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BIOGENESIS OF SILVER NANOPARTICLES BY *PENICILLIUM* SP. OF *CALOPHYLLUM APETALUM* WILLD. AND THEIR CHARACTERIZATION.

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ABSTRACT

The present work managed the combination of silver nanoparticles by the concentrate of *Penicillium sp.* disconnected from *Calophyllum apetalum*. Blended silver nanoparticles have been described by UV-vis spectroscope, scaning electron microscope instrument and XRD. Surface plasmon reverberation spectra of silver nanoparticles were acquired at 420nm and SEM thinks about uncovered strength of round shape particles. X-ray diffraction contemplates affirm that the nanoparticles orchestrated by endophytic concentrate were crystalline silver. This strategy is one of the basic, proficient and quick strategies to combine silver nanoparticles at surrounding temperature without utilization of lethal chemicals.

KEYWORDS: *Penicillium*, *Calophyllum apetalum*, silver nanoparticles, UV-vis, SEM, XRD.

1. INTRODUCTION

Presently the nanotechnology is one of the essential exploration regions which includes combination and advancement of nanoparticles by different strategies and having an

assortment of utilizations (Vardhana and Kathiravan 2015; Swetha and Valli Nachiyar 2012; Swetha and Valli Nachiyar 2012). Endophyte can be characterized as a microorganism (microscopic organisms and parasite) dwelling in the tissues of plant and can be created a portion of the imperative metabolites (Muna and Josphat 2014; Rekha *et al.*, 2013). Silver Nanoparticles are the most widely recognized nanoparticles which can be utilized as antimicrobial, cancer prevention agent operators to cure different infections and utilized as a part of catalysis (Mohamed *et al.*, 2014), lable-free colorimetric test (Wei *et al.*, 2008), photocatalysts (Jiangtian *et al.*, 2013), medicinal diagnostics (El-Sayed *et al.*, 2005; Elechiguerra *et al.*, 2005), sun oriented vitality (Moulin *et al.*,2008), cytotoxic (Safaepour *et al.*, 2009) impacts. Microorganisms, chemicals and plants have been recommended as organic and eco-accommodating course of union of nanoparticles (Li *et al.*, 2010; Konishi *et al.*, 2007; Willner *et al.*, 2006). There are some reports on biosynthesis of silver nanoparticles utilizing endophytes *Alternaria alternata*, *Aspergillus clavatus*, *Fusarium acuminatum* and *Penicillium* sp have demonstrated the effectiveness of the endophytic parasites to orchestrate silver nanoparticles having antimicrobial movement (Philip *et al.*, 2011; Gajbhiye *et al.*, 2009; Verma *et al.*, 2010; Ingle *et al.*, 2008; Singh *et al.*, 2014).

In the present study, an endeavor has been made to explore endophytic organisms *Penicillium* sp confined from leaf of an ethnomedicinal plant *Calophyllum apetalum* for their capacity to combine silver nanoparticles and the portrayal of the biosynthesized nanoparticles were done utilizing UV-vis spectroscope, scanning electron microscope instrument and XRD.

2. MATERIALS AND METHODS

2.1. Identification and collection of *Calophyllum apetalum*

The plant *Calophyllum apetalum* was gathered in the month of January, 2015 from the Agumbe ghats, Udupi area, Karnataka, India and was recognized and validated by Dr S G K Bhat, Taxonomist, Udupi District, Karnataka. Naturally gathered leaf materials were washed altogether under running faucet water took after by sterile refined water to evacuate the followed flotsam and jetsam. Leaf was subjected for surface cleansing under aseptic condition in consecutive strides by inundating in mercuric chloride ($1mgml^{-1}$) for 10min and 70% ethanol for one more moment took after by washing at long last with refined water.

2.2. Inoculation of implants (*Calophyllum apetalum*)

After progressive surface cleansing of leaf of *Calophyllum apetalum*, it was cut into little cushions ($0.5 \times 0.5\ cm^2$) and set 5–6 pieces on hardened sterile potato dextrose agar (PDA)

media. The inoculated plant inserts were brooded till the development of discernable parasitic endophytes had been watched.

