysis (XRD), Field emission scanning electron microscopy (FE-SEM), and particles size distribution analysis. The biosynthesized silver nanoparticles have been evaluated in vitro for antimicrobial activities and found to have higher antimicrobial activities.

KEYWORDS: antimicrobial activities; FE-SEM; *Psoralea corylifolia;* Silver Nanoparticles; XRD

SUNITA D. DANAI-TAMBHALE
Department of Botany, Annasaheb Magar Mahavidyalaya, Pune-411028, India.

INTRODUCTION

The field of nanotechnology is mainly concerned with synthesis of nanoparticles of different sizes, shapes, chemical compositions and controlled dispersity and their potential use for human health. An important aspect of current nanotechnology research is the development of an environmental friendly process for synthesis of nanomaterials. Nanoparticles have received considerable attention in recent years due to their wide range of applications in the field of catalysis, photonic, optoelectronics, biological tagging, pharmaceutical applications, environmental pollution control, drug delivery system and material chemistry[1,2,3]. In recent years, plant mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Plants are relatively safe, easily available and affordable to the masses. Green synthesis of nanoparticles has been achieved using environmentally acceptable plant extract and eco-friendly reducing and capping agents. Uptill now, biological methods of nanoparticle synthesis using plants and plant extract such as *Azadirachta indica*[4], *Euphorbia hirta*[5], *Boswellia ovalifoliata* and *Shorea tumbuggaia*[6], *Desmodium triflorum*[7], *Ellatoria cardamomom*[8], *Dioscoria bulbifera*[9] *Cardiospermum helicocacabum*[10] have been proven to be eco-friendly alternatives to chemical and physical methods. The use of plants for the synthesis of nanoparticles does not require high energy, temperatures and it is easily scaled up for large scale synthesis[11, 12]. However medicinal potential value of many plants along with their role in synthesis of nanoparticles is yet to be studied in detail. Especially silver nanoparticles have been used as a healing and antibacterial agent for thousands of years[13].

Psoralea corylifolia L. commonly known as Bawachi is a variety of family Leguminosae with bluish purple colored flowers. It is widely distributed herbaceous plant commonly found in the tropical and subtropical regions of the world, especially in China and Southern Africa[14]. It grows throughout the plains of India, especially in the semi-arid regions of Rajasthan and eastern districts of Punjab, adjoining Uttar Pradesh. It is also noted in some valley in Bihar, Himalayas, Dehra Dun, Bundelkhand, Bengal, Deccan, Karnataka and Bombay[15, 16]. Seeds of *Psoralea corylifolia* are brownish black in color, oblong and flattened, thick, hard, smooth, exalbuminous with straw colored testa, with agreeable aromatic odor and pungent-bitter taste[17,18]. It is one of the most popular Traditional Chinese Medicine[19] and used to treat a variety of skin diseases such as psoriasis, leucoderma and leprosy[20] and it possesses antitumor, antibacterial, and cytotoxic and antihelmenthic properties[21,22]. Seed powder extract is proven to be useful as diuretic, anthlminthic, laxative and wounds curative[23]. Seed powder has also been used by Vaidyas internally for leprosy and leucoderma and externally in the form of paste and ointment[18,24] for bilious disorders[18,24]. Moreover, the pigments monoterpenoid phenol named bakuchiol, raffinose, coumarin[14], and chemicals corylifolean, corylifolin, corylifolinin[25], angelicin[26], psoralidin and isopsoralen[20] with some active components such as benzofuran, corylifonol, isocorylifonol[27] were isolated from seeds of Psoralea corylifolia. Its seeds are extensively used to make perfume oil. Herein we report the synthesis of silver nanoparticle by using the seed extract of *Psoralea corylifolia* L. The obtained silver nanoparticles were characterized by UV-Visible spectroscopy, X-ray Diffraction, Field emission-scanning electron microscopy and particle size distribution analysis. The antimicrobial activity of the prepared sample was evaluated against a wide range of Gram negative and Gram positive pathogenic bacteria.

MATERIALS AND METHODS

Fresh plant material was collected from field Usturi village, District Latur, (Maharashtra) India. The specimen was identified and certified by the Botanical Survey of India (BSI) Pune. The seeds were used for synthesis of nanoparticles. All the chemicals were purchased from Sigma Chemicals.

