Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(9):340-346

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Green synthesis of silver nanoparticles and their antimicrobial property of endophytic fungi isolated from *Mentha arvensis* L. and *Psidium guajava* L

Senthamarai Manogaran, Navaneetha Krishnan, Vinitha Sree Senthilkumar, Sowmiya Manivannan, Vijayalekshmi Veerababhu and Kilavan Packiam Kannan

Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Erode District, Tamil Nadu, India

---

ABSTRACT

Nanotechnology is the emerging modern research gained more attention in the field of Biotechnology. In the present research, total of six endophytic fungi such as Aspergillus fumigatus, Cladosporium cladosporioides, Colletrotrichum Sp, Fusarium oxysporum, and Phomopsis Sp were isolated from the healthy leaves and stem of Mentha arvensis L.and Psidium guajava L. and screened for the production of silver nano particles. The presence of silver nano particles formation was confirmed by the change of color in the fungal extracts and further confirmed by UV-Vis spectroscopy. Furthermore the antimicrobial activity of the silver nano particles was studied by disc diffusion method against Escherichia coli, Salmonella typhi, Klebsiella pneumonia and Staphylococcus aureus. This result will pay the way to use these silver nanoparticles for the treatment of fungal infections.

Key words: Silver nano particles, Endophytic fungi, *Mentha arvensis* L., *Psidium guajava* L, Antimicrobial activity

---

INTRODUCTION

Nanotechnology is a multidisciplinary field that encompasses the fusion of Physics, Chemistry and biology for the synthesis of nano particles at nanometer scale. Nano particles to its high surface to volume ratio enlighten the field of medicine in traversing the biological barriers. In the recent research the synthesis of silver nano particles has been the focus of intense because of their promising applications in a number of areas such as bio imaging, biosensors, bio labels, biomedicines and drug carriers for targeted delivery [1,2,3,4]. The biosynthesis of Nano scale materials using the microorganisms and plant materials have emerged as an alternative for the chemical synthesis. Medicinal plants are rich sources of endophytic fungi which are the precursor for the production of silver nano particles.

The use of microbial cells for the synthesis of nano sized materials has recently emerged as a novel approach for the synthesis of silver nanoparticles [5]. These methods provide firmly controlled, highly reproducible synthesis and water-soluble, biocompatible particles. In the present scenario pharmaceutical and biomedical sector was facing numerous problems due to the challenge of continuous increase in the emerging pathogens, with their antibiotic resistance profiles, with fear about the emergence and re-emergence of multi-drug resistant pathogens and parasites [6]. Due to these challenges, there was a need for an alternative approach to explore for new bioactive compounds to meet the high demand of nano particles. There are many reports on the microbial production of nano particles either intracellular or extracellular and thus given insights to utilize endophytic fungi for the production of silver nano particles [7].

Since very few reports are available for the synthesis of silver nano particles using endophytic fungi, the effort was taken to explore the research in this field. The reduction of the Ag^{+}ions occurs due to reductases released by the fungi into the solution; explore novel fungal strain for synthesizing AgNPs based on the biodiversity [8, 9]. Moreover, it could also facilitate the deeper understanding of molecular mechanisms for AgNPs biosynthesis. Hence, in the present research, was undertaken with the objectives of to isolate the endophytic fungi from the tissues of *Mentha arvensis* L. and *Psidium guajava* L. and to screen and synthesis the silver nano particles from the selected endophytic fungi.

EXPERIMENTAL SECTION

Isolation of endophytic fungi

The healthy leaves and stem of *Mentha arvensis* L and *Psidium guajava* L were collected from Bannari Amman Institute of Technology, Staff Quarters, and Sathyamangalam and washed with sterile distilled water and air dried before they are being processed. Leaf and stem parts of each sample was segmented to 1cm (approximately) from the middle portion and was surface sterilized using modified protocols [10].The segments were dipped in 70% ethanol for 5seconds, immersed in 4%sodium hypo chloride for 90 seconds and washed with sterile distilled water for 10 seconds. 50 segments of petiole from the plants were placed in PDA medium amended with ampicillin of 150mg on Petri dishes and were sealed using paraffin [11]. The Petri dishes were incubated at room temperature for a period of four weeks. The fungi which grew out from the tissues were sub cultured to fresh PDA slants.

