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Green synthesis of silver nanoparticles from Endophytic fungus *Aspergillus niger* isolated from *Simarouba glauca* leaf and its Antibacterial and Antioxidant activity

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ABSTRACT
The field of nanotechnology is the most promising area of the research. In our present study we report the biological method of synthesis of silver nanoparticles by endophytic extracts isolated from the leaf of *Simarouba glauca*. The surface Plasmon resonance characteristic of silver nanoparticles was revealed by the UV-Vis spectrum at 400 nm. The crystalline nature of silver nanoparticle was confirmed by X ray diffraction studies. Spherical shaped and monodispersed nanoparticles were found in Scanning electron micrograph. The average size of silver nanoparticles was 41.9 nm as determined by dynamic light scattering. The peak in silver region confirming the presence of elemental silver was determined by Energy dispersive X-ray spectroscopy analysis. Antibacterial activity of silver nanoparticles utilized in this study was found to be more significant than standard Taxim antibiotic against multidrug resistant bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumonia*. Endophytic based silver nanoaprticles were found to possess significant antioxidant activity.

***Keywords*:** silver nanoparticles, endophyte, multidrug, Taxim, Antioxidant.

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I. INTRODUCTION

Nanotechnology has acquired colossal propulsion in harmonizing metals into nanosized, shapes and controlled disparity owing to their prospective use for human benefits [1]. Due to cost effective and environment friendly nature, biological method of nanoparticles synthesis takes advantage over physical and chemical synthesis [2, 3]. For the synthesis of nanoparticles, many micro organisms such as bacteria (4), fungi (5), yeasts (6), plant extracts (7), and biological particles (8) have been exploited. As the fungi can secrete large amount of enzymes, in the synthesis of metal nanoparticles they are preferred as ideal candidates [9]. Silver is an effective antimicrobial agent with diverse *in vitro* and *in vivo* applications with greatest advantage to humans [10]. Currently topical dressing with silver based is widely used in treating chronic ulcers and open wounds [11]. Silver nanoparticles have found potential application in many fields such as, antibacterial, drug delivery, biological sensors, textiles and filters [12, 13]. *Simarouba glauca* is one of the important traditional medicinal plants due to the presence alkaloids, flavonoids, carbohydrates, glycosides, a phenolic compound, tannins, terpenoids, cardenolides, saponins, fixed oils which can usually account for their therapeutic action including Antibacterial, antiviral, anti-inflammatory, antiprotozoal and antitumor activities [14]. But never synthesized and characterized silver nanoparticles by the extracts of *Simarouba glauca.* Therefore, In the present article we emphasizes on the synthesis of silver nanoparticles from the endophytes contained in *Simarouba glauca* leaf, as these endophytes harbour inter or intra cellularly with the similar characters and also using these endophytes helps in maintaining ecological balance as if the plants are used in bulk synthesis of silver nanoparticles there is the risk of endangering of the particular plant thus entering into endophytic synthesis of nanoparticles can overcome this problem. In this study we prioritize on antimitotic assay which is not yet reported for the

silver nanoparticles synthesized from endophytic extracts.

II. MATERIALS AND METHODS

Isolation of endophytic fungi

Healthy leaves of *Simarouba glauca* were collected from Shridevi Institute of Engineering and Technology campus in month of April 2016. They were surface cleaned with running tap water to remove contaminants and incised into 0.5 mm pieces and surface sterilized using 0.01% mercuric chloride followed by double distilled water. The leaves were transferred onto PDA (Potato Dextrose Agar) medium and incubated for 7 days for the growth of endophytic fungi. Stock cultures and subcultures were maintained for future processes.

Identification of endophytic fungi

Endophytic fungus isolated from leaf of *Simarouba glauca* was identified as *Aspergillus niger* based on morphological characteristics, colony growth and hyphae (Fig-1).

Preparation of endophytic extracts

The mycelia of *Aspergillus niger* from growing edge was inoculated into 1000 mL Erlenmeyer flask containing 500 mL of Potato Dextrose broth medium and incubated for one week on a rotary shaker at 25° C. The collected culture filtrate was filtered through a Whatman filter paper followed by centrifugation at 10,000 rpm for 15 min and supernatant was collected and stored for further analysis.

Synthesis of silver nanoparticles

5 ml of endophytic extracts was treated with 45 ml of aqueous 5 mM silver nitrate in Erlenmeyer flask which was kept for incubation for a period of 24 hours at room temperature in dark condition. The endophytic extract with silver nitrate was centrifuged at 10,000 rpm for 10 min where supernatant was discarded and pellate was washed repeatedly with double distilled water to washout un reacted silver nitrate and endophytic extracts. The left over pure pellate was stored for further experiments.