(i) Sample preparation

Seeds of P*soralea cor*ylifolia (Figure-1a) were air dried and ground to make fine powder. 4 g of powder was taken into beaker and 100 ml of sterile distilled water was added to it. This solution was boiled for 20 min at 100^{o}C. The seed extract thus formed was filtered through Whatman filter paper no -01 and collected separately in conical flask (Figure-1b).

(ii) Synthesis of silver nanoparticles

The procedure for the preparation of the silver nanoparticles has been adopted from[28,29]. 5 mM silver nitrate ($AgNO_3$) solution was prepared and stored in amber colored bottle. 10 ml of seed extract was taken in beaker. To this seed extract solution 50 ml of 5 mM $AgNO_3$ was added drop wise with constant stirring at 50-60^{o}C and colour change was observed. The colour change was checked periodically and the beaker was incubated at room temperature for 24 hours. The colour of seed extract changes from faint brown to dark brown indicating the formation of silver nanoparticles. The extract was then centrifuged at 10,000 rpm for 20 min. The supernatant was used for the physicochemical characterization and for the evaluation of antimicrobial activity.

(iii) Physicochemical characterization

The optical absorption studies of synthesized nanoparticles were performed by UV-Visible spectrophotometer (Jasco V-570 UV-Visible Spectrophotometer). The absorption spectrum of the reduced reaction mixture was recorded after 24 h. incubation by diluting a small amount of aliquot of the sample with distilled water. The phase analysis of synthesized nanoparticles was done by powder X-ray diffraction (model: Brucker D8, Advanced X-ray powder diffractometer) using CuKα radiation (1.54 Å), Nickel filter, 40 kV voltage and 40 mA current in 2θ range of 20 to 80^{o}. The resultant reaction mixture after reduction was drop casted on a glass slide for enabling the XRD measurements. The surface morphology was examined using a Field emission scanning electron microscopy (model: Hitachi S4800 Scanning electron microscope). The particle size distribution analysis was done using a dynamic light scattering method (Zetasizer Nano-ZS, Model: ZEN 3600, Malvern Instruments Ltd. UK).

(iV) Antimicrobial activity

Pure cultures of *Salmonella paratyphi,* B *Psuedomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive) were used to check the antimicrobial activity. The antimicrobial activities of Psoralea corylifolia Silver Nanoparticles (PcSNPs) were carried out by disc diffusion method[30]. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification of agar medium, fresh bacterial culture suspensions were swabbed on these plates. The sterile discs of Whatman filter paper no. 1 (5 mm diameter) were prepared and impregnated with 10μl of 5mM concentrations of PcSNPs solution. The discs were placed on the nutrient agar medium surface after dried for 10 -15 minutes and kept for incubation at 37^{o}C for 24 h. Zone of inhibition was measured and compared with standard Chloromphenicol disc and silver nitrate solution. The experiments were repeated thrice and mean values of inhibition zone diameter were calculated.

RESULTS

The change in the color of the *P. corylifolia* seed extract from light brown to yellowish brown after the addition of 5 mM $AgNO_3$ has been observed. The bio-reduction of aqueous silver salt into silver nanoparticles was first monitored by measuring the optical absorption spectra. Figure-2 depicts the UV-Visible absorption spectrum of the reaction solution taken after incubation period of 24 h. The absorption spectrum shows the maximum absorption peak at~425 nm. This characteristic peak is due to the oscillation of conduction band electrons of silver (also known as surface plasmon resonance). The observed peak position is red shifted as compared with bare silver nanoparticles, which exhibits sharp absorption peak at 390 nm[31]. The position of plasmon absorption band depends on particle size, aspect ratio and diameter of nanowires. In the present work, the shift in the λmax with broadening in the peak is observed due to the nano size of the silver particles. Formation of the silver nanoparticles is further supported by the X-ray diffraction analysis. Figure-3 exhibits the X-ray diffraction pattern of resultant silver nanoparticles. The presence of major diffraction peak are observed at 2θ = 38.03, 44.12, 64.64 and 77.30^o corresponded to (100), (200), (220) and (311) planes respectively. All prominent peaks pertaining to respective 2θ values can be readily assigned to zero-valent fcc silver. The observed broadening of the peaks clearly hints the formation of nano-sized structures. Moreover, it is interesting to observe that the (100) diffraction peak is much stronger than that of the other diffraction peaks suggesting preferential orientation facilitating the one dimensional directional growth of nanorods. The primary grain size calculated (using Scherrer's Equation) is in the range of 20 to 25 nm.

