WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 21, 734-748. Research Article ISSN 2277–7105

GREEN SYNTHESIS OF SILVER NANOPARTICLES AND CHARACTERIZATION USING PLANT LEAF ESSENTIAL OIL ROSEMARINUS OFFICINALIS AND THEIR ANTI FUNGAL ACTIVITY AGAINST HUMAN PATHOGENIC FUNGI

R. R. Thanighai Arassu, Balwin Nambikkairaj* and D. R. Ramya

PG and Research Department of Zoology Eco-Biology Wing, Voorhees College, Vellore-632001, Tamilnadu, India.

Article Received on
11 October 2023,

Revised on 31 Oct. 2023,
Accepted on 21 Nov. 2023

DOI: 10.20959/wjpr202321-30391

***Corresponding Author**
Balwin Nambikkairaj
PG and Research Department of Zoology Eco-Biology Wing, Voorhees College, Vellore-632001, Tamilnadu, India.

ABSTRACT

Present study, we utilized the bio–reductive potential of the plant leaf essential oil Rosemarinus officinalis, for the synthesis of silvernanoparticles (AgNPs). Thegreen synthesized AgNPs was achievedat 80°C and found to be highlystablein roomtemperature for some time. The Ag NP swasfoundto besmall dotround in shape with an averagesize of ~52 nmindiameter. The AgNPs were characterize dusing ultraviolet-visible absorption spectroscopy fourier transform Infrared spectroscopy (FTIR), transmission electron microscopy (TEM), Scanning electronmicroscopy and Edx (SEM). The anti-fungal activities of the synthesized AgNPs were tested against a few human fungal pathogens Aspergillus niger, Aspergillus flavus, Candidaalbicans, Candida tropicalis and Candida kefyrshowed high activity againstthese microorganisms. The results showed that thesize and the shape canbe easily controlled by employing the optimum concentrations ratio of AgNO3 solution and also varying temperature and incubation time. Thesynthesized AgNPs from Rosemarinus officinalis plantleaf oil can be used as a potent therapeutic agentand for anti-canceractivity.

KEYWORDS: Aspergillus niger, Green synthesis, XRD, Silver nanoparticles, Tem.

INTRODUCTION

The discovery of the fact that nano silver (Ag) can fightagainst the microbes, manufacturers have incorporated Ag nanoparticles (Ag NPs) into more than 200 consumer products, includingclothing and cosmetics. They are quickly gaining popularity for theirmultitudeof uses. Despite tremendous benefits, theinevitable releaseofengineered NPs in the environment with the development ofnanotechnology is a serious case of concern of environmental biologistsworld wide [Bhatt and tripathi, 2011]. As chemical synthesis isapopular approach to theproduction of metal Ag; however, it requiresthe use of toxic chemicals as reducing and/or capping substances [Songand kim, 2008; Gole and Murphy, 2004; Meltzer et al., 2001]. Therefore, there is a growing need to simultaneously develop eco-friendlybiological processes for the synthesis of Ag that do not use toxicchemicals in the synthesis protocol.

Green processes with the use of economic, efficient and ecofriendly catalysis are gaining much importance due to the benefits associated with their use. The major advantage of green synthesis of nano materials is their important role in protecting the environment[Saifuddin et al., 2009]. All the green and clean technologies are expected to minimize things that contribute to environmental problems: air and water emissions, greenhouse gases, non-renewable or toxicsubstances or materials. The utilization of products and materials from various plants peciesaswell as several other biological materials for the synthesis of nano Ag particles are directly related to the mechanisms of nanotechnology and green chemistry. More and more research inputsare rendered for this and there has been an upsurge of interest in the biological synthesis of nano materials by using several plants, plant pure compounds and plant biochemical compounds in the past few years[Egorova and Revina, 2000; Mukherjee et al., 2002; Zhang et al., 2011].

Nanotechnology has been one of the rapidly growinginterdisciplinary areas of science and technology that integratesmaterial science and biology. This technology has broad applications inthe fields including electronics, biomedical sciences, pharmaceuticalindustry, cosmetics preparation, water filtration, and catalytic systems. Developments in nanotechnology lead to the emergence of newmaterials which contribute to the environmental burden of engineerednanoparticles (NP). This has resulted in a growingenvironmentalimpact of direct exposures to NP, which thoroughly established(Brayner, 2008). It is, therefore, imperative to explore both the cost-effective and eco-friendly methods of synthesizing NP and to furtherevaluatetheir safety fromthe standpointofeco-friendliness(Navarro etal., 2008a; Singh et al., 2009). Plant leaf extract mediated synthesisevaluation of NP is one such green technology that has the potential toaddress the public concern in the above perspectives.