Characterization of silver nanoparticles

The reduction of silver ions was primarily monitored using UV visible spectroscopy (Agilent Cary 60). The analysis was performed by continuous scanning between the wavelengths of 300 – 700 nm. The diffracted intensities were recorded for 2θ angles from 10^0 to 80^0 by XRD diffractometer Rigaku-SmartLab with monochromatized Cu-Kα radiation. FT- IR spectra were recorded with Bruker- alpha. 100 mg of dried sample was mixed with 100 mg of spectral grade KBr and pressed into discs under hydraulic pressure.The size and surface morphology was studied using scanning electron microscopy (Ultra 55 Model-II, Carl Zeiss SEM machine). EDX analysis was carried out to check the elemental composition of nanoparticles that gives the elemental knowledge of sample.

Anti- bacterial assay

The disc diffusion method was used to carried out antibacterial activity of silver nanoparticles on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia*. Mackonkey broth (HiMedia) medium was used to sub culture bacteria and was incubated at 37°C for 24h. Overnight fresh cultures were collected and spread on Mackonkey agar plates to cultivated bacteria. 5 mm sterile discs were saturated with double distilled water, Taxim (standard antibiotic), AgNPs and endophytic extracts were placed in each plate and incubated again at 37°C for 24h. Antibacterial activity was measured based on zone of inhibition around the disc impregnated with double distilled water, Taxim (standard antibiotic), AgNPs and endophytic extracts.

In vitro antioxidant activity

DPPH radical scavenging assay

To determine the scavenging ability, 1 ml of various concentrations of silver nanoparticles and ascorbic acid (standard) were prepared with 4 ml of DPPH solution separately (15). The mixture was mixed vigorously and kept in the dark for 30 min before measuring the absorbance at 517nm against blank. Then the scavenging activity was calculated using the following equation.

Scavenging effect (%) = [1-Absorbance of sample/ Absorbance of Control] × 100

Antimitotic assay

The antimitotic activity of synthesized silver nanoparticles was studied using *Allium cepa* root tips (16). *Allium cepa* root tips were treated for 48 hours with synthesized nanoparticles, quercetin (10mg/ml, 5mg/ml; quercetin – 1mg/ml) and distilled water were used. The root tips were fixed with 1:3 acetoalcohol and squash with acetocarmine stain was prepared and monitored for cellular, nucleolar, and chromosomal abnormalities. The mitotic indices were determined for each root tip manually, scoring approximately 500 cells under high resolution bright field light microscopy (100 x oil immersions) and the phases were observed. Dividing cells include prophase, metaphase, anaphase, and telophase. The cells were examined for the abnormalities like chromosomal fragments, vagrant chromosomes, chromosomal gaps, anaphase, multipolar anaphases, and telophases and stick chromosomes. The mitotic index was calculated by using the formula:

Mitotic index = [Number of dividing cell/Total number of cells] x 100%

III. RESULTS AND DISCUSSION

Isolation of endophytic fungi

The isolated endophytic fungi were identified as *Aspergillus niger* based on microbial observation, colony growth, morphological characteristics and hyphae, was identified as *Aspergillus niger* (Fig 1). *Withania somnifera* leaf is also been used in the isolation of endophytes and synthesis of silver nanoparticles [17]. *Aspergillus terreus, Aspergillus fumigatus* and *Aspergillus Niger* were used in the synthesis of Silver Nanoparticles [18-20].

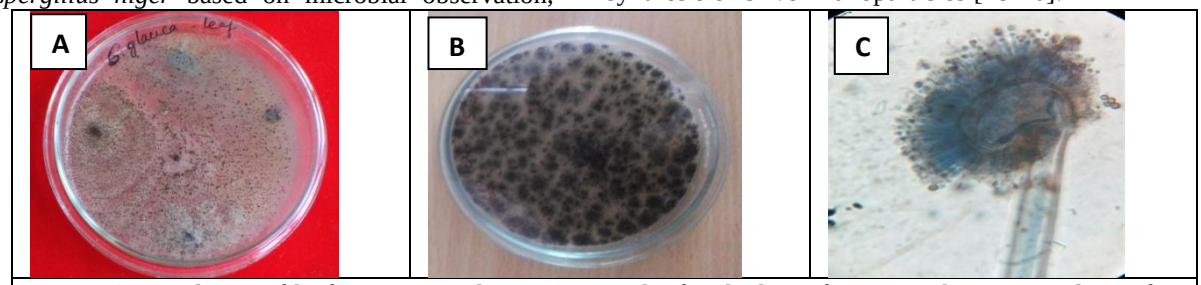


Fig 1. A) Inoculation of leaf on PDA medium. B) Growth of endophytic fungus on the PDA medium after the incubation period of 7 days. C) *Aspergillus niger.*