The morphology of the sample was observed by FE-SEM. Figure-4 displays selected FE-SEM image of synthesized silver nanostructures. From the figures, it is observed that the product is mainly composed of silver nanorods and the dimensions of nanorods are between 50–200 nm in width and few microns in length. However the dimensions of these rod-like structures are not uniform. Along with these rod-like structures the existence of numerous nanoparticles was also observed. It is opined that the rod-like morphology is formed presumably due to the slow reduction process leading to the agglomeration of the silver nanoparticles. Particle size distribution analysis from the dynamic light scattering technique Figure-5 of silver nanoparticles indicated the mean diameter of 100 to 110 nm.

DISCUSSION

The current study was undertaken to explore the *Psoralea corylifolia* L. seed resources in the development of silver nanoparticles. It is a traditional medicinal plant used in this first ever report on the synthesis of silver nanoparticles from its seed extracts. It has potential to reduce silver nitrate ions to silver nanoparticles. The light brown color of aqueous extract of P. corylifolia was changed to faint brown to yellowish brown after 24 hours of incubation due to the excitation of surface plasmon vibrations in silver nanoparticles[32]. The appearance of yellowish- brown colour in the reaction vessel suggests the formation of silver nanoparticles (SNPs) [4,6,29] (Linga Rao, and Savitharamma 2012) figure-1(c). The synthesis of silver nanoparticles was also confirmed from the UV-Vis Spectrometer of PcSNPs where the maximum absorbance was found ~450 nm the broadening of peak indicated that the particles are poly-dispersed (Figure-2) after 24 hours of incubation. The results of the investigation showed that silver nanoparticles synthesized from *P. corylifolia* a novel medicinal plant. It possesses discrete antibacterial activity against pathogenic bacteria at a concentration of 10µl. The antimicrobial activity of the silver nanoparticles was studied against *K. pneumoniae, E. coli*, *B. subtilis, S. paratyphi* B, *P. aeruginosa* and *S. aureus* by using standard zone of inhibition microbiology assay. The nanoparticles showed inhibition zone against all the studied bacteria [Figure- 6]. Maximum zone of inhibition was found to be (25 mm) in *K. pneumoniae,* (20 mm) *E. coli* and (19 mm) *B. subtilis* and minimum with *S. paratyphi* B, *P. aeruginosa* and *S. aureus* it was represented in table-1 and graph. Similar observation was found in papaya fruit[33], *Clitoria ternatea*[34] *and Euphorbia hirta*[5]. The silver nanoparticles were compared favourably with silver nitrate solution, plant extract and standard antibiotic chloramphenicol at a 10 µml (Table -1). Silver nanoparticles exhibited more activity than silver nitrate and as good as equal activity with standard chloramphenicol.

The green synthesis of silver nanoparticles is a traditional method and the use of plant extract has new awareness for the control of disease, besides being safe and no phytotoxic effect[29]. Biologically prepared silver nanoparticles using seed extract were found to be highly toxic to bacterial species selected. Silver nitrate is used as reducing agent due to its distinctive properties such as good conductivity, catalytic and chemical stability. Silver nitrate which is readily soluble in water has been exploited as an antiseptic agent for many decades[35, 36]. The time duration of change in colour of plant mediated silver nanoparticle synthesized aqueous solution varies from plant to plant. In case *P. corylifolia* silver nanoparticles synthesized within 20 minutes (Plate-1). The biosynthesized *P. corylifolia* silver nanoparticles (PcSNPs) are found to have high antimicrobial activity. The present work supports the medicinal values of this plant. It also provides a simple, rapid and economic method for synthesis of silver nanoparticles. The synthesized PcSNPs promotes medicinal efficacy and strengthen the medicinal values of *P. corylifolia*.