2.3. Identification of endophytes

Settlements rising out of the surface cleaned leaf, endophyte *Penicillium* sp. were chosen in light of the morphological attributes, colony development, hyphae and conidia (Ellis 1971; Barnett and Hunter 1972).

2.4. Isolation and mass culture of *Penicillium* sp.

The *Penicillium* sp. were sub cultured on PDA plates and mass refined in cone shaped flagons containing Potato dextrose broth (PDB). Following 15–20 days of incubation, the parasitic mycelia mat was gathered.

2.5. Preparation of endophytic concentrate

Fluid concentrate of *Penicillium* sp. was set up by pounding the mycelia mat with twofold refined water and sifted through the Whatman filter paper no 1. The filtrate was centrifuged at 6000 rpm for 10 min and the supernatant was gathered for further investigations.

2.6. Synthesis of silver nanoparticles

10 ml of concentrated *Penicillium* sp. concentrate was added to 25 ml of freshly prepared 5 mM silver nitrate contained in an Erlenmeyer jar and kept it in an orbital shaker under dim conditions for brooding for 24 h at 37 °C. The concentrate alone (without silver nitrate) and immaculate silver nitrate arrangement (without concentrate) were utilized as positive and negative controls, separately. A silver nitrate treated sample was centrifuged at 10,000rpm for 10min. Supernatant was disposed of and the pellate was washed thrice with deionized water to evacuate unreacted $AgNO_3$ and endophytic extract. The immaculate pellate was gathered, air dried and safeguarded for further portrayal.

2.7. Characterization of silver nanoparticles

An aliquot of this pellate containing silver nanoparticles was utilized for UV–Vis spectroscopic studies (Shimadzu organization model-UV3600) and SEM (Ultra 55 Model-II, Carl Zeiss SEM machine). For XRD considers, dried silver nanoparticles were covered on a XRD framework and the spectra were recorded by utilizing a Rigaku diffractometer at a voltage of 40keV and a current of 30mA with Cu-Kα radiation with a wavelength of 1.5418 Å.

3. RESULTS

The concentrate of *Penicillium* sp. was arranged and utilized as a diminishing operator for the syntheisi of AgNPs (figure 1).

Fig 1. (A) Emerging endophytes from leaf of *Calophyllum apetalum*, (B) mass culture of *Penicillium* species.

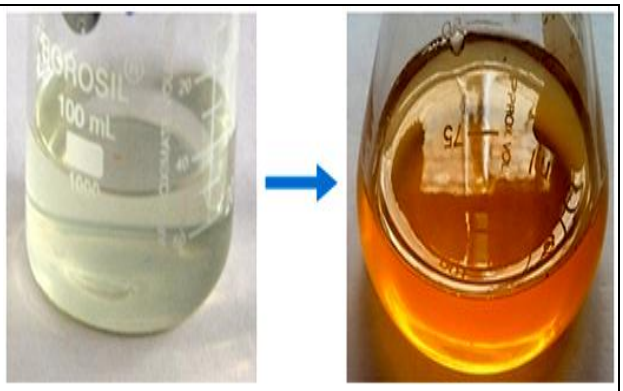

Fig 2. Extracts before and after treating with silver nitrate

3.1. Blend of silver nanoparticles

Biosynthesis of AgNPs was described by the shading change response from shading less endophytic concentrate to light yellow shade of $AgNO_3$ upon 24h of incubation (figure 2).

3.2. Characterization of silver nanoparticles: The solid assimilation crest at 420nm with UV–Vis spectroscopic studies affirmed the combination of AgNPs which was further affirmed by XRD and SEM concentrates on. Because of the polydispersed nanoparticles, an expansive top was watched (figure 3). The aftereffects of SEM plainly showed that cubic AgNPs of size extending from 65.41nm–102.3nm were combined.