Synthesis of Silver nanoparticles

Silver ions were reduced to silver nanoparticles when added endophytic extracts and it was observed that the color of the mixed solution turned from pale yellowish to dark brown which indicated the formation of silver nanoparticles due to surface Plasmon resonance after 24 h of incubation (Fig 2) [21-24]. The formation and stability of silver nanoparticles in the solution are confirmed by UV-vis spectrophotometer studies.

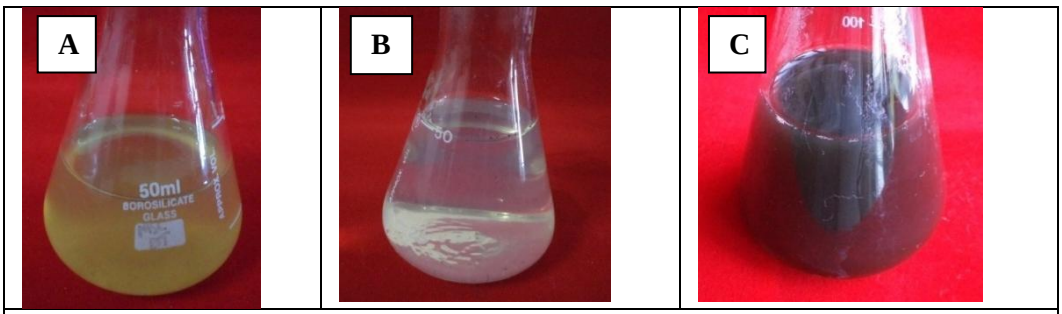


Fig 2. A) Endophytic extract, B) just after the addition of silver nitrate, C) After 24 hours of incubation in dark condition.

Characterization of silver nanoparticles

Synthesis of silver nanoparticles from endophyte *Aspergillus* extract isolated from *Simarouba glauca* has showed astounding strong broad spectra of 400 nm. [25-28]. This was further confirmed by X ray diffraction analysis, the lattice plane indexed to the (111), (200), (220), (311) with the Bragg reflections with 2θ values of 38^0, 44^0, 64^0, 77^0, (JCPDS file no. 4-783). G Rajakumar et al also reported the XRD patterns for which XRD pattern of our sample resembles which suggests that the synthesized silver nanoparticles from endophyte *Aspergillus niger* isolated from *Simarouba glauca* are biphasic in nature. [29-33].

The functional group was confirmed by FT-IR analysis, a broad band between 3289 cm^{-1} is due to strong O-H stretching intermolecular bond of alcohol, the band at 2916 cm^{-1} corresponds to medium C-H stretching Alkenes, the medium band at 1597 cm^{-1} corresponds to N-H bending amine, the medium band at 1387 cm^{-1} corresponds to C-H bending aldehyde, the strong band at 1062 cm^{-1} corresponds to C-O stretching primary alcohol, the strong band at 536 indicates C-L stretching halo compound (Fig 3) [34,35].

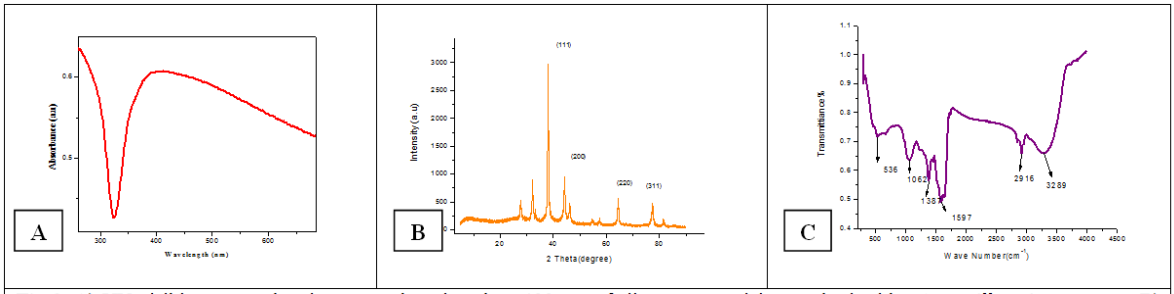


Fig 3. A) UV visible spectra showing strong broad peak at 400 nm of silver nanoparticles synthesized by *Aspergillus niger* extracts. B) X-ray crystallographic studies of silver nanoparticles. C) FT- IR spectra of silver nanoparticles.