Identification of endophytic fungi

The culture was stained with lacto phenol cotton blue staining technique. Lactophenol cotton blue contains 50 mg of cotton blue, 25 g of phenol, 25g of lactic acid, 50g of glycerol (pure) and 100 ml of distilled water. Lacto phenol was prepared by warming phenol until it gets dissolved, then lactic acid and glycerol was added to it. This was then made up to 100 ml and then cotton blue was added. After the preparation of lacto phenol cotton blue, slide preparation was done.

A drop of lacto phenol cotton blue solution was placed on a slide followed by smearing of fungal culture over it. The coverslip was placed over it. The slide with the fungal culture was heat fixed by passing it over a flame and viewed under microscope. Sporulating isolates were isolated with the help of standard manual [12, 13]. Further confirmation of the fungal species was made after observation under a light microscope and pictures were taken using a Nikon digital camera.

Biosynthesis of silver nanoparticles

The identified fungi were grown in 100ml of Potato Dextrose Broth for 5 days at 120rpm. After 72 hours the cell mass was collected using a muslin cloth and washed with double distilled water in order to remove the medium components. Medium free biomass were transferred to 100ml conical flask containing distilled water and then incubated at room temperature for 150 rpm for 24 hours. This was followed by filtration using Whatman Filter Paper No 1 and 1mM silver nitrate was added to the filtrate in the ratio of 1:1 and kept in a shaker (Figure 1) for 160rpm it changes to dark brown colour [14].

Characterization of silver nanoparticles

UV- Vis Spectrophotometric analysis

The reduction of silver nitrate was observed visually by the colour change as well as by UV-Vis Spectrophotometric measurement was recorded from the wavelength range of 200nm-700nm and absorbance was plotted on a graph by graph pad prism 5.0 software [15, 16].

Harvesting of nanoparticles

To remove, the micro particles, the sample mixture was centrifuged at 2000rpm for 5min for the reduced filtrate containing silver nanoparticles. The supernatant was transferred to a fresh centrifuge tube and again centrifuged at 12000 rpm for 10min for the separation of nano particles.

Antimicrobial Properties of synthesized nanoparticles

The antimicrobial property of the harvested nanoparticles from *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Colletrotrichum* Sp, *Fusarium oxysporum*, and *Phomopsis* Sp and were tested against *Escherichia*

coli, *Salmonella typhi*, *Klebsiella pneumonia* and *Staphylococcus aureus* using the standardized dilution method. The zone of inhibition was measured and the values were recorded [17, 18, 19, 20].

RESULTS AND DISCUSSION

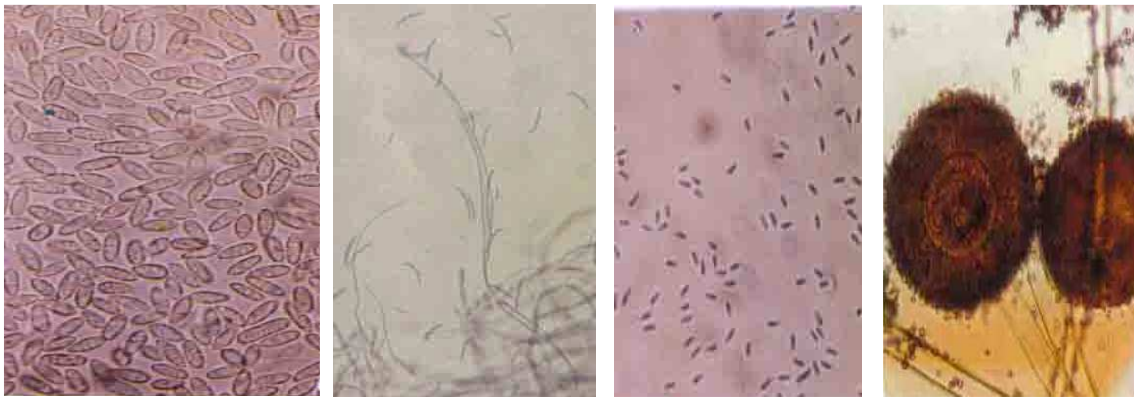
The isolated fungal colonies were stained by Lacto Phenol cotton blue and identified by morphological, microscopic and through referring standard manuals. List of endophytic fungi identified from *Mentha arvensis* L. and *Psidium guajava* L (Figure 1), were *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Colletrotrichum* Sp, *Fusarium oxysporum*, and *Phomopsis* Sp (Figure 2, 3).