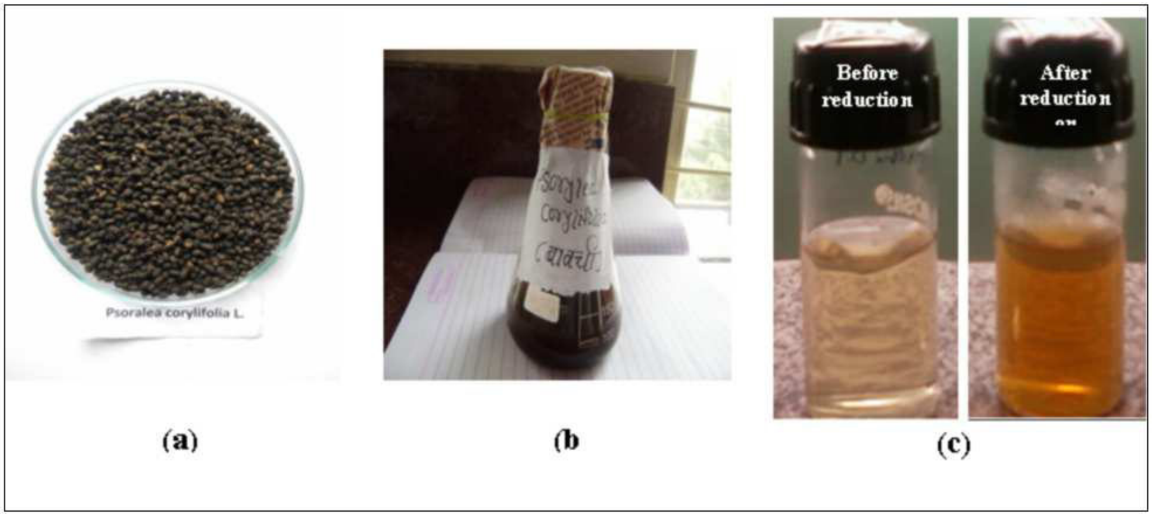

Figure 1
Photographs showing seeds of a) P. corylifolia b) seed extract c) color changes after adding $AgNO_3$ before reaction and after reaction time of 24 hours.

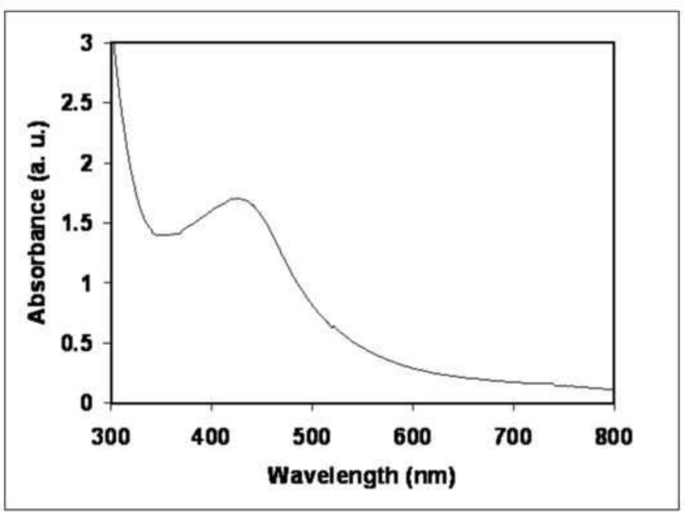

Figure 2
Showing UV-Visible absorption spectrum of P. corylifolia silver nanoparticles after 24 hours of reaction time

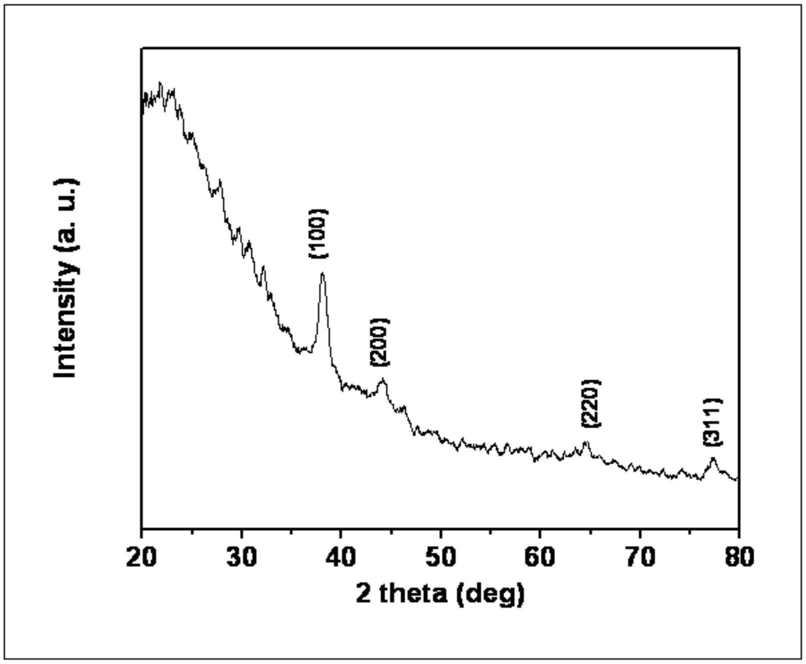

Figure 3
XRD patterns of silver nanoparticles of plant extract of P. corylifolia.