Scanning Electron Microscopy analysis was performed to study size and surface morphology of the silver nanoparticles synthesized from endophyte *Aspergillus niger* extracts. The average particle size was 41.9 nm calculated using Debye- Scherrer equation (Fig 4) [36, 37]. EDX analysis suggested that silver and oxygen are present in the sample, also it confirmed that the sample contains silver nanoparticles (Fig 5).

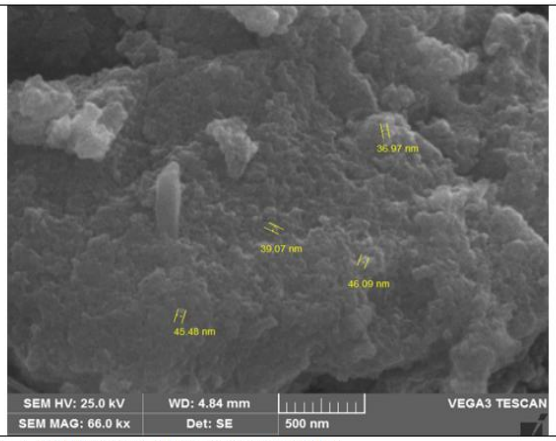


Fig - 4. SEM images of silver nanoparticles

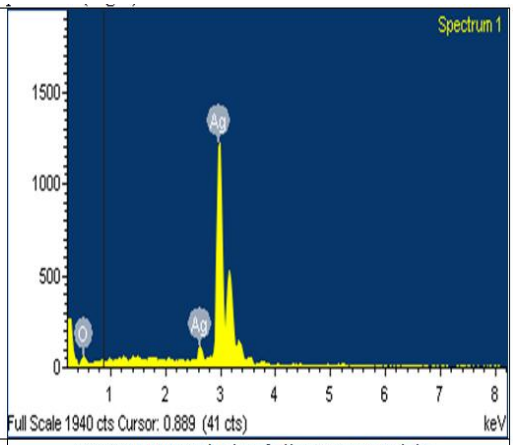
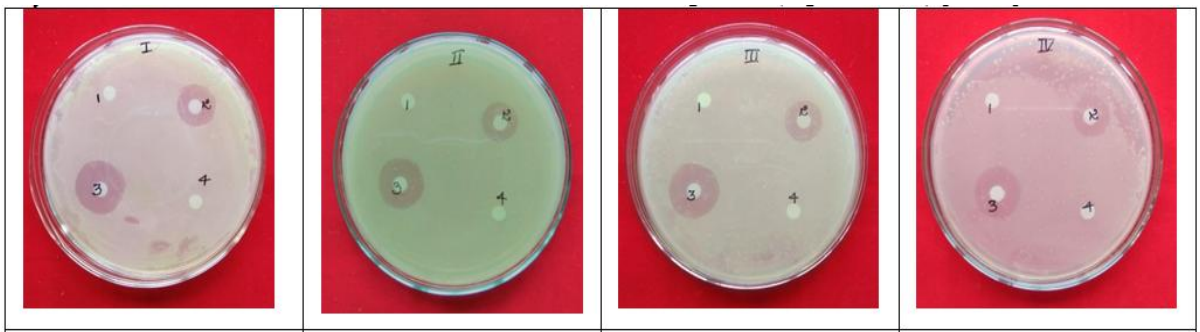


Fig 5. EDS analysis of silver nanoparticles

ANTI- BACTERIAL ASSAY

The antibacterial activity of silver nanoparticles and endophytic extract was performed by disc diffusion method. The results indicated that the endophytic extract alone was failed to exhibit antibacterial effect against the tested microorganisms. Nevertheless, silver naoparticles showed the significant antibacterial activity against the tested microorganisms (Fig 6, Table-1) [38, 39].



Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumonia*

Fig - 6. Antibacterial activity of silver nanoparticles synthesized from endophyte *Aspergillus niger* extract isolated from *Simarouba glauca* leaf.
I. *Escherichia coli,* II. *Pseudomonas aeruginosa,* III. *Staphylococcus aureus,* IV. *Klebsiella pneumonia.* 1. Double distilled water, 2. Taxim (antibiotic), 3. AgNPs, 4. Endophytic extract.