***Mentha arvensis* L.**

***Psidium guajava* L.**

Figure 1. Habitat of Medicinal Plants

Figure 2.Endophytic fungal propagules from *Mentha arvensis*L .and *Psidium guajava* L.



*Colletrotrichum*Sp. *Fusarium oxysporum* *Phomopsis*Sp. *Aspergillus niger*

Colletrotrichumfalcatum *Cladosporium cladosporoides* *Aspergillus fumigatus*

Figure 3. List of endophytic fungi identified from *Mentha arvensis* L. and *Psidium gujava* L

From the list of identified fungi, *Aspergillus fumigatus*, *Cladosporium cladosporoides*, *Colletrotrichum* Sp, *Fusarium oxysporum*, and *Phomopsis* Sp, were screened for the synthesis of silver nanoparticle. The colour change to dark brown indicates the presence of silver nanoparticles and further centrifugation at 12,000 rpm for 10 min provided the silver nanoparticle as a pellet. All the fungal extracellular metabolites showed the reduction of $AgNO_3$ to silver nanoparticles, which was determined by changes in colour of the interaction mixture due to the reducing agent present in the extracellular metabolites and the role of enzymes present in the filtrate (Figure 4).

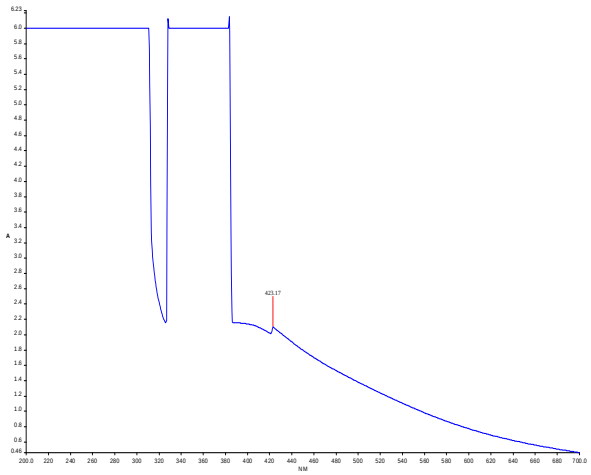
Reducing Ag^+ ion to Ag° using fungi as reducing agent

$$AgNO_3 \longrightarrow Ag^+ + NO_3^-$$

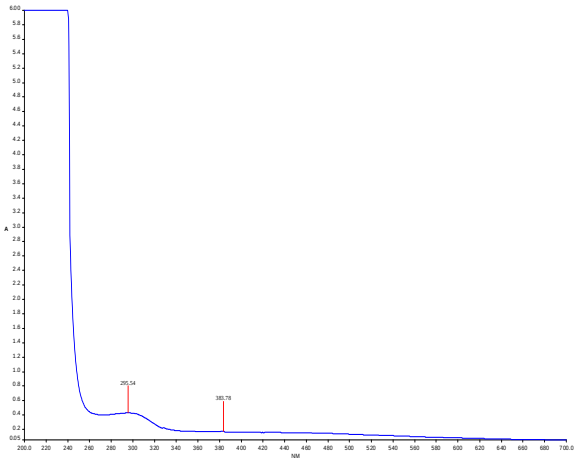
$$Ag^+ + e^- \longrightarrow Ag^\circ$$

Figure 4 . 1. Reducing Ag^+ ion to Ag° using fungi as reducing agent *Aspergillus fumigatus*, 2. *Phomopsis* Sp, 3.*Fusarium oxysporum, 4.Cladosporium, cladosporoides, 5.Colletrotrichum* Sp