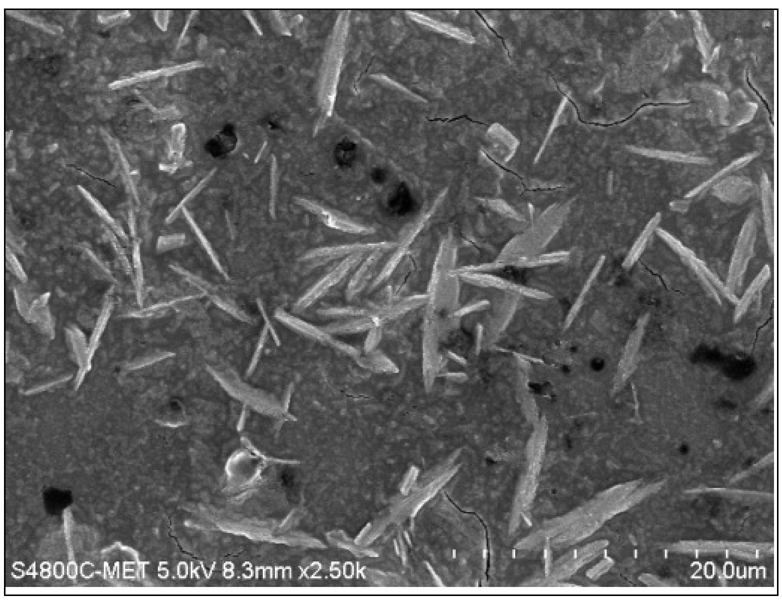

Figure 4
FE- SEM images of silver nanoparticles formed by P. corylifolia.

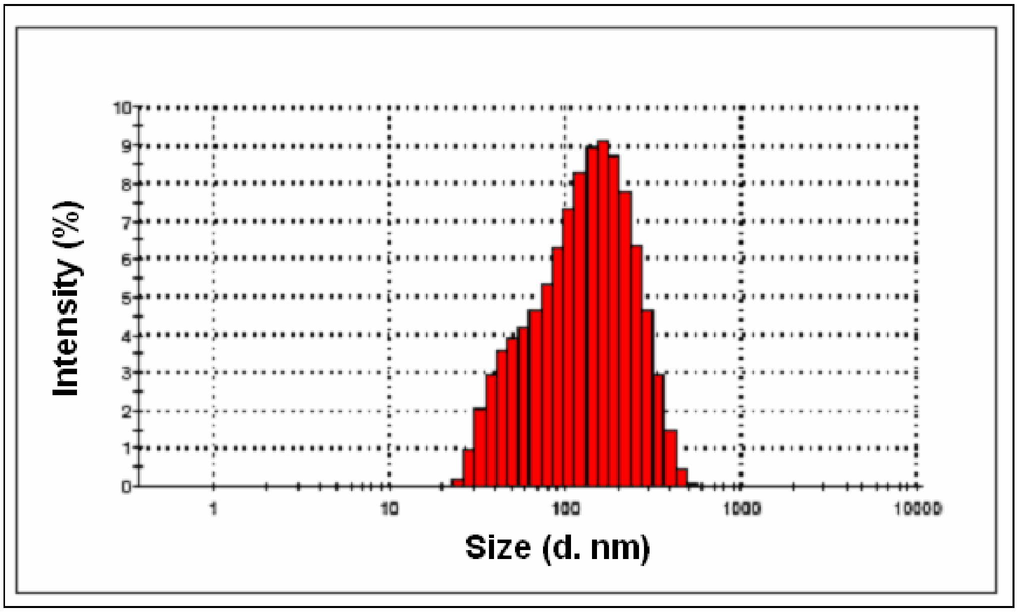

Figure 5
Particle size distribution analysis graph of P. corylifolia silver nanoparticles

Figure 6
Growth inhibition zones of bacterial species after 48 hours of incubation period.