Table-1. Antibacterial activity of silver nanoparticles synthesized from endophyte *Aspergillus niger* extracts isolated from *Simarouba glauca* leaf.

		Zone of inhibition of test organisms (mm)			
Sl. No	Test organisms	Control	Standard	Sample	Endophytic extract
I	*Escherichia coli*	0	19±1.7[b]	26±0.9[b]	0
II	*Pseudomonas spp*	0	15±1.3[a]	21±1.6[b]	0
III	*Staphylococcus spp*	0	18±1.5[a]	24±1.2[b]	0
IV	*Klebsiella spp*	0	17±1.0[a]	20±1.4[a]	0

Values were expressed as the means of three replicates ± SD.

ANTIOXIDAT ASSAY DPPH ASSAY

The synthesized nanoparticles and Ascorbic acid showed the percentage inhibition graphically and the IC_{50} value was found to be 58µg/ml and 147 µg/ml for ascorbic acid and silver nanoparticles respectively (Fig7) [40]. Many authors suggested that synthesized silver nanoparticles can be used as strong antioxidants (41).

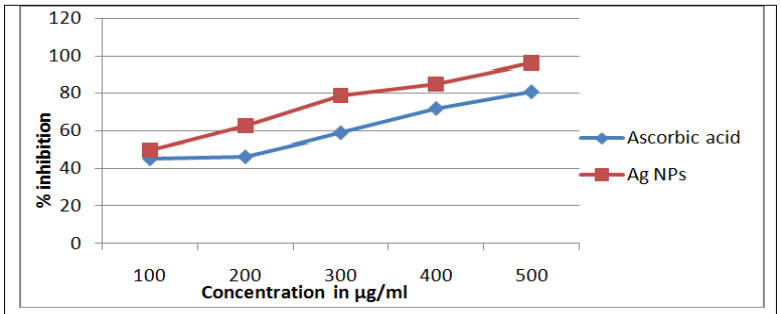
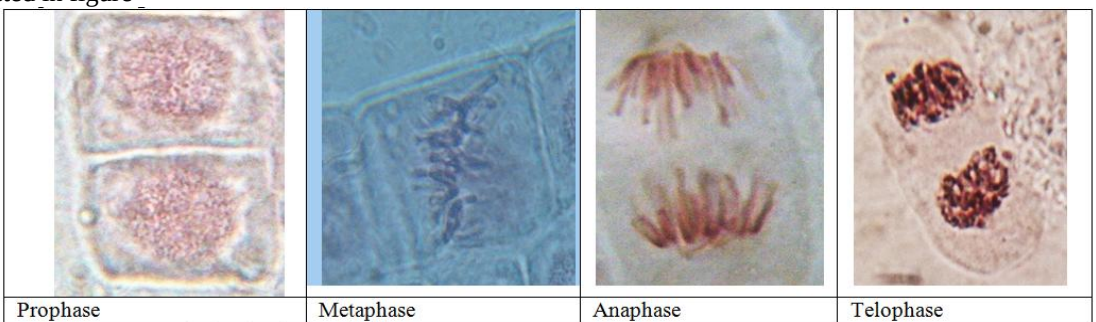


Fig - 7. Free radical scavenging of Ascorbic acid and Silver nanoparticles synthesized from endophyte *Aspergillus niger* extract isolated from *Simarouba glauca* leaf against DPPH at 517nm.

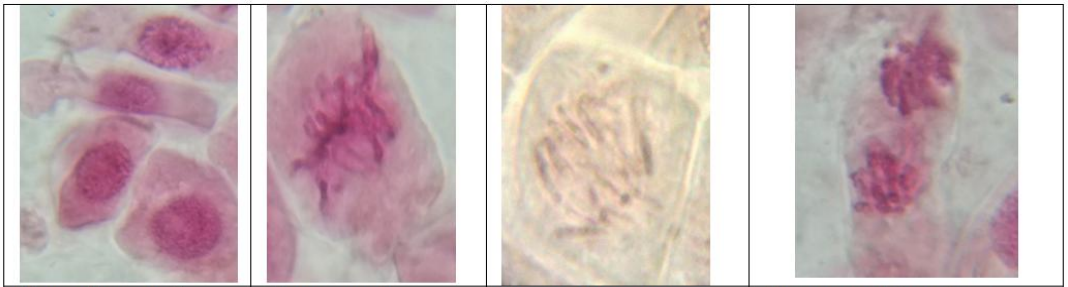
ANTIMITOTIC ASSAY

The outcomes of antimitotic assay for synthesized silver nanoparticles, distilled water, Quercetin in different stages of cell cycle are depicted in figure-10, 11, 12 and table 2. The meristem division of the *Allium cepa* is similar to the cancer cell division and hence we have selected the same for evaluation of anticancer activity of silver nanoparticles [42].



