International Journal of Technical Research and Applications e-ISSN: 2320-8163,
www.ijtra.com Volume 2, Issue 4 (July-Aug 2014), PP. 108-111

ANTIFUNGAL ACTIVITY OF EXTRACELLULARLY SYNTHESIZED SILVER NANOPARTICLES FROM MORINDA CITRIFOLIA L.

P Ravikumar[1*], S.Sathish Kumar[2]

[1]Associate Professor, PG and Research Department of Botany,
Government Arts College (Autonomous) Coimbatore 641018 TN India

[2]Research Scholar, St. Joseph's College (Autonomous),
Tiruchirappalli 620002, TN India.

***Abstract*— Enlargement of biologically stimulated investigational processes for the synthesis of nanoparticles is budding into an important branch of nanotechnology. Eco responsive methods of green mediated synthesis of nanoparticles are the present research in the extremity of nanotechnology. The bioreduction behavior of leaf extracts of Morinda citrifolia L. (Rubiaceae) in the green synthesis of silver nanoparticles was investigated employing UV/Visible Spectrophotometry, Particle size analyzer, Zeta potential, Filed emission scanning electron microscopy, Energy Dispersive X-ray Analysis and Fourier-Transform Infrared Spectroscopy. The antifungal property of the silver nanoparticles was tested against Candida albicans, Candida tropicalis and Candida krusei. The Antifungal assay tests Zone of inhibition revealed the concentrations of more than 10µl of silver nanoparticles were inhibited the growth of fungal pathogens.**

***Key words*— Silver nanoparticles, Morinda citrifolia, antifungal activity.**

I. INTRODUCTION

The nanotechnology was one of the majority dynamic areas of research in modern nanomaterial sciences demonstrates completely new and different in normal or enhanced properties based on specific characteristics such as size, shape, intensity, charges, distribution and morphology (1). These extraordinary and exclusive properties could be recognized to their nano sizes and large surface areas based on the surface fixation. For these reasons, metallic nanomaterials have found uses in wind range of applications in different fields, such as catalysis, photonics, and electronics (2). Nanobiotechtechnology has emerged up as integration and interaction between the two advanced areas of biotechnology and nanotechnology for developing biosynthetic and eco-friendly technology for synthesis of nanomaterial in biological methods (3). Among the different type of nanomaterials Copper (4), Zinc (5), Titanium (6), Magnesium (7), Gold (8), Alginate (9) and Silver, Silver nanoparticles have proved to be most effective as it has good efficacy against fungi, bacteria, viruses and other eukaryotic micro-organisms (10). Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, compositions and controlled disparity of their potential use for human benefits for the modern developed technology (11). Bioinspired synthesis of silver nanoparticles provides advancement over chemical and physical methods as it is a cost effective and eco-friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (12). Synthesis of silver nanoparticles using plant extracts have been reported in Boswellia ovalifolilata, Shorea tumbuggaia Svensoina hyderobadensis, Thespesia populnea, Vinca rosea (13), Cassia auriculata (14). Morinda citrifolia L. (Noni) has been extensively used in folk medicine for over 2,000 years. It has been reported to have broad therapeutic effects, including cancer activity, in both clinical practice and laboratory animal models (15). Noni has traditionally been used for colds, flu, diabetes, anxiety, and high blood pressure, as well as for depression and anxiety. The green fruit, leaves, and root/rhizomes were traditionally used in Polynesian cultures to treat menstrual cramps, bowel irregularities, diabetes, liver diseases, and urinary tract infections (16). The mechanism for these effects remains unknown (17-21). The hypothesis that Morinda citrifolia possesses an antifungal preventive effect at the initiation stage of carcinogenesis was studied (35). Hence, in the present investigation, green synthesis of silver nanoparticles using the leaf extract of Morinda citrifolia L. and their antifungal activities are presented and discussed.

II. MATERIALS AND METHODS

Morinda citrifolia L. leaves were collected from Farm House near Erode, Periyar District, Tamilnadu and authenticated by Botanical Survey of India, Southern Regional Centre, Coimbatore 641003 Tamilnadu. The fungal test strains were procured from MTTC, Chandigarh. Silver Nitrate (AgNO3) was purchased from HiMedia, Mumbai.