Application of green technology has reduced the use of hazardous reagents andsolvents, improved the material and energy efficiency of chemical processes, and enhanced the design of non-toxic products.

This present study the silver nanoparticles were synthesized from plant leaf essential oil Rosemarinus officinalis and thesenanoparticles was characterized. Antifungal activity test was done to know the biological activity of synthesized silver nanoparticles against the most pathogenic fungi.

MATERIALSANDMETHODS

1. Plantleafessentialoils

Theplantleafessential oils were purchasedfromCommercialcenter Aromax Trading Company, Chennai, Tamil Nadu (India). Thesilver nitrate ($AgNO_3$) was purchased from Hi Media (Mumbai, India).

2. Fungal Strains

The fungi used in this assay Aspergillus niger, Aspergillusflavus, Candida albicans, Candida tropicalis and Candida kefyr wasprovided from Christian medical college vellore Tamil Nadu, India.

3. UV–visiblespectraanalysis

The biosynthesis of Ag nanoparticles was monitoredperiodically in a UV–visible spectrophotometer (Shimadzu, UV-2550, Japan). For the analysis, 0.1mL of the sample in a cuvette andwasdiluted to 2mL with deionized water. The UV–visible spectra of theresulting diluents were monitored as a function of reaction time andbiomaterial dosage at a resolution of 1 nm.

4. Biosynthesisofsilvernanoparticles

Inthe typically synthesis process of silver nano particles, add10ml of plant essential oil into the 90ml of 1mM ofsilvernitratesolution in 250 ml conical flask. The reaction mixture was kept at roomtemperature under mechanically stirring. The colour change was notedand nano particles formation was monitored using UV-visSpectrophotometer periodically.

5. TEMobservationofsilvernanoparticles

Thirty minutes after the reaction, the biomass had settled atthe bottom of the conical flasks and the suspension abovetheprecipitate was sampled for transmission electron microscopy

(TEM)observation. TEM samples of the aqueous suspension of Ag nanoparticles were prepared by placing a drop of the suspension on carbon-coated copper grids and the films on the TEM grids were allowed tostand for 2 min, after which the extra solution was removed using ablotting paper and the grid was allowed to dry prior to measurement. TEM observations were performed on a HITACHI-JP/H7600 instrument(Japan) operated at an accelerating voltage of 100 kV. The sizedistribution ofthe resulting nanoparticles was estimated on the basis of TEM micrographs with the assistance of Sigma Scan Pro software (SPSSInc., Version 4.01.003). Energy dispersive X-ray (EDX) analyses wereperformed on a JEOL JSM-6400 microscope (Japan) fitted with Oxford-6506 (England)EDX analyser.

6. FTIR Analysis

Perkin-Elmer spectrometer FTIR Spectrum ONE in therange4000–400 cm−1 at a resolution of 4 cm−1. was used. The samplewas mixed with KCl procured from Sigma. Thin sample disc wasprepared by pressing with the disc preparing machine and placed in Fourier Transform Infra Red[FTIR]fortheanalysisof the nanoparticles.

7. XRDAnalysis

The silver nano particles was coated onto a glass substrateand the XRD measurements were carried out using a Philips X'Pert ProX-Ray diffractometer, with the following working conditions: CuK_ Ni-filtered radiation; 40 kV, 30 mA; divergence slit 0.47◦.

8. SEM-EDX Analysis

To determine the morphology of the synthesized silvernanoparticles using seed powder extract, the sample was analysed with Zeiss 700 Scanning electron microscope (SEM). The redispersednanoparticles were dried in an oven to obtain a powdered form. Then, 10 mg of the sample was redispersed in ethanol and the sample wasprepared in thin films on carbon coated copper grid. EDX was used forelemental analysis in the sample.

9. Fluorescence Spectraanalysis

Fluorescence Spectra Analysis was done to confirmthe silvernanoparticles. The reduction of silverionswas monitored by measuringthe fluorescence spectrum by taking small amount of the sample in the U-bottom micro titter plate. Thistechnique has provedto be very usefulfor the analysis of nano particles.