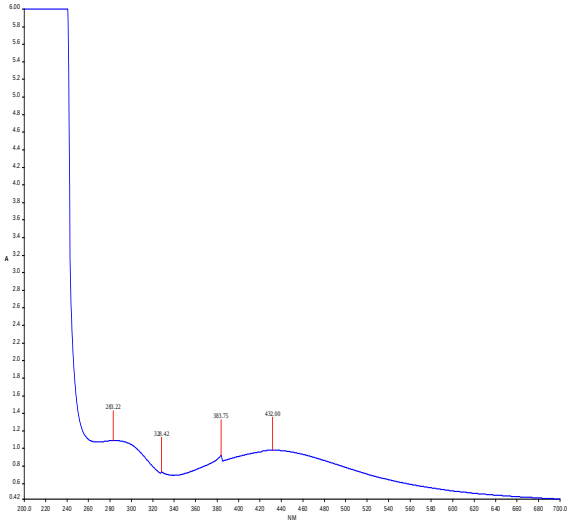
The synthesized silver nanoparticles were purified by adding methanol and further characterized by UV spectrometry. The UV-Vis spectra were recorded at 450 nm and absorbance was plotted on a graph by graph pad prism 5.0 software (Figure. 5).



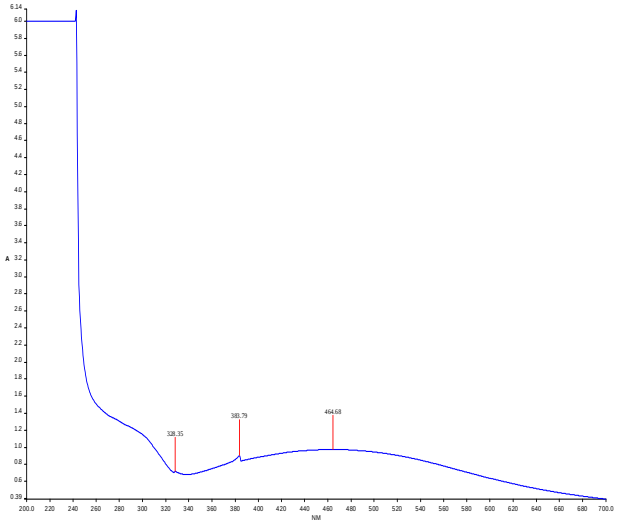
***Phomopsis* Sp**



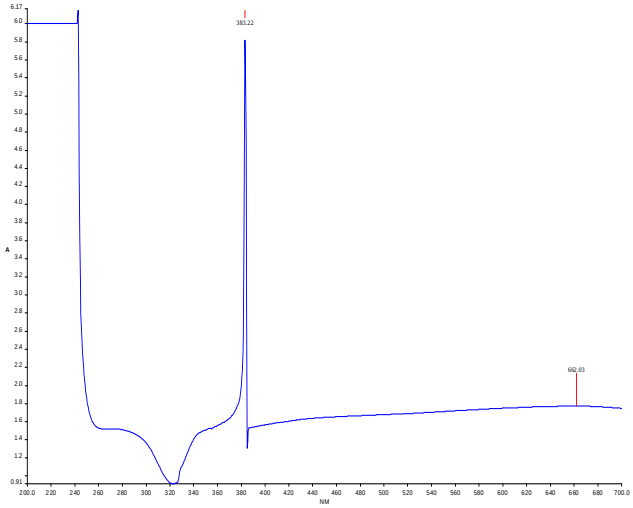
Cladosporium cladosporoides



Fusarium oxysporum



Aspergillus fumigatus



***Colletotrichum* Sp**

Figure 5. UV-Vis spectra of fungal synthesized nanoparticles at 450 nm

The antimicrobial properties of the nanoparticles were tested against *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia* and *Staphylococcus aureus.* and the zone of inhibition was listed (Table 1).