III. SYNTHESIS OF SILVER NANOPARTICLES

Shade dried leaves of Morinda citrifolia were pulverized into fine powder. 1 gram of the leaf powder was taken in a 250 ml Erlenmeyer flask and to it 100ml of distilled water was added. The mixture was kept on a heating mantle at 700C for 20 minutes. The extract was filtered by Whatmann No.1 filter paper and use. For the synthesis of silver nanoparticles (AgNPs), leaf powder extract was added to the silver nitrate solution in the ratio 95:5 ml and kept in sunlight for 120 seconds to synthesize the nanoparticles.

IV. CHARACTERIZATION STUDIES:

The leaf powder extracts containing silver nanoparticles were characterized by the following methods:

A. Visual Observation:

A change of colour from pale yellow to reddish brown was observed in the solution after visible irradiation.

B. UV Spectrophotometric analysis:

The formations of leaf extract mediated silver nanoparticles were confirmed by the spectral analysis. The UV spectra of the biosynthesized silver nanoparticles were recorded using Lambda 35, Perkin Elmer UV Spectrophotometer by continuous scanning from 190nm to 1100nm and the leaf powder extract was used as the reference for the baseline correction.

C. Fourier Transform Infra Red Spectroscopy Analysis:

FTIR analysis was used to investigate and predict the physicochemical interactions between different components in a formulation. FTIR spectroscopy measurements were taken for the AgNPs synthesized after 24 hrs of reaction. These measurements were carried using a FTIR SHIMADZU 8400S instrument with a wavelength range of 4000 to 400 nm where the samples were incorporated with KBr pellets to acquire the spectra. The results were compared for shift in functional peaks.

D. Field Emission Scanning Electron Microscopy

FESEM was used to characterize the mean particle size, morphology of the AgNPs. The powder sample and freeze dried sample of the AgNPs solution was sonicated with distilled water; small drop of this sample was placed on glass slide allowed to dry. A fine powder was coated to make the samples conductive Jeol JSM-6480 LV FESEM machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10-20 kV.

E. Particle Size Analyzer (DLS method)

In most applications theoretical calculations predict the relative effects of particle size, particle composition, composition of the surrounding medium and wavelength of light. In order to find out the particles size distribution the Ag powder was dispersed in water by horn type ultrasonic processor (Vibronics, VPLP1). The data on particle size distribution were extracted in Zetasizer Ver. 6.20 (Mal1052893, Malvern Instruments).

F. Energy Dispersive X-ray Analysis:

The elemental composition of the synthesized nano particles by M. citrifolia were dried; drop coated on to carbon film and tested using EDAX analysis (S-3400N Japan).

G. Antifungal activity study

Antifungal activity of the synthesized silver nanoparticles was indomitable, using the agar well diffusion assay method (22). Approximately 20 ml of Muller Hinton Agar was poured in sterilized Petri dishes. The fungal test organisms were grown in potato dextrose broth for 24 h. Agar wells of 6 mm diameter were prepared with the help of a sterilized stainless cork borer. Two wells were prepared in the agar plates. The wells were labeled as A and B. 'A' well was loaded with 50 µl of silver nanoparticles, 'B' well was loaded with 50 µl of Distilled water and were incubated at 37oC. The plates were examined for confirmation of zones of inhibition, which become visible as a clear area around the wells (23). The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

V. RESULTS AND DISCUSSION

The aqueous leaf extract of M. citrifolia was used to synthesis silver nanoparticles in the eco friendly and green synthesis method. Based on the reduction process the yellow color was turned into reddish brown color in the source of sunlight. The sunlight was act as the energy producer of the nanomaterials synthesis process (24). Colors of the silver nanoparticles were recognized based on the Surface Plasmon Resonance (SPR) arising due to the free conduction electrons induced by an interacting electronic field (25). UV spectra absorption shows that the silver nanoparticles high peck at 497 nm and the spectrum is show in fig. 1.