Determination of antifungalactivity

Agarwelldiffusionmethod

In this study standard agar well diffusion method wasfollowed (Perez et al., 1990; Perez et al., 1999; Erdemogllu et al., 2003; Bagamboula et al., 2004). Each fungal isolate was suspended in PotatoDextrosebroth HimediaMumbai, Maharashtra(India) brothand dilutedto approximately 105 colony forming unit (CFU) per mL. They were “flood inoculated onto the surface of Potato Dextrose agar and thendried. Five-millimeter diameter wells were cut from the agar using asterile cork-borer, and 25 µl of the samples solutions weredeliveredinto the wells. The plates were incubated for 48 h at room temperature. Antimicrobialactivity was evaluated by measuringthezoneofinhibitionagainst the test microorganisms. Ethanol was used as solvent control. Amphotericin B was used as reference antifungal agent for molds andfluconazole was used for yeast like fungi. The tests were carried out intriplicate.

RESULTS

1. Invitroantifungalassay

Theplant essentialoil Rosemarinusofficinalis showednotableantifungal activity against Aspergillus niger, Aspergillus flavus, Candidaalbicans, Candidatropicalis and Candidakefyrin (Table. 1). Theessentialoil Rosemarinus officinalis was very highly active against Candidatropicalis (12.36±0.48) and least against Candida albicans (4.35±0.35). Silver nitrate solution was highly active against Candida tropicalis(6.27±0.44) and least against Candida albicans (3.01±0.33). The silvernanoparticle Rosemarinus officinalis was also highly active againstCandida tropicalis (22.23±0.78) and least against Aspergillus flavus(14.59±0.58). All fungiwere found to be sensitive to all test essential oilRosemarinus officinalis andsynthesized silver nanoparticle Rosemarinusofficinalis and mostly comparable to the standard reference antifungaldrug Amphotericin B and fluconazole to some extent.

Table No.1: AntifungalactivityofsynthesizedsilvernanoparticleRosemarinusofficinalisoil.

Microorganisms	R.officinalisoil	Agno3	silvernanoparticleR. officinalis	Antifungalagents
Aspergillusniger	11.38±0.17a	4.22±0.09a	16.31±0.14a	7.09±0.67a Amphoterecin-B
Aspergillusflavus	5.37±0.16b	3.35±0.16b	14.59±0.38b	9.06±0.77b Amphoterecin-B
Candidaalbicans	4.35±0.35c	3.01±0.33c	15.35±0.05c	11.25±0.19c Fluconazole
Candidatropicalis	12.36±0.48d	6.27±0.44d	22.23±0.78d	13.04±0.21d Fluconazole

Candidakefyr	5.89±0.59e	4.11±0.67e	16.13±0.24e	12.08±0.88e Fluconazole

The values are represented as the Mean±S Dofessential oil Rosemarinus officinalis and synthesizedsilvernanoparticle Rosemarinus officinalis. Theseessential oil Rosemarinus officinalis and synthesizedsilver nanoparticle Rosemarinusofficinalis havesignificant effect at 0.05 levels.

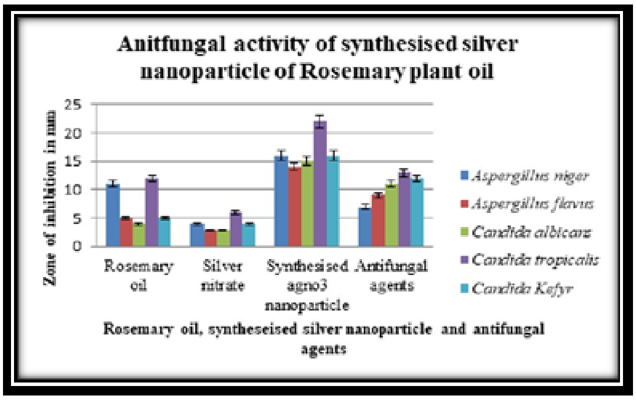


Fig.1:InhibitionofgrowthofselectedbacteriabysynthesizedsilvernanoparticleRosemarinus officinalis.

2. Biosynthesisofsilvernanoparticles

The plant leaf essential oil Rosemarinus officinalis of20 mlwas added to 80 ml of 2 mM Agno3 solution in a conical flask, and themagnetic stirrer startedvigorously stirredinside the conicalflaskwhichis on the hot plate the biosynthesis reaction started after continuousstirring with magnetic stirrer for twenty five minutes and the colorreaction was observed in which clear Agno3 solution changed into very dark brown color which indicatesthat formation of correspondingsilvernanoparticles shown in (Fig. 2a and 2b). The UV–Vis spectra of silvernanoparticles synthesized by Rosemarinus officinalis leaf oil are showednarrow peak observed at 450 nm was seen in (Fig. 2c).

3. SEMandEDXanalysis

The scanning electron microscope showed the structure ofsynthesized silver nanoparticles was observed which is in condensedform (Fig 3a). The analysis of energy dispersive spectroscopy (EDS) ofthe silver nanoparticles the presence of elemental silver signal wasconfirmed (Fig 3b).

