



RESEARCH ARTICLE

**Preliminary Investigation of Synthesizing Silver Nanoparticles from the Different
Biological Source: A Modern Eco-friendly Tool**

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Manuscript No: IJPRS/V4/I1/00023, Received On: 06/02/2015, Accepted On: 16/02/2015

ABSTRACT

Nanotechnology is nothing about designing the particles or materials, in nanoscale production. It involves a lot of chemical and physical method to control the size of nanoparticles produced, which creates pressure and toxicity to the environment, but the rise of biological method to synthesis will overcome all the drawbacks that have been reported earlier. Silver nanoparticles were successfully synthesized from silver nitrate through a simple, green and natural route using the different parts of a plant, lichen and mushroom. Silver Nanoparticle synthesis is proved under UV-Vis absorption spectroscopy, synthesized nanoparticles were quite stable and no visible changes were observed even after a month or so. Nanoparticles have wider application in the medical world like gene therapy, Cancer therapy, drug delivery, etc. and medical world also accepts the biological world of non-polluted, Eco-friendly and non-expensive nature of synthesizing nanoparticles.

KEYWORDS

Eco friendly, biological method, mushroom, plants, *Ganoderma lucidum*, *Phellinus igniarius*, *Parmelia sulcata*, *Parmelia perlata*, *Poystichen aerostichoides*, *Garcinia cambogia*, *Agaricus bisporous*, *Santalum album*, *Courouptia guianensis*, *Tridax procumbens*, *Myristica fragrans*, *Calotropis gignatea*, *Kigelia pinnata*

INTRODUCTION

Nanotechnology is the art of manipulating the particles in nanometer. Nano size range of particle has been found in the universe for millions of years¹, it has been known for ages. This has been used in the preparation of medicines especially in siddha, eg churanam-it will be in size of nanoparticles, and still today it's in the world wide market. Nowadays nanoscale materials have been found in use in different areas such as electronics, biomedical, pharmaceutical, cosmetics, catalyst and material application.

Due to the advancement of technology, there has been a worldwide increase in investment in nanotechnology². Biological method of synthesizing metal nanoparticles brings a "green revolution" in the field of nanotechnology. Especially synthesizing of silver nanoparticles by biological sources from prokaryotes and Eukaryotes³⁻⁹ like bacteria, fungi¹¹, mushroom^{12,13} and plants¹⁴⁻¹⁷. Then the formation of nanoparticles and size could be varied by altering parameters such as temperature, pH, substrate, concentration and exposure time in synthesizing. Various techniques, chemical¹⁸⁻²¹ and mechanical method have been used in the production of nanoparticles²²⁻²⁴.








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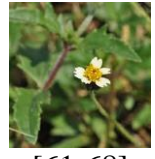


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Table 1: Mushroom, Plants and lichens used for the synthesizing of silver nanoparticles

S. No	Binomial Name	Common name	Family	Part Used	Activity	Images/ References
1	<i>Ganoderma lucidum</i>	Reishi, lingzhi	Ganodermataceae	Fruiting body	Anti-hyperglycemic effect, immunomodulating, Immunostimulating, anti-mutagenic, anti-