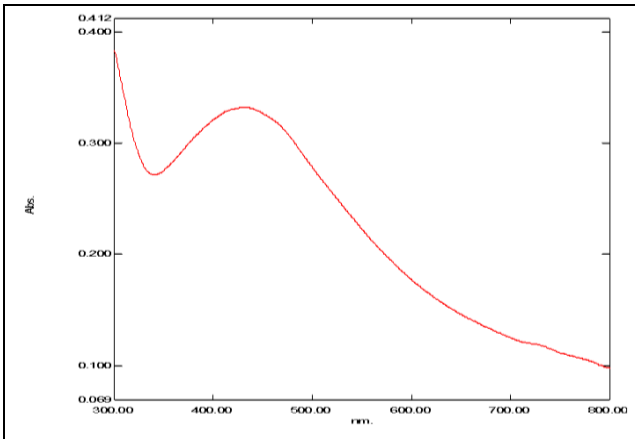

Fig 3. A strong broad peak located at 420nm of silver nanoparticles synthesized by *Penicillium* sp extract isolated from leaf of *Calophyllum apetalum* .

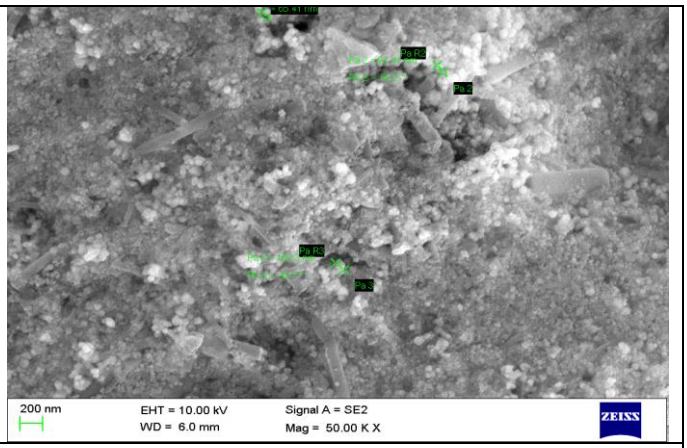

Fig 4. SEM images of silver nanoparticles synthesized by the extract of *Penicillium* sp isolated from leaf of *Calophyllum apetalum*

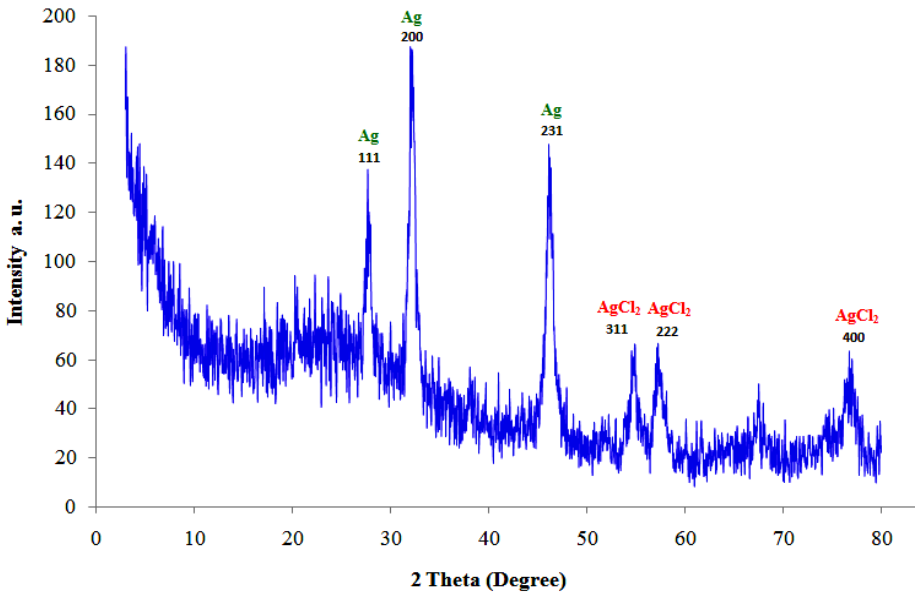

Fig 5. XRD patterns of silver nanoparticles synthesized by the extract of *Penicillium* sp. isolated from leaf of *Calophyllum apetalum*.