4. TEManalysis

The transmission electron microscope image of silvernanoparticle was noted in (Fig 4). The picture suggests that thesmalldot round shaped silver nanoparticles is distributed widely with adiameter of 52 nm.

5. XRDanalysis

The X-ray diffraction pattern of silver nanoparticle wasshown in (Fig 5). The XRD pattern thus clearly illustrates that the silvernano particles present green synthesis method are powdery in nature.

6. FTIR Analysis

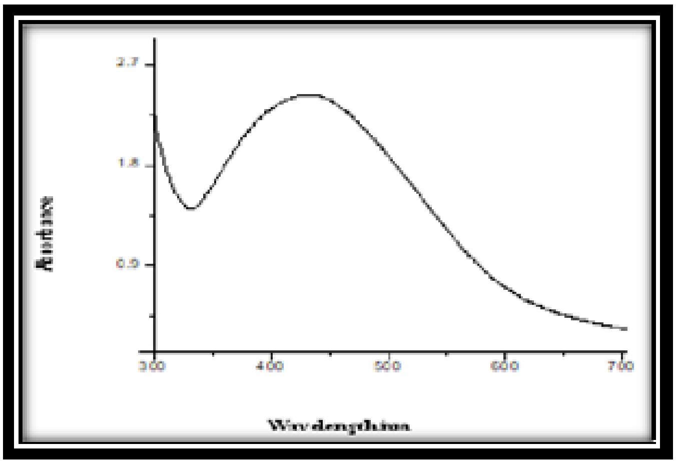
FT IR spectroscopic studieswere carriedout to investigate tofind possible bioreducing agents present in the plant oil. The spectra ofplant oil were recorded before and after adding the silver nitratesolution (Fig. 6). The spectrum indicates major peak at 3472 cm-1. The FTIR spectrum also reveals the presence of alkenes C=Cstretchvibration(1743cm-1), ether C-O stretch(1087cm-1). Inadditionto this, other peakswereobtainedat 1645cm-1, 1087cm-1and minor peaksat2933cm-1, 1373 cm-1, 560 cm-1.

The major peak at 3472 cm-1 indicates the presence of OHgroup and Alkenyl C=C Stretch, Alcoholic C - O Stretch, which plays amajor role in the synthesis of seed powder, mediated silvernanoparticles.

7. FluroscenceSpectroscopyanalysis

The fluroscent spectroscopy of silver nanoparticle wereshown in (Fig 7). A broad emission band having prominentpeakcentered at 185∼ nm is observed for the plant oil as it is excited at 500nm. In this study emission intensity gradually increases with thedecreasing concentration of Agno3. This decreasing intensitysuggest that due to the close proximity of emissive species with nanoparticles, quenching of emissiontaketakes place through energy transfer process.

(A) **(B)**



(C)

Fig.2:A)Silvernitrate solution;B)SilvernanoparticleRosemarinusofficinalis oil;C)UV–Vis spectrum analysis of silvernanoparticlereduced by Rosemarinusofficinalis plant leaf oil at 450 nm.

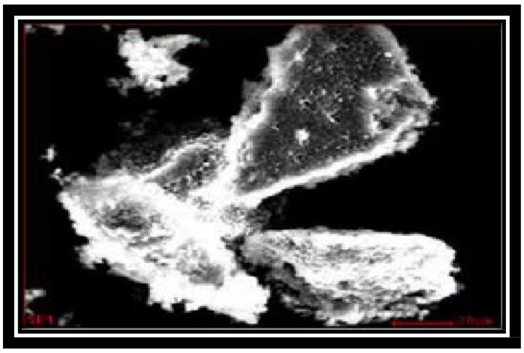


Fig.3a:Scanningelectronmicroscopeimageofsilvernanoparticlesynthesizedbyplant leaf oil Rosemarinus officinalis.