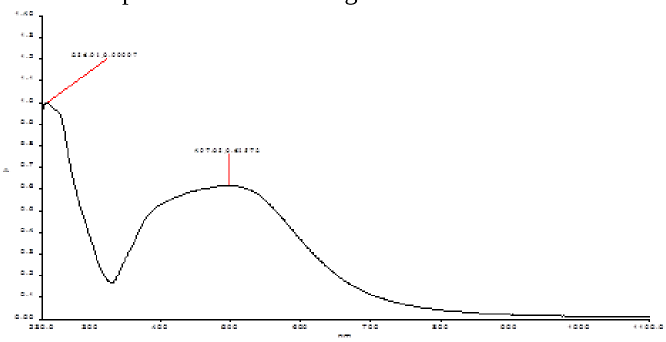

Figure 1. UV Spectral analysis of silver nanoparticles synthesized from Morinda citrifolia

FTIR analysis expose that the biological compounds present in the nanoparticles. The biomolecules were identified after the Ag+ reduction and the spectra are as shown in fig. 2. The spectra revealed that the maximum major peak at 3401.64 cm-1 which corresponded in N-H group and presence of amine compounds. The minor groups were also formed of alkynes and aromatics. The morphological analysis of silver nanoparticles carried out by FESEM recorded the size and shape of the silver nanoparticles, fig. 3. It is illustrated that the silver nanoparticles were predominantly spherical in shape with uniform distribution. EDX pattern shows the crystalline and elemental composition of silver nanoparticles synthesized from leaf extract of M. citrifolia (Fig. 4.) The strong signal in the silver region was observed at 3 keV for silver nanoparticles due to the Surface Plasmon Resonance (26, 27). 73% of silver present in the nano silver colloid. The weak O atom present in K series. The particle size distribution exposed the particle diameter in the nano silver liquid. The maximum size of the nanosilver was 193.5 dnm and the minimum size was 22.34dnm (Fig. 5.). Toxicity studies on pathogenic fungi open an access for nanotechnology applications in medicine. In this study, M. citrifolia leaf extract and the AgNPs syn¬thesized with M. citrifolia leaf extract were tested for their antifungal activities against Candida albicans, Candida tropicalis and Candida krusei. The AgNPs recorded the maximum activity against the C. tropicalis (28.0 ± 0.57 mm), C. albicans (24.0 ± 1.52 mm), and the minimum inhibitory zone for C. krusei was (21.0 ± 1.52) (Fig 6). Biological and green synthesis of metal AgNPs is a traditional method and the use of plant extracts has a new awareness for the control of sickness, moreover being safe with no phytotoxic side effects (28). AgNPs are expansively used in the pharmaceutical indus¬try and have inhibitory activities on various microorganisms. They have also been used in balms and ointments to avert infections following burns and wounds (29). Silver ions released by the silver nanoparticles may attach almost to negatively charged particles to the bacterial cell wall and shatter it, thereby chief to protein denaturation and cell death (30). Ag+ ions uncouple the respiratory shackle from oxidative phosphorylation or collapse the proton-motive force across depending on the size and shape (31- 32). Many of the studies reported that AgNPs preferentially spring to the cytoplasmic membrane leading to cell damage in the lock and key or induced fit method. In addition to that, the pitting caused by AgNPs in the bacterial or fungal cell wall is also responsible for the death of bacteria or fungi (33).

	Frequency range (cm^{-1})	Bond	Compound type
Composition $AgNO_3$: Extract 95 : 5	3401.64 ~	N–H stretch	primary, secondary amines, amides
	2107.90 ~	–C(triple bond)	alkynes
	1572.35 ~	C–C stretch (in–ring)	aromatics
	1126.01 ~	C–N stretch	aliphatic amines
	760.98 ~	–C(triple)C–H: C–H bend	alkynes
	696.80 ~	C–Br stretch	alkyl halides

Table 1. Summary of FTIR interpretation

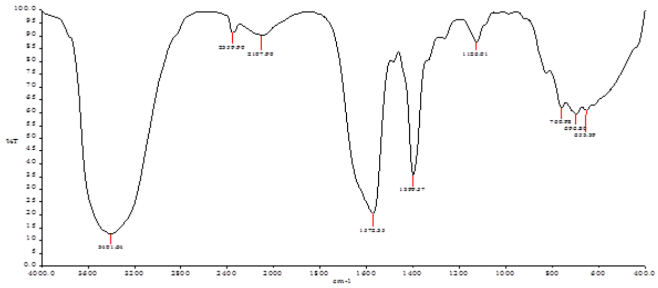

Figure 2. FTIR Spectral analysis of silver nanoparticles synthesized from Morinda citrifolia Leaf.