The consequences of XRD studies were paramount in affirming the biosynthesis of silver nanoparticles (AgNPs) by the concentrate of *Penicillium* sp. separated from leaf of *Calophyllum apetalum*. Cubic crystalline silver nanoparticles were created by the bioreductive procedure. The particular pattern of XRD tops at 2θ diffraction has given six confirmative tops. Grid planes of face focused cubic (fcc) precious crystal at 2θ qualities were filed with separate degrees, 27.726° (111), 32.0983° (200), 46.1538° (231), 54.8034° (311), 57.3419° (222) and 76.6154° (400) and contrasted with the information of JCPDS (Joint Committee on Powder Diffraction Standards, record No. 87-0720). The normal molecule sizes were ascertained by Debye–Scherrer equation (Klug and Alexander 1959) (figure 5).

4. DISCUSSION

Silver nanoparticles blended by the concentrate of *Penicillium* sp. confined from leaf of *Calophyllum apetalum* have portrayed by UV–Vis spectroscopy, SEM and XRD ponders and which have been given noteworthy data. A solid top at 420nm compares to the surface Plasmon reverberation of silver nanoparticles has been seen in UV Spectra. Prior it is as of now reported that absorbance at around 430nm for silver is an element of the honorable metal particles (Logeswari *et al.*, 2013). The silver nanoparticles are of 65.41nm–102.3nm quantifiable extent and round fit as a fiddle. The SEM picture of silver nanoparticles was because of cooperations of hydrogen bond and electrostatic associations between the

bioorganic topping atoms bound to the silver nanoparticles (Preetha *et al.*, 2013). The aftereffects of UV, SEM and XRD of silver nanoparticles orchestrated by the concentrate of *Penicillium* sp. secluded from leaf of *Calophyllum apetalum* were contrasted and affirmed with late works did by different creators in the region of nanoscience (Paulkumar *et al.*, 2013; Stalin *et al.*, 2014).

Pt, Zr, Ag, Au, Cd, Pb and Ti nanoparticles were combined by *Fusarium oxysporum* (Bansal *et al.*, 2004; Sanyal *et al.*, 2005; Bansal *et al.*, 2005; Duran *et al.*, 2005) and silver nanoparticles by *Penicillium* sp. secluded from soil (Sadowski *et al.*, 2008; Hemath *et al.*, 2010). Auxiliary metabolites of plants and fungi, for example, naphthoquinones (Medentsev and Alimenko 1998; Duran *et al.*, 2002; Bell *et al.*, 2003) and anthroquinones (Baker and Tatum 1998; Misko *et al.*, 1993; Kumar and McLendon 1997) were observed to be required in diminishment of metals.

5. CONCLUSION

Blend of silver nanoparticles by the concentrate of *Penicillium* sp. segregated from *Calophyllum apetalum* is one of the biogenic techniques. The consequences of UV, SEM and XRD studies were bolstered the present work. Improvement of a viable media for mass development of the endophyte may be required. We could likewise build up the strategy to control the hereditary data for modern scale generation. Without a doubt, it can be guaranteed that the combination of silver nanoparticles by endophytes is a danger free and eco-accommodating strategy.

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REFERENCES

1. Vardhana, J and Kathiravan G. Biosynthesis of Silver Nanoparticles by Endophytic Fungi *Pestaloptiopsis pauciseta* Isolated From the Leaves of *Psidium guajava* Linn., Int. J. Pharm. Sci. Rev. Res., 2015; 31(1): 29-31.