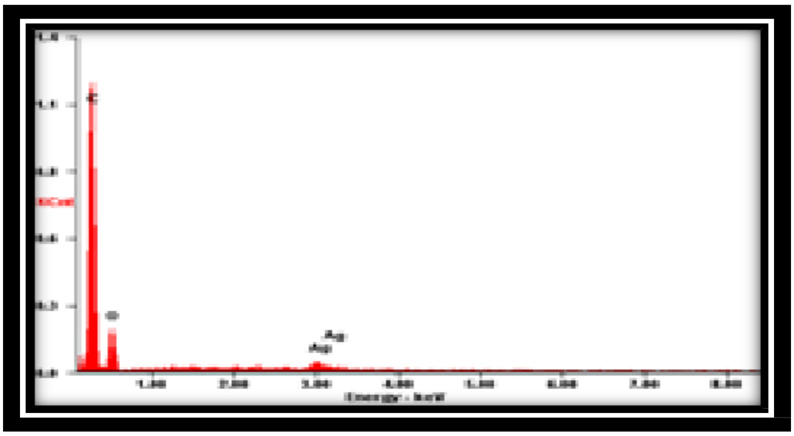


Fig.3b: Sem-EDSspectrumshowedthepresenceofsilversignal.

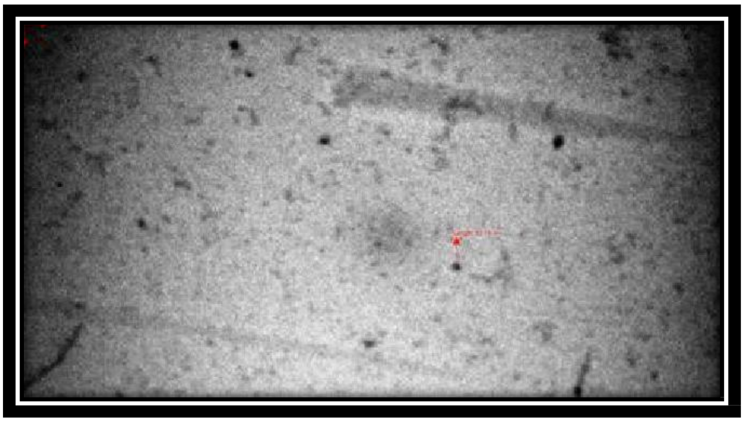


Fig.4: Transmissionelectronmicroscopeimageofsilvernanoparticle synthesizedby plant leaf oil Rosemarinus officinalis.

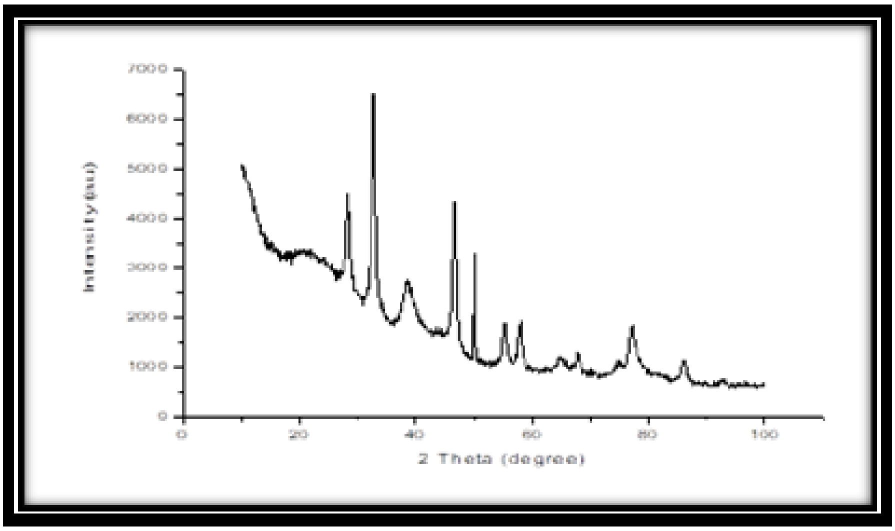


Fig.5:XRDpatternsofsilvernanoparticlessynthesizedbyplantleafessentialoilRosemarinus officinalis.

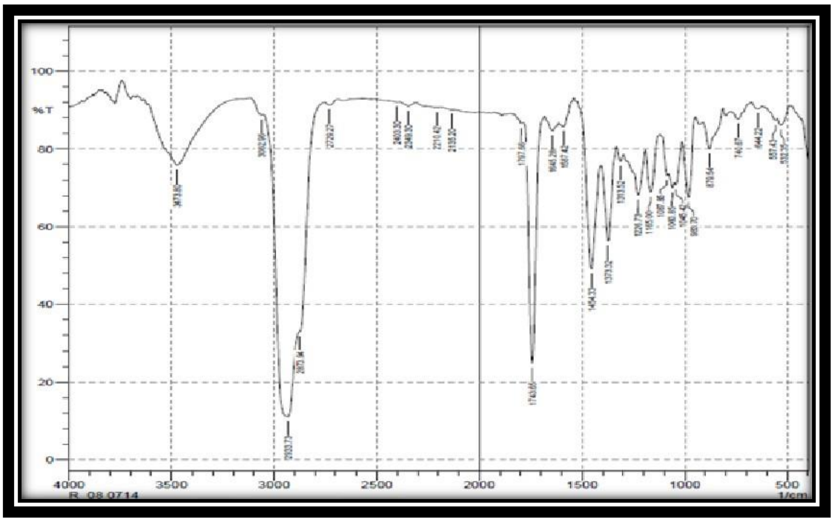


Fig.6:FTIRspectrumofvacuumdriedpowderofsilvernanoparticlessynthesizedbyplant leaf essential oil Rosemarinus officinalis.