Prophase	Metaphase	Anaphase	Telophase

Figure-10. Normal mitotic phases of *Allium cepa.*



Mega cells and cell shrinkage	Chromosomal clumping at metaphase	Vagrant chromosome at anaphase	Chromosomal clumping at telophase

Fig -11. Mitotic abnormalities of *Allium cepa.*

Table 2. Antimitotic activity of silver nanoparticles			
Sl. No.	Sample	Concentration	Mitotic index
1	Distilled water	---------	94.22
2	Ag NPs	10 mg/ml	25.19
3	Ag NPs	5 mg/ml	32.52
4	Quercetin (Standard)	1mg/ml	23.98

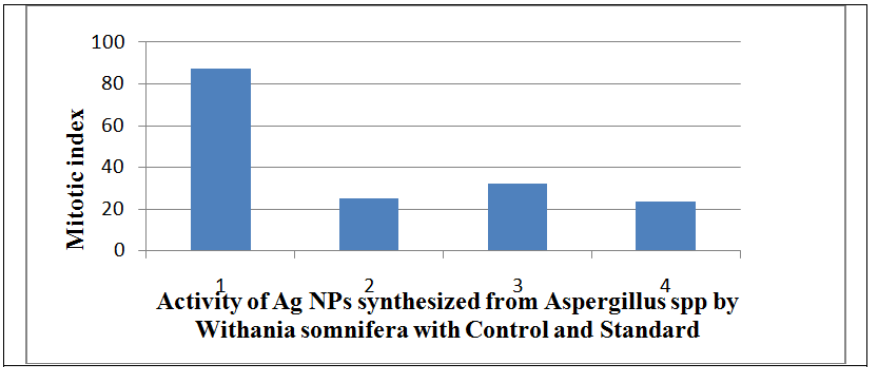


Fig 12. Antimitotic activity of Ag NPs synthesized from *Aspergillus spp* by *Withania somnifera*

IV. CONCLUSION

The present study revealed that silver nanoparticles can be synthesized by aqueous extract of *Aspergillus niger* isolated from *Simarouba glauca* leaf. SEM analysis showed that the sizes of the synthesized AgNps ranged from 36.97 to 46.07 nm. Synthesized silver nanoparticles displayed excellent antibacterial activity against tested microorganisms comparatively with standard antibiotic used. A significant antioxidant activity was also shown by silver nanoparticles and it woukd be appropriate for the development of new drugs for various ailments.

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REFERENCES

[1] D. Singh, V. Rathod, S. Ninganagouda, J. Herimath, P. Kulkarni, - Biosynthesis of silver nanoparticles by endophytic fungi Pencillium sp. Isolayed from Curcuma Longa (turmeric) and its antibacterial activity against pathogenic gram negative bacteria, Journal of Pharmacy Research, pp. 448- 453, 2013.

[2] R. Veerasamy, T. Zi. Xin, S. Gunasagaran, T. F. Wei Xiang, E.F Chou Yang, N. Jeyakumar, S.A. Dhanaraj, - Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities, Journal of Saudi Chemical Society, pp. 113–120, 2011.

[3] H.M.M. Ibrahim, - Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms, Journal of Radiation Research and Applied Sciences, pp. 265-275, 2015.

[4] A.G. Ingale, A.N. Chaudhari, - Biogenic synthesis of nanoparticles and potential applications: An eco friendly approach, J. Nanomed. Nanotechol, pp.1-7, 2013.

[5] K. Kathiresan, S. Manivannan, M.A. Nabeel, B. Dhivya, - Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment, Colloids Surf. B Biointerfaces, pp. 133-137, 2009.

[6] M.A. Farooqui, P.S. Chauhan, P. Krishnamoorthy, J. Shaik, - Extraction of silver nanoparticles from the leaf extracts of clerodendrum inerme, Digest Journal of Nanomaterials and Biostructures, pp. 43- 49, 2010.

[7] K.P. Upendra, S.S. Preeti, S. Anchal, - Bioinspired synthesis of silver nanoparticles, Digest Journal of Nanomaterials and Biostructures, pp. 159-166, 2009.