2. Swetha, S and Valli Nachiyar C. Biogenesis of antibacterial silver nanoparticles using the endophytic bacterium *Bacillus cereus* isolated from *Garcinia xanthochymus*., Asian Pacific Journal of Tropical Biomedicine., 2012; 2(12): 953-959.
3. Swetha, S and Valli Nachiyar C. Microbial Synthesis and Characterization of Silver Nanoparticles Using the Endophytic Bacterium *Bacillus Cereus*: A Novel Source in the Benign Synthesis., Global journal of medical research., 2012; 12(2): 8-9.
4. Muna, A. A and Josphat C.M. Endophytes as Producers of Peptides: An Overview about the Recently Discovered Peptides from Endophytic Microbes., Nat. Prod. Bioprospect., 2014; 4: 257–270.
5. Rekha., Kumari Jyoti., Manju Bala., Vedpriya A. "Endophytic fungus: a potential source of biologically synthesized nanoparticle" Basic Research Journal of Microbiology. 2013; 1-1: 01-07.
6. Mohamed, S., Abdel, A., Mohamed, S., Shaheen., Aziza A. El-N and Mosaad A. A.W. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using Chenopodium murale leaf extract., Journal of Saudi Chemical Society., 2014; 18: 356–363.
7. Wei, H., Chen, C., Han, B and Wang E. Enzyme colorimetric assay using unmodified silver nanoparticles., Anal. Chem., 2008; 80: 7051–7055.
8. Jiangtian, L., Scott, K.C., Joeseph, B., Fanke, M., Tess, R.S., Peng, Z., Alan, D.B and Nianqiang W. Ag@Cu2Ocore–shell nanoparticles as visible-light plasmonic photocatalysts., ACS Catal., 2013; 3: 47–51.
9. El-Sayed, I.H., Huang, X and El-Sayed M.A. Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer., Nano Lett., 2005; 5: 829–834.
10. Elechiguerra, J.L., Burt, J.L., Morones, J.R., Camacho-Bragado, A., Gao, X., Lara, H.H and Yacaman M.J. Interaction of silver nanoparticles with HIV-1., J. Nanobiotechnology., 2005; 3-6.
11. Moulin, E., Sukmanowski, J., Schulte, M., Royer, F.X and Stiebig H. Thin-film silicon solar cells with integrated silver nanoparticles., Thin Solid Films., 2008; 516: 6813–6817.
12. Safaepour, M., Shahverdi, A.R., Shahverdi, H.R., Khorramizadeh, M.R and Gohari A.R. "Green synthesis of small silver nanoparticles using geraniol and its cytotoxicity against fibrosarcoma-Wehi 164., Avicenna J. Med. Biotechnol., 2009; 1(2): 111–115.

13. Li, W.R., Xie, X.B., Shi, Q.S., Zeng, H.Y., Ou-Yang, Y.S and Chen Y.B. "Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*., Appl Microbiol Biotechnol., 2010; 85 (4): 1115–1122.
14. Konishi, Y., Ohno, K., Saitoh, N., Nomura,T., Nagamine, S., Hishida, H., Takahashi, Y and Uruga T.. Bioreductive deposition of platinum nanoparticles on the bacterium Shewanella algae., J Biotechnol., 2007; 128(3): 648–653.
15. Willner, I., Baron, R and Willner B. Growing metal nanoparticles by enzymes., Adv Mater., 2006; 18(9): 1109–1120.
16. Philip, D., Unni, C., Aromal, S and Vidhu V.K. Murraya Koenigii leaf-assisted rapid green synthesis of silver and gold nanoparticles., Spectrochim Acta A., 2011; 78 (2): 899–904.
17. Gajbhiye, M., Kesharwani, J., Ingle, A., Gade, A and Rai M. Fungus mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole., Nanomedicine., 2009; 5(4): 382-386.
18. Verma, V.C., Kharwar, R.N and Gange A.C. Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*., Nanomedicine., 2010; 5(1): 33-40.
19. Ingle, A., Gade, A., Pierrat, S., Sonnichsen, C and Rai M. Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria., Curr Nanosci., 2008; 4: 141-144.
20. Singh, D., Rathod, V., Ninganagouda, S., Hiremath, J., Singh, A.K and Mathew J. Optimization and characterization of silver nanoparticle by endophytic fungi *Penicillium* sp. isolated from *Curcuma longa* (turmeric) and application studies against MDR *E. coli* and *S. aureus*., Bioinorg Chem Appl., 2014; 1-8.
21. Ellis, M. B. Dematiaceous Hypomycetes (England: Kew Surrey)., 1971.
22. Barnett, H.L and Hunter B.B. Illustrated Genera of Imperfect Fungi., 2nd ed (Minnesota: Burgess Publishing Company). 1972.
23. Klug, H.P and Alexander L. E. X-ray Diffraction Procedures (New York: Wiley)., 1959.
24. Logeswari, P., Silambarasan, S and Abraham J. Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties., Scientia Iranica Transactions F: Nanotechnology., 2013; 20(3): 1049–1054.
25. Preetha, D., Prachi, K., Chirom, A and Arun R. Synthesis and characterization of silver nanoparticles using Cannonball leaves and their cytotoxic activity against MCF-7 cell line., Journal of Nanotechnology., 2013; 2013: 1-5.