Table 1. Zone of inhibition of fungal nanoparticles

S. No	Nano particles synthesized endophytic fungi	Zone of inhibition (mm)			
		Escherichia coli	***Salmonella typhi***	***Klebsiella pneumonia***	***Staphylococcus aureus***
1	*Phomopsis* Sp.	5.3	4.7	6.4	5.0
2	*Cladosporium cladosporioides*	4	5.2	4.3	4.3
3	*Fusarium oxysporum*	6.1	5.9	7.1	5.4
4	*Aspergillus fumigatus*	6.3	6.0	5.5	4.1
5	*Colletrotrichum* Sp.	4.3	2.6	2.5	4.5

The synthesis of silver nanoparticles particle from the endophytes of *Mentha arvensis* and *Psidium guajava* were performed. The endophytes such as *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Colletrotrichum* Sp, *Fusarium oxysporum*, and *Phomopsis* Sp, were characterized and the spectra were recorded by UV- Vis spectrophotometer. This method of synthesizing nanoparticle from endophytic fungi is highly useful in developing medicines for fungal infections and an alternative approach to explore for new bioactive compounds to meet the high demand of nanoparticles [21, 22].

CONCLUSION

Hence this study reveals that the synthesis of silver nanoparticles from endophytic fungi can also be performed and the cost of the resources used will also be minimized compared to already existing work. This paves the way to synthesize and scale up the silver nanoparticles, which are having several applications in biotechnology

Acknowledgements
The authors are grateful to Chairman, Director, Chief executive and Principal of Bannari Amman Institute of Technology, Sathyamangalam for providing the necessary facilities.

REFERENCES

[1] M Han; X Gao; JZ Su; S Nie., *Nat. Bio. technol*., **2001**, 19, 631–635.
[2] X Huang; IH El-Sayed; W Qian; MA El-Sayed. *J.Am. Chem. Soc*., **2006**, 128, 2115–2120.
[3] CA Mirkin; RL Letsinger; RC Mucic; JJ Storhoff. *Nature.*, **1996**, 382, 607-609.
[4] AK Salem; PC Searson, KW Leong. *Nat.Mater*., 2003, 2, 668–671.
[5] E Boisselier; D Astruc . *Chem. Soc. Rev.*, **2009**, .38, 1759-1782.
[6] FC Tenover. Am. *J.Medicine*., **2006**, 119, 119 3-10.
[7] M Kalaiselvam. *International Journal of Pharmaceutical & Biological Archives*, **2013**, 4(3), 481 – 487.
[8] JHP Watson; DC Ellwood; AK Soper; J Chamock, *Magn Mater* **1999**, 203, 69-72.
[9] M Schuller; M Laude; J Bodewaldt, H Hamm, HC Korting. *Skin Pharm. Physiology*, **2004,** 17, 31.
[10] JK Dobranic; JA Johnson; QR Alikhan. *Can J Microbiol*., **1995**, 4, 194-198.
[11] PJ Fisher; O Petrini; LE Petrini; BC Sutton. *New Phytologist*, **1994**, 127(1). 133-137.
[12] EE Guba; RE Young; T Ui. *Plant Dis.Rptr*, 1961, 45, 102-105.
[13] J Frohlich; K.D Hyde; O Petrini. *Mycological Research*, 2000, 104, 1202-1212.
[14] CFAlappat; KP Kannan; NS Vasanthi. *Engineering Science and Technology: An International Journal*, **2012**, 2 (3), 2250-3498.
[15] K Kalimuthu; R Suresh Babu; D Venkataraman; M Bilal; S Gurunathan, *Colloids and Surfaces B: Biointerfaces* **2008**, 65, 150-153.
[16] M Kowshik; SH Ashtaputre; SHKharazi. *Nanotechnology*. **2003**, 14, 95-100.
[17].MN Devi; SP Dheepan; S Sutha. *Int.l J of Biomed. Adv. Res..*, **2012,** 3(5), 409-415
[18] FCA Tenover. *J.Medicine*. **2006**, 119, 3-10.
[19] RW Glomm. *J Dispersion Sci.Technology*, 2005, 26, 389-314.
[20] O Petrini. *In: Microbial ecology of leaves*, edited by JH Andrews; SS Hiran. *Springer Verlag*, New York, USA **1991**.
[21] W Suthep; S Nongluksna; P Wattana; T Nuntawan; D. Kannawat; R. Nijsiri; M. Vithaya. *World Journal of Microbiology and Biotechnology*, **2004,** 20, 265-272

[22] SM Fathima; RM Balakrishnan. *Journal of Medical and Bioengineering*. **2014**, 3, 162-166