[9] V.L. Das, R. Thomas, T.V. Rintu, E.V. Soniya, J. Mathew, E. K. Radhakrishnan, - Extracellular synthesis of silver nanoparticles by the Bacillus strain CS 11 isolated from industrialized area, 3 Biotech, pp. 121–126, 2014.

[10] Guangquan Li, Dan He, Y. Qian, B. Guan, Song Gao, Yan Cui, K. Yokoyama, Li Wang, - Fungus mediated green synthesis of silver nanoparticles using *Aspergillus terreus*, International Journal of molecular science, pp. 466-476, 2012.

[11] M. Kowshik, S. Ashtaputre, S. Kharrazi, W. Vogel, J. Urban, S.K. Kulkarni and K. M. Paknikar, - Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3, Nanotechnology, pp. 95-100, 2002.

[12] S. Ahmed, M. Ahmad, B.L. Swami, S. Ikram. - A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise, Journal of Advanced Research, pp. 17-28, 2016

[13] S. Iravani, H. Korbekandi, S.V. Mirmohammadi, B. Zolfaghari, - Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci. pp. 385–406, 2014.

[14] J.L. Elechiguerra, J. L. Burt, J.R. Morones, A.C. Bragado, X. Gao, H. H Lara, M.J. Yacaman, - Interaction of silver nanoparticles with HIV-I, Journal of Nanobiotechnol, pp. 1-10, 2005.

[15] L.K. Santhana, D. Sangeetha, S. Sivamani, M. Tamilarasan, T.P. Rajesh, B. Anandraj, - *In vitro* antibacterial, antioxidant, haemolytic, thrombolytic activities and phytochemical analysis of *Simarouba glauca* leaves extracts, International Journal of Pharmaceutical Sciences and Research, pp. 432-437, 2014.

[16] A.K. Singh, V. Rathod, D. Singh, S. Ninganagouda, Prema Kulkarni, Jasmine Mathew, Manzoor ul Haq, - Bioactive Silver nanoparticles from endophytic fungus *Fusarium* sp. Isolated from an ethnomedical plant *Withania somnifera* (Ashwagandha) and its antibacterial activity, International Journal of Nanomaterials and Biostructures, pp. 15-19, 2015.

[17] C.P. Chandrappa, M.Govindappa, N. Chandrasekar, Sonia Sarkar, Sepuri Ooha, R. Channabasava, - Endophytic synthesis of silver chloride nanoparticles from *Penicillium* sp. of *Calophyllum apetalum* Advances in Natural Sciences: Nanoscience and Nanotechnology, pp. 1-5, 2016.

[18] C. Kuber, S.F.Bhainsa. D'Souza, - Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*, Colloids and surfaces B: Biointerfaces, pp. 160-164, 2006.

[19] A. Braca, N. De Tommasi, L. Di Bari, C.Pizza, M. Politi, I. Morelli, - Antioxidant principles from Bauhinia terapotensis, J. Nat. Prod, pp. 892–895, 2001.

[20] C.P.Chandrappa, M.Govindappa, N.V.Anil Kumar, - *In vitro* antimitotic, antiproliferative and DNA fragmentation assay of ethanol extract of *Carmona retusa* (Vahl.) Masam, ACB, pp. 52-57, 2013.

[21] Y.K. Mohanta, S.K. Panda, R. Jayabalan, N. Sharma, A.K. Bastia, T.K. Mohanta, - Antimicrobial, Antioxidant and Cytotoxic Activity of Silver Nanoparticles Synthesized by Leaf Extract of Erythrina suberosa (Roxb.), Frontiers in Molecular Biosciences, pp. 14. 1-9, 2017.

[22] A. K. Gade, P. Bonde, A. P. Ingle, P. D. Marcato, N. Duran, M. K. Rai, - Exploitation of *Aspergillus Niger* for Synthesis of Silver Nanoparticles, Journal of Biobased Materials and Bioenergy, pp. 1–5, 2008.

[23] A. Bankar , B. Joshi , A. Ravi Kumar, S. Zinjarde, - Banana peel extract mediated novel route for the synthesis of silver nanoparticles, Colloids and Surfaces A: Physicochemical and Engineering Aspects, pp. 58–63, 2010.

[24] M. Rai , A. Yadav, A. Gade, - Silver nanoparticles as a new generation of antimicrobials, Biotechnology Advances, pp. 76–83, 2009.