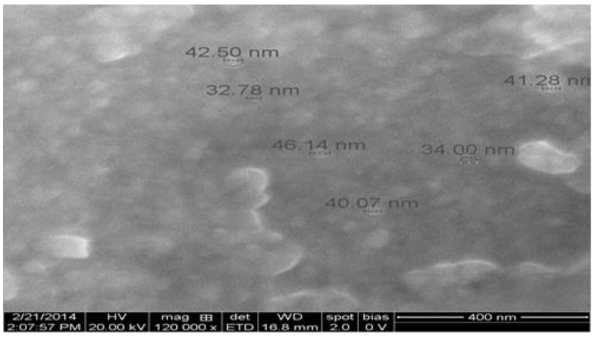

Figure 3. FESEM analysis of silver nanoparticles synthesized from Morinda citrifolia leaf.

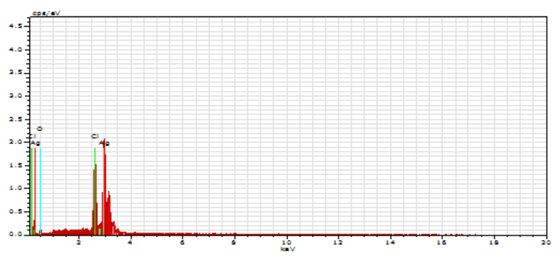

Figure 4. EDAX Spectral analysis of silver nanoparticles synthesized from Morinda citrifolia leaf.

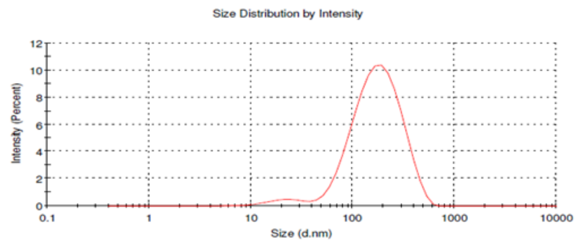

Figure 5. Particle size analysis of silver nanoparticles synthesized from Morinda citrifolia

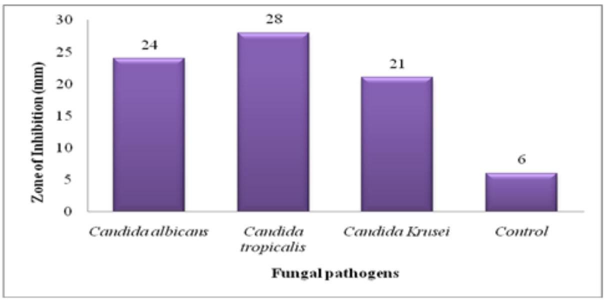

Figure 6. antifungal activity of silver nanoparticles synthesized from Morinda citrifolia

VI. CONCLUSION

Rapid and green synthesis shows that the environmentally benign silver nanoparticles have been synthesized using Morinda citrifolia leaf. The synthesis is found to be efficient in terms of reaction time as well as stability of the synthesized AgNPs. Throughout the globe, interest for pronouncement of this bioactive compounds increases because of the pathogenic resistance to available drugs. The present study emphasizes the use of plants mediated synthesis of AgNPs with potent antifungal effect.

REFERENCES

[1] Geethalakshmi R. and Sarada D.V.L. 2010. Synthesis of plant-mediated silver nanoparticles using Trianthema decandra extract and evaluation of their anti-microbial activities. In J of Eng Science and Technology. 2(5); 970-975.

[2] Mehrdad Forough, Khalil Farhadi. 2010. Biological and green synthesis of silver nanoparticles. Turkish J. Eng. Env. Sci. 34, 281 – 287.