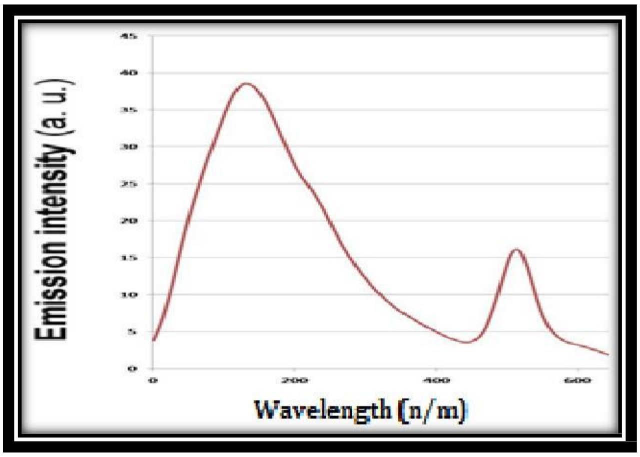


Fig.7:Fluorescenceemissionspectra(excitationat500nm)ofsynthesizedsilvernanoparticlesf romplant leaf essential oil Rosemarinus officinalis.

DISCUSSION

The green syntheses of nanoparticles opena new possibilityof conveniently synthesizing pure metallic or bimetallic nanoparticlesusing natural products. However, the mechanism of green route to synthesis of nanoparticles is not completely understood. Biologicalmolecules are interact with metal salts through these functional groupsand mediate their reduction to nanoparticles [Dickson, 1999]. A recentresearch stated the additional valuable note that Caffeine and theophyllinethesurface-activemoleculesstabilizingthenanoparticle catalyzes synthesis of fewer nanoparticles [Groning et al., 2001]. With Rosemarinus officinalis oil, it was reported that terpenoids are believedto be and reaction of the metal ions is possibly facilitated by reducingsugars and/or terpenoids present in the plant oil [Shankar et al., 2004].

Recent results with Rosemarinus officinalis plant oil indicated that theproteins which have amino groups played a reducing and controllingrole during the formation of AgNPs in the solution and the secondarystructure of the proteins changed after reaction with silver ions[Chandran et al., 2006]. The result from a study alleged that the enzymereductase together with electron shuttling compound and otherpeptides/proteins may be responsible for the reduction of Ag ions andthe continues formation of silver nanoparticles [Saifuddin et al., 2009]. Extracellular synthesis of nanoparticles could be highly advantageousfrom the point of view synthesis in large quantities and easydownstream processing [Ingle et al., 2008]. More elaborate studies arerequired to elucidate the exact mechanism of biological nanoparticlessynthesis.

One of the most interesting aspects of Ag nanoparticle is thattheir optical properties depend strongly upon the particle size & shape. These optical properties are dominated by the collective oscillation ofconduction electrons resulting from the interaction withelectromagnetic radiation. Controlling the spontaneous precipitation ofsilver nanoparticles occurs in which medium, is called solgel medium. This controlling procedure is fully successful by different plant latex. When light absorbancecapacity of solgel medium is increased, thensizeof nano particle is increased & when peak height for UV-Vis absorption(nm) is increased, then concentration of nano particles is increased. Nano particles have a large surface area compared with thetotalvolume. The surface area to volume ration is interesting becausechemical reactions typically occurs on surfaces, so nano particles that have a high surface to energy ration can be used in many interestingways such as in catalysis.