26. Paulkumar, K., Rajeshkumar, S., Gnanajobitha, G., Vanaja, M., Malarkodi. C and Annadurai G. Biosynthesis of silver chloride nanoparticles using *Bacillus subtilis* MTCC 3053 and assessment of its antifungal activity., ISRN Nanomaterials., 2013; 2013: 1-8.
27. Stalin D, T., Ganesh, K.V., Karthick, V., Jini, A.K and Govindaraju K. Facile synthesis of silver chloride nanoparticles using marine alga and its antibacterial efficacy., Spectrochim. Acta. A: Mol. Biomol. Spectr., 2014; 120: 416-420.
28. Bansal,V., Rautaray, D., Ahmad, A and Sastry M. Biosynthesis of zirconia nanoparticles using the fungus *Fusarium oxysporum*., J. Mater.Chem., 2004; 14: 3303-3305.
29. Sanyal, A., Rautaray, D., Bansal, V., Ahmad, A and Sastry M. Heavy-metal remediation by a fungus as a means of production of lead and cadmium carbonate crystals., Langmuir., 2005; 21(16) : 7220-7224.
30. Bansal, V., Rautaray, D., Bharde, A., Ahire, K., Sanyal, A and Ahmad A. Room-temperature biosynthesis of ferroelectric barium titanate nanoparticles., J. Mater. Chem., 2005; 15(26): 2583-2589.
31. Duran, N., Priscyla, M. D, Oswaldo, A.L., Gabriel De Souza, I.H and Esposito E. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains., J. Nanobiotechnol., 2005; 3(8): 1-7.
32. Sadowski, Z., Maliszewska, I.H., Grochowalska, B., Polowczyk, I and Kozlecki T. Synthesis of silver nanoparticles using microorganisms., Materials Science-Poland., 2008; 26(2): 419-424.
33. Hemath, N.K.S., Kumar, G., Karthik, L., Bhaskara R.K.V. Extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Penicillium* sp., Arch. Appl. Sci. Res., 2010; 2(6): 161- 167.
34. Medentsev, A.G and Alimenko V.K. Naphthoquinone metabolites of the fungi., Photochemistry., 1998; 47(6): 935-959.
35. Duran, N., Teixeira, M.F.S., De Conti, R and Esposito E. Ecological-friendly pigments from fungi., Crit. Rev. Food Sci. Nutr., 2002; 42(1): 53-66.
36. Bell, A.A., Wheeler, M.H., Liu, J., Stipanovic, R.D., Puckhaber, L. S and Orta H. United States Department of Agriculture-Agricultural Research Service studies on polyketide toxins of *Fusarium oxysporum* f sp vasinfectum: potential targets for disease control., Pest. Manag. Sci., 2003; 59(6-7): 736-747.
37. Baker, R.A, and Tatum J.H. Novel anthraquinones from stationary cultures of *Fusarium oxysporum*., J. Ferment. Bioeng., 1998; 85(4): 359-361.

38. Misko, T.P., Schilling, R.J., Salvemini, D., Moore, W.M and Currie M.G. A fluorometric assay for the measurement of nitrite in biological samples., Anal. Biochem., 1993; 214(1): 11-16.
39. Kumar, C.V and McLendon G.L. Nanoencapsulation of cytochrome c and horseradish peroxidase at the galleries of α-zirconium phosphate., Chem. Mater., 1997; 9(3): 863-870.