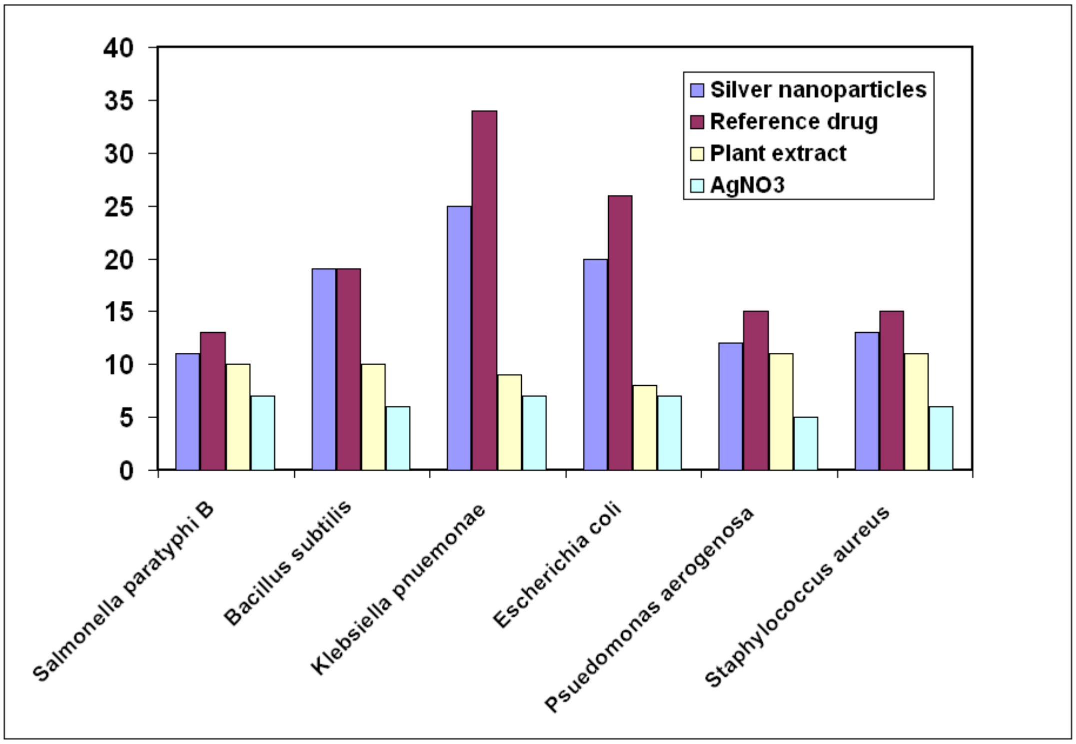

Figure 7
Graph showing effect of P. corylifolia silver nanoparticles, reference drug, plant extract and boiled aqueous silver nitrate solution on growth inhibition zone of various bacterial sp.

Bacterial sp	Inhibition zone			
	GsSNPs	Chloram.	Plant extract	$AgNO_3$
S. paratyphi B	18	17	11	8
Bacillus subtilis	19	19	18	8
K. pneumoniae	15	32	14	9
E. coli	19	22	11	9
P. aeruginosa	14	11	12	8
S. aureus	13	15	14	7

Table 1
Showing effect of P. corylifolia silver nanoparticles, reference drug, plant extract and boiled aqueous silver nitrate solution on growth inhibition zone of various bacterial sp.

CONCLUSION

In conclusion, this study has successfully demonstrated for the first time the use of *Psoralea corylifolia* seed for extracting silver nanoparticles. This study included the biosynthesis of silver nanoparticles through seed extracts of *P. corylifolia*. Biosynthesized silver nanoparticles using *P. corylifolia* seed might be useful for the development of newer and more potent ointment against skin diseases caused by various pathogens. The aqueous silver ions exposed to the extracts, the synthesis of silver nanoparticles were confirmed by the change of colour of plant extract. Silver nanoparticles revealed to possess an effective antimicrobial against *K. pneumoniae,* (20 mm) *E. coli* and (19 mm) *B. subtilis.* The results proved that silver nanoparticles showed maximum activity at 5 mM concentration, which revealed silver nanoparticles as novel antimicrobial agent.

Abbreviations
PcSNPs- Psoralea corylifolia Silver Nanoparticles; XRD- X-ray diffractometer; SEM- Scanning electron microscopy; Uv-Vis- Spectro, Ultra violet Visible Spectrophotometer.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
PV characterized the XRD, Scanning electron microscopy of nanoparticles analysis.
SD designed the study, synthesized nanoparticles, Particle size Distribution Graph, UV Vis. Spectrophometer and wrote the paper. Both authors read and approved the final manuscript.