Use of silver nanoparticle should emerge as one of the novelapproaches in cancer therapy and when the molecular mechanism oftargeting is better understood, the applications of silver nanoparticlesare likely to expand further (Satyavani et al. 2011). The silvernanoparticles show efficient antimicrobial activity compared to othersalts. Therefore, silver is ideally suited for effective control of germs, molds andfungus. Its benefit over theuseof antibiotics can be used as apowerful strategy to combat the increasing spread of multidrugresistance resulting from broad use of antibiotics. Therefore, clinicalefficiency of antibiotics has been compromised (Ghosh et al. 2012). Themain biomolecules responsible for nanoparticle synthesis werepolyphenols or flavonoids (Ghosh et al. 2012). In one of ourrecentstudy, thesynthesisof silver nanoparticles usingwhole plantextracts of Rosemarinus officinalis has been reported (Malabadi et al. 2012). Antibacterial activity of silver nanoparticles was assessed by using discdiffusion method against Aspergillus niger, Aspergillus flavus, Candidaalbicans and Candida tropicalis. The results of this study also clearlyindicated that silver nanoparticles synthesized from plant extracts of Rosemarinus officinalis has many pharmaceutical applications for thecontrol of deadly pathogens (Malabadi et al. 2012). The significant andhigher anti fungicidal activity of Aspergillus flavus are probably due tothepresenceofflavonoidsintheplant(Malabadietal. 2005; Malabadietal. 2012). Shanker et al. (2003) demonstrated the rapid synthesis ofstable silver nanoparticles in high concentration usingproteins/enzymes extracted from Rosemarinus officinalis leaf. Thereduction of metal ions and stabilization of the silver nanoparticles isbelieved to occur by an enzymatic process (Shankar etal. 2003).

Biological properties of nanoparticles are largely influencedbysize, distribution and morphology (Satyavani et al. 2011; Malabadi etal. 2012a, 2012b; Farooqui et al. 2010). There are many applications ofnanoparticles in medicine and they play an important role in drugdelivery(Malabadi etal. 2012a, 2012b).ReductionofAg(I)to Ag(o)canbe achieved by chemical, electrochemical, and phytochemical reductionas well as thermal, ultrasound, microwave, gamma and electronirradiation (Hettiarachchi and Wickramarachchi, 2011). Silvernanoparticlesare novel silver compounds composedof clustersofsilveratoms developed using nanotechnology.

CONCLUSION

At first time, we reported that Rosemarinus officinalis plantleaf oil is found to be suitable for the synthesis AgNPs with 20 min and found that the optimum quantity of plant leaf oil, silver nitrateconcentration, temperature and time for the rapid and controlledformationofAgNPs Thespectroscopic characterizationfrom UV-Vis, FT-IR, TEM and SEM-EDX support the stability of green synthesizedAgNPs. Very importantly, the antifungal study also discovers the toxic nature of AgNPs against human fungal pathogens. This study supports the simple, rapid and economical green route synthesis of AgNPs with eco-friendlymanner and their capability of rendering the antifungal efficacy. Moreover the synthesized AgNPs enhance the therapeutic efficacy andstrengthen the medicinal values of these plants.

ACKNOWLEDGMENTS

The authors wish to thank Indian Institute of Technology Sophisiticated Analytical and instrumentation facility ChennaiTamilnadu, India for their full support to complete this nanotechnologyprojectwork.

REFERENCES

1. Bagamboula CF, Uyttendaele M, Debevere J. Inhibitory effect ofthyme and basil essential oils, carvacrol, thymol, estragol, linalool and p- cymene towards Shigella sonnei and S. flexneri. Food Microbiol, 2004; 21: 33–42.
2. Bhatt I, Tripathi BN. Interaction of engineerednanoparticleswith various components of the environment and possiblestrategiesfortheirriskassessment. Chemosphere, 2011; 82: 308-317.
3. Brayner R. The toxicologicalimpact of nanoparticles. Nanotoday, 2008; 3: 48–55.
4. Chandran SP, M. Chaudhary, R. Pasricha A. Ahmad and M. Sastr. Synthesis of gold nanotriangles and silver nanoparticles usingAloe vera plant extract. Biotechnol Prog, 2006y; 22: 577-583.
5. Dickson DPE. Nanostructured magnetism in living systems. JMagn Magn Mater, 1999; 203: 46-49.
6. Egorova EM, Revina AA. Synthesis of metallic nanoparticles inreverse micelles in the presence of quercetin. Colloid Surf A, 2000; 168: 87-96.
7. Erdemogllu N, Ku¨ peli E, Yes ilada E. Anti-inflammatory andantinociceptive activity assessment of plants used as remedy in Turkish folk medicine. J Ethnopharmacol, 2003; 89: 123-129.
8. Farooqui AMD, Chauhan PS, Moorthy SN, Shaik PK (2010)Extraction of silver nanoparticles from the left extracts of Clerodendrum incerme. Digest J Nanomater Biostruct, 2010; 5: 43-49.
9. Ghosh S, Patil S, Ahire M, Kitture R, Kale S, Pardesi K, CameotraSS, Bellare J, Dhavale DD, Jabgunde A, Chopade BA. Synthesis of silver nanoparticles using Dioscorea bulbifera tuber extract andevalution of its synergistic potential in combination withantimicrobial agents. Int J Nanomed, 2012; 7: 483-496.
10. Gole A, Murphy CJ. Seed-mediated synthesis of gold nanorods: role of the size and nature of the seed. Chem Mater, 2004; 16: 3633-3640.
11. GroningR, J. Breitkreutz, V. BarothandRS.Muller. Nanoparticlesin plant extracts: factors which influence the formation ofnanoparticles in black tea infusions. Pharmazie, 2001; 56: 790-792.
12. Hettiarachchi MA, WickramarachchiPASR. Synthesisof chitosanstabilized silver nanoparticles using gamma ray radiation andcharacterization. J Sci University of Kelaniya, 2011; 6: 65-75.
13. IngleA, M.Rai, A.Gade and M. Bawaska.Fusariumsolani: a novelbiological agent for the