[25] R.Nithya, R.Ragunathan, - Synthesis of silver nanoparticle using pleurotus sajor caju and its antimicrobial study, Digest Journal of Nanomaterials and Biostructures, pp. 623 – 629, 2009.

[26] Y. rong Ma,H. yun Niu, X.le Zhang, Y. qi Cai, - Colorimetric detection of copper ions in tap water during the synthesis of silver/dopamine nanoparticles, ChemComm, pp. 12643–12645, 2011.

[27] Y.Kobayashi, H. Katakami, E. Mine, D. Nagao, M. Konno, L.M. Liz-Marzan, - Silica coating of silver nanoparticles using a modified Stober method, Journal of Colloid and Interface Science, pp. 392–396, 2005.

[28] K. C. Song, S.M. Lee, T.S.Park, B.S.Lee, - Preparation of colloidal silver nanoparticles by chemical reduction method, Korean J. Chem. Eng., pp. 153-155, 2009.

[29] A. Frattini, N. Pellegri, D. Nicastro, O. de Sanctis, - Effect of amine groups in the

synthesis of Ag nanoparticles using aminosilanes, Materials Chemistry and Physics, pp. 148–152, 2005.

[30] S.A. Cumberland, J.R. Lead, - Particle size distributions of silver nanoparticles at environmentally relevant conditions, Journal of Chromatography A, Journal of Chromatography A, pp. 9099–9105, 2009.

[31] S. Shiv Shankar, A. Ahmad, M. Sastry, - Geranium Leaf Assisted Biosynthesis of Silver Nanoparticles, Biotechnol. Prog, pp. 1627−1631, 2003.

[32] H. Bar, D.K. Bhui, G.P. Sahoo, P. Sarkar, S.P. De, A. Misra, - Green synthesis of silver nanoparticles using latex of Jatropha curcas, Colloids and Surfaces A: Physicochemical and Engineering Aspects, pp.134–139339, 2009.

[33] K. Kalimuthu, R.S. Babu, D. Venkataraman, Mohd. Bilal, S. Gurunathan, - Biosynthesis of silver nanocrystals by Bacillus licheniformis, Colloids and Surfaces B: Biointerfaces, pp. 150–153, 2008.

[34] S. Basavaraja, S.D. Balaji, A. Lagashetty, A.H. Rajasab, A. Venkataraman, Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum, Materials Research Bulletin, pp. 1164–1170, 2008.

[35] M.Raffi, F.Hussain, T.M.Bhatti, J.I.Akhter, A.Hameed, M.M.Hasan, - Antibacterial Characterization of Silver Nanoparticles against E. Coli ATCC-15224, J. Mater. Sci. Technol., pp. 192-196, 2007.

[36] Prietop, M. Pineda, M. Aguilar, - Spectrophotometeric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex specific application to the Determination of vitamin, E Anal Biochem, pp. 337-341, 1999.

[37] G Rajakumar, A.A. Rahuman, - Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrate* leaf extract against filariasis and malaria vectors, Acta Tropica, pp. 196-203, 2011.

[38] M.M.H. Ibrahim, - Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms, Journal of Radiation Research and Applied Sciences, pp. 265-275, 2015.

[39] N.N. Devi, D.Shankar, Sutha, - Biomimetic synthesis of silver nanoparticles from an endophytic fungus and their antimicrobial efficacy, International Journal of Biomedical and Advance Research, pp. 309-315 , 2012.

[40] D. Jain, H. K. Daima , S. Kachhwaha, S. L. Kothari, - Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities, Digest Journal of Nanomaterials and Biostructures, pp. 557 – 563, 2009.

[41] C.P.Chandrappa, M. Govindappa, N Chandrashekhar, S. Sonia, S. Ooha, R. Channabasava - Endophytic synthesis of silver nanoparticles from Penicillium sp. of *Calophyllum apetalum*, Adv. Nat. Sci.: Nanosci. Nanotechno, pp. 1-5, 2016.

[42] V. Dhand, L. Soumya, S. Bharadwaj, S. Chakra, D. Bhatt, B. Sreedhar, Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity, Materials Science and Engineering, pp. 36-43, 2016.

[43] T.A. Nagalakshmi, A. U.S.R. Mahadeva, Study of Cytotoxic and Antimitotic Activities of *Solanum nigrum* by Using *Allium cepa* Root Tip Assay and Cancer Chemo preventive Activity Using MCF-7-Human Mammary Gland Breast Adenocarcinoma Cell Lines, International Journal of Science & Technology, pp. 26-48, 2011.

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