Authors' information
SD Ph.D. and Assistant Professor and researcher in Botany
PV Ph.D. and Scientist, Centre for Materials for Electronics Technology

ACKNOWLEDGEMENT

SDDT would like to acknowledge to Dr. Pisal S. S., Serum Institute of India, for providing the Particle size distribution graph facility. The authors also thank to Pol Poonam, Dagade Trupti and Gholap Rupali, Mukta Kumari (M.Sc.-II) students of 2013 batch, AM Mahavidyalaya, Pune for experimental assistance.

REFERENCES

1. Gittins D. I., Bethell D., Schiffrin D. J., Nichols R. J., A nanometre-scale electronic switch consisting of a metal cluster and redox-addressable groups. *Nature*. (2000), 408 (6808):67–69.
2. Darroudi M., Ahmad M. B., Abdullah A. H., Ibrahim N. A., Green synthesis and characterization of gelatin-based and sugar-reduced silver nanoparticles. *Int J Nanomedicine*. (2011), 6:569–574.
3. Wise K., Brasuel M., The current state of engineered nanomaterials in consumer goods and waste streams: the need to develop nanoproperty quantifiable sensors for monitoring engineered nanomaterials. *Nanotechnol Sci Appl*. (2011), 4: 3–86.
4. Shankar S. S., Rai A., Ahmad A., Sastry M., Rapid synthesis of Au, Ag, and bimetallic Au core Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *J Colloid Interface*. (2004).
5. Elumalai E. K., Prasad T. N., Hemachndran V. K., Viviyan Therasa J. S., Thirumalai T.,. David E, Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antimicrobial activities. Journal of Pharmaceutical Sciences and Research, (2010), ISSN: 0975-1459, 549-554.
6. Savitharamma N., Linga Rao M, Rukmini K., Suvrnalata Devi P., Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants, International Journal of Chem Tech Research (2011), 3 (3): 1394- 1402.
7. Ahmad N., Sharma S., Singh V. N., Shamsi S. F., Fatma A., Mehta B. R., Biosynthesis of silver nanoparticles from Desmodium triflorum: a novel approach towards weed utilization. Biotechnol. Res Int: (2011), 454090.
8. Gnana Dhas GnanaJobitha, Gurusamy Annadurai, Chellapandian Kannan, Green synthesis of silver nanoparticle using *Elettaria cardamomom* and assessment of its antimicrobial activity. International Journal of Pharma Sciences and Research. (2012), ISSN: 0975-9492, Vol. 3 No3, 323-330.
9. Ghosh Sougata, Sumersingh Patil, Mehul Ahire, Rohini Kitture, Sangeeta Kale, Karishma Pardesi, Swaranjit S Cameotra, Jayesh Bellare Dilip D Dhavale, Amit Jabgunde and Balu A Chopade, Synthesis

of silver nanoparticles using Dioscoria bulbifera tuber extract and evaluation of its synergetic potential in combination with antimicrobial agents. Int Journal Nanomedicine, (2012),7: 483-496s.