[3] Sivakumar J., Premkumar C., Santhanam P. and Saraswathi N. 2011. Biosynthesis of Silver Nanoparticles Using Calotropis gigantean Leaf. African Journal of Basic & Applied Sciences 3 (6): 265-270.

[4] Vinod Vellora Thekkae Padil and Miroslav Černík. 2013. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. Int J Nanomedicine. 8: 889–898.

[5] Vidya C, Shilpa Hiremath, M N Chandraprabha, M A Lourdu Antonyraj, Indu Venu Gopal, Aayushi Jain, and Kokil Bansal. 2013. Green synthesis of ZnO nanoparticles by Calotropis Gigantea. In Journal of Current Engineering and Technology, 1: 118-120.

[6] Retchkiman-Schabe, P.S., G. Canizal, R. Becerra- Herrera, C. Zorrilla and H.B. Liu, 2006. Biosynthesis and characterization of Ti/Ni bimetallic nanoparticles. Opt. Mater., 29: 95-9.

[7] Kaul KRK, Umar K, Burman U, Joshi P, Grawal AA, Raliya R and Arafdar JCT. 2012. Magnesium and iron nanoparticles production usingmicroorganisms and various salts. Materials Science-Poland, 30(3); 254-258.

[8] Gu, H., P.L. Ho, E. Tong, L. Wang and B. Xu, 2003. Presenting vancomycin on nanoparticles to enhance antimicrobial activities. Nano Lett., 3(9): 1261-3.

[9] Ahmad, Z., R. Pandey, S. Sharma and G.K. Khuller, 2005. Alginate nanoparticles as antituberculosis drug carriers: formulation development, pharmacokinetics and therapeutic potential. Ind J Chest Dis Allied Sci., 48: 171-6.

[10] Kasivelu Govindaraju, Sabjan Khaleel Basha, Vijayakumar Ganesh Kumar, Ganesan Singaravelu. 2008. Silver, gold and bimetallic nanoparticles production using single-cell protein (Spirulina platensis) Geitler, J Mater Sci., 43:5115–5122.

[11] Sathish Kumar S. and Melchias G. 2014. Characterization of Biologically Synthesised Silver Nanoparticles from Euphorbia hirta. Asian J. Pharm. 4(1); 01-05.

[12] Yamini SudhaLakshmi.G. 2011. Green Synthesis of Silver Nanoparticles from Cleome Viscosa: Synthesis and Antimicrobial Activity. International Conference on Bioscience, Biochemistry and Bioinformatics IPCBEE vol.5

[13] Savithramma N., Linga Rao M., Ankanna S. and Venkateswarlu P. 2012. Screening of medicinal plants for effective biogenesis of silver nano particles and efficient anti-microbial activity. IJPSR, 3(4): 1141-1148.

[14] Velavan S, Arivoli P and Mahadevan K. 2012. Biological reduction of silver nanoparticles using cassia auriculata flower extract and evaluation of their in vitro antioxidant activities. Nanoscience and Nanotec.,: An Int Journal. 2(4): 30-35.

[15] Wang MY, Su C. 2001. Cancer preventive effect of Morinda citrifolia (Noni). Ann N Y Acad Sci. 952:161-8.

[16] Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, Anderson G (2002). "Morinda citrifolia (Noni): a literature review and recent advances in Noni research". Pharmacol Sin 23 (12): 1127–41

[17] Hiramatsu T, Imoto M, Koyano T, Umezawa K. 1993. Induction of normal phenotypes in ras-transformed cells by damnacanthal from Morinda citrifolia. Cancer Lett.;73 :161-166.

[18] Hirazumi A, Furusawa E. 1999. An immunomodulatory polysaccha-ride-rich substance from the fruit juice of Morinda citrifolia (noni) with antitumor activity. Phytother Res.; 13:380-387.

[19] Millonig G, Stadlmann S, Vogel W. 2005. Herbal hepatotoxicity: acute hepatitis caused by a Noni preparation (Morinda citrifolia). Eur J Gastroenterol Hepatol.;17:445-447.