extracellular synthesis of silvernanoparticles. J Biobased Mater Bioenergy, 2008r; 2: 243-7.
14. Malabadi RB. Antibacterial activity in the rhizome extract ofCostus speciosus (Koen.). J Phytol Res, 2005; 18(1): 83-85.
15. Malabadi RB, Lokare- Naik S, Meti NT, Mulgund GS, Nataraja K, Vijaya Kumar S. Synthesis of silver nanoparticles from in vitroderived plants and callus cultures of Clitoria ternatea; Evaluation of antimicrobial activity. Res in Biotech, 2012a; 3(5): 26-38.
16. Malabadi RB, Mulgund GS, Meti NT, Nataraja K, Vijaya Kumar S.Antibacterialactivityofsilvernanoparticlessynthesizedby usingwholeplantextracts of Clitoriaternatea. Resin Pharm, 2012b; 2(4): 10-21.
17. Meltzer S, Resch R, Koel BE, Thompson ME, Madhukar A, Requicha A, Will P. Fabrication of nanostructures byhydroxylamine seeding of gold nanoparticle templates, Langmuir, 2001; 17: 1713-1718.
18. Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan M, Kumar R,Sastry M. Extracellular synthesis of gold nanoparticles by thefungus Fusarium oxysporum. Chem Bio Chem, 2002; 5: 461-463.
19. Navarro E, BannA, BehraA, Hartman NB, Filser J, Miao AJ, QuiggA, Santischi PH, Sigg L. Environmental behavior andecotoxicology of engineered nanoparticles. Ecotoxicol, 2008a; 17: 372–386.
20. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Acta Biol Med Exp, 1990; 15: 13-115.
21. Perez C, Agnese AM, Cabrera JL. The essential oil of Seneciograveolens (Compositae): chemical composition andantimicrobial activity tests. J Ethnopharmacol, 1999; 66: 91-96.
22. Saifuddin N, Wong CW, Nur-Yasumira AA. Rapid biosynthesis ofsilver nanoparticles using culture supernatant of bacteria withmicrowave irradiation. E-J Chem, 2009; 6: 61-70.
23. Satyavani K, Gurudeeban S, Ramanathan T, Balasubramanian T.Biomedical potential of silver nanoparticles synthesized fromcalli cells of Citrullus colocynthis (L.) Schrad. J Nanotech, 2011; 43(9): 1-8.
24. Shankar SS, Ahmad A, Pasricha R, Sastry M. Bioreduction ofchloroaurate ions by geranium leaves and its endophytic fungusyields gold nanoparticles of different shapes. J Mater Chem, 2003a; 13: 1822-1826.
25. Shankar SS, A. Rai, A. Ahmad and MJ. Sastry. Rapid synthesis of Au, Agandbimetallic Aushell nanoparticlesusing Neem.J CollidInterface Sci, 2004; 275: 496-502.

26. Singh N, Manshian B, Gareth JS, Jenkins GJS, Griffiths SM, Williams PM, Maffeis TGG, Wright CJ, Doak SH.Nanogenotoxicology: the DNA damaging potentialof engineerednanomaterials. Biomaterials, 2009; 30: 3891-3914.
27. Song JY, Kim BS. Rapid biological synthesis of silvernanoparticles using plant leaf extracts. Bioproc Biosyst Eng, 2008; 32: 79-84.
28. Zhang X, Yan Y, Tyagi RD, Surampalli RY. Synthesis ofnanoparticles by microorganisms and their application inenhancing microbialreactionrates. Chemosphere, 2011; 82: 489-494.