10. Vishnudas D., Bhaskar Mitra, Sant Sudhindra B., Annamalai A., Green–synthesis and characterization of silver nanoparticles by aqueous leaf extract of Cardiospermum helicocacabum L. Drug Invention Today (2012),4 (2), 340-344
11. Shankar S. S., Ahmed A., Sastry M., Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnology Process. (2003), 19: 1627-1631.
12. Geethalaxmi R., Sarada D. V. L.: Synthesis of plant mediated silver nanoparticles using Trianthema decandra extract and evaluation of their anti microbial activities. International Journal of Engineering Science and Technology. (2010), 2 (5): 970-975.
13. Panacek A., Kvitek L., Prucek R., Silver colloid nanoparticles, synthesis, characterization, and their antibacterial activity. *J Phys Chem B:* (2007), 110 (33):16248–16253.
14. Krishnamurthi A. K., Manjunath B. L., Sastri B. N., Deshaprabhu S. B., Chadha Y. R. Vol. 7. New Delhi: CSIR; The wealth of India: Raw Materials, (1969) pp 295-8
15. Sah P., Agrawal D., Garg S. P.,. Isolation and identification of furocoumarins from the seeds of *Psoralea corylifolia* L. Indian J Pharma Sci (2006), 68:768-71.
16. Sharma P.C., Yelne M. B.,. Dennis T. J, Central Council for Research in Ayurveda and Siddha, Database on Medicinal Plants used in Ayurveda; Vol. 2 New Delhi: (2001). pp 89-93.
17. Chopra R. N., Chopra I.C.. 2nd ed. Kolkatta: Academic Publishers;. Indigenous Drugs of India; (1958), pp 391-4.
18. Panda H., New Delhi: National Institute of Industrial Research;. Herbs, Cultivation and Medicinal uses; pp 479-81. Kapoor LD. Boca Raton, Florida: CRC Press; 2001. Handbook of Ayurvedic Medicinal Plants; (2000) pp274-5.
19. Khushboo P.S., Jadhav V.M., Kadam V.J., Development and validation of a HPTLC method for determination of psoralen in *Psoralea corylifolia* (Bavachi). *Intl. J. Pharm Tech. Res*. (2009), 1 (4): 1122-1128.
20. Gidwani B., Alsapure R. N. and Duragkar N. J., Pharmacognostic and standardization and physico-chemical evaluation of *Psoralea corylifolia* linn seeds. Imperial J. Pharmacog. Natural Prod. (2011), 1 (1): 145-151
21. Quio C. F., Han Q. B., Song J. Z., Mo S. F., Kong L. D., Kung H. F., Quality assessment of Fructus Psoraleae. Chem Pharm Bull. (2006), 54:887-90.
22. R. Liu, A. Li, A. Sun, L. Kong, preparative isolation and purification of psoralen and isopsoralen from Psoralea corylifolia by high speed counter- current chromatography. J. Chromatogr A. (2004); 1057:225-8.
23. Khastgir H. N, Duttagupta P. C, Sengupta P, Psoralen and Isopsoralen from Psoralea corylifolia Indian J Appl Chem. 1959;22:82-5.
24. K. M. Nadkarni Vol.1. Mumbai: Popular Prakashan Pvt. Ltd.. Indian Materia Medica; (1976) pp 1019-22
25. R. P. Rastogi, B. N. Mehrotra vol. 2 Lucknow, CDRI, and New Delhi, NISCiR,. Compendium of Indian Medicinal Plants, (1998) pp 332-4.
26. K. Gupta, T. Neeraj, S. Madhu, Vol. 3. New Delhi; ICMR;. Quality Standards of Indian Medicinal Plants; (2005) pp 290-8.
27. Rajpal V. Vol. 2 New Delhi: Eastern Publishers; 2005. Standardization of Botanicals; pp.284-95.
28. J. L. Gardea-Torresdey, E. Gomez, J. Peralta- Videa, J. G. Parsons, H. E. Troiani, Jose- Yacaman, Synthesis of Gold Nanotriangles and Silver Nanoparticles Using *Aloe vera* Plant Extract. Langmuir, (2003), 13:13-57.
29. M. Linga Rao, N. Savitharamma, Antimicrobial activity of silver nanoparticles

synthesized by using stem extract of Svensonia hyderobadensis (Walp.) molds a rare medicinal plant. Research in Biotechnology. (2012), 3 (3): 41-47.

30. R. Cruickshank 11th (ed) Medical microbiology: a guide to diagnosis and control of infection. Edinburghand London: E & S Livingston Ltd. (1968), p.888.
31. S. Prashanth, R. Menaka, Muthezhilan and Navin Kumar Sharma Synthesis of plant mediated silver nanoparticles using medicinal plant extract and evaluation of its antimicrobial activities. International Journal of Engineering science and Technology (2011), Vol. 3 No. 8.
32. Thirumurugan, N. A. Tomy, Jai Ganesh, S. Gobikrishnan, Biological reduction of silver nanoparticles using plant leaf extracts and its effect on increased antimicrobial activity against clinically isolated organisms. De phar. Chem. (2010), 2, 279-284.
33. D. Jain, H. kumar Daima, S. Kachhawaha and S. L. Kothari, Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities**.** Digest Journal of Nanomaterials and Biostructures, (2009), Vol. 4, No. 3, p. 557-563.
34. B. Ravindra Malabadi, S. Gangadhar, Malgund, Neelambika T, Meti K, Natraja and S. Vijaya Kumar, Antibacterial activity of silver nanoparticles synthesized by using whole plant extracts of *Clitoria ternatea.* Research in Pharmacy (2012), 2 (4): 10-21.
35. B. Lansdown, I. Silver, Its antibacterial properties and mechanism of action, J. Wound Care, 11 (2000), 125-130.
36. R. W. Raut, N. S. Kolekar, J.R. Lakkakula, V. D. Mendhulkar and S. B. Kasid, Extracellular synthesis of silver nanoparticle using dried leaves of *Pongamia pinnata* (L) Pierre, Nano-Micro Letter, 2 (2010) 106-113.