[20] Mueller BA, Scott MK, Sowinski KM, Prag KA. 2000. Noni juice (Morinda citrifolia): hidden potential for hyperkalemia? Am J Kidney Dis.;35:310-312.

[21] Yüce B, Gülberg V, Diebold J, Gerbes AL. 2006. Hepatitis induced by Noni juice from Morinda citrifolia: a rare cause of hepatotoxicity or the tip of the iceberg? Digestion.; 73:167-170

[22] Hsin-Lun Huang, Chien-Hui Ko , Yeong-Yu Yan , and Chin-Kun Wang. 2014. Antiadhesion and Anti-inflammation Effects of Noni (Morinda citrifolia) Fruit Extracts on AGS Cells during Helicobacter pylori Infection. J. Agric. Food Chem., 62 (11); 2374–2383

[23] Perez C; Paul M; Bazerque P. 1990. Anti-inflammation Effects of Noni (Morinda citrifolia). Acta Biol. Med. Exp, 15, 113.

[24] Cheesbrough M. 2000. District Laboratory Practice in Tropical Countries. Low price edition. The press syndicate of the University of Cambridge, Trumpington Street Cambridge part, 2, 157.

[25] Prathna T. C., Ashok M. Raichur, Chandrasekaran N., Amitava Mukherjee. 2014. Sunlight Irradiation Induced Green Synthesis of Stable Silver Nanoparticles Using Citrus limon Extract. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 84(1); 65-70.

[26] Karunakar Rao Kudle, Manisha R Donda, Prashanthi Y, Ramachander Merugu, Pratap Rudra MP. 2013. Synthesis of silver nanoparticles using the medicinal plant Allmania nadiflora and evolution of its anti-microbial activities. Int. J Res. Pharma. Sci., 4(4); 504-511.

[27] Rajeshkumar S., Malarkodi C., Paulkumar K., Vanaja M., Gnanajobitha G., Annadurai G. (2013) Intracellular and extracellular biosynthesis of silver nanoparticles by using marine bacteria Vibrio alginolyticus. Nanoscience and Nanot.,: An In Journal, 3(1): 21-25

[28] Li S., Shen Y., Xie A., Yu X., Qiu L., Zhang L., Zhang Q. 2007. Green synthesis of silver nanoparticles using Capsicum annuum L. extract. Green Chem 9: 852–858.

[29] Gardea-Torresdey J.L, Gomez E, Peralta- Videa J, Parsons J.G, Troiani H.E, Jose- Yacaman. 2003. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Langmuir, 13, 1357.

[30] Arunachalam KD, Annamalai S, Hari S. 2013. One-step green synthesis and characterization of leaf extract-mediated biocompatible silver and gold nanoparticles from Memecylon umbellatum. Int J Nanomedicine. 8:1307–1315.

[31] Nalwade A.R.and Jadhav A. 2013. “Biosynthesis of silver nanoparticles using leaf extract of Daturaalba Nees. And evaluation of their antibacterial activity”. Applied science research, 5(3); 45-49.

[32] Kim K. J., Sung W. S., Moon S. K., Choi J. S., Kim J. G., and Lee D. G. 2008. “Antifungal effect of silver nanoparticles on dermatophytes,” J of Microbi and Biot., 18(8); 1482–1484.

[33] Gokak I.B. and Taranath T.C. 2014. Cansjera rheedii J. F. Gmel. a medicinal plant-mediated synthesis of silver nanoparticles and their antibacterial activity. International Journal of Scientific Engineering and Technology. 3(3): 293 – 296.

[34] Morones J. R., Elechiguerra J. L. and Camacho A. 2005. “The bactericidal effect of silver nanoparticles”, Nanotechnology, 16(10); 2346– 2353.

[35] Tyagi SP, Sinha DJ, Garg P, Singh UP, Mishra CC, Nagpal R. 2013. Comparison of antimicrobial efficacy of propolis, Morinda citrifolia, Azadirachta indica (Neem) and 5% sodium hypochlorite on Candida albicans biofilm formed on tooth substrate: An in-vitro study. J Conserv Dent.16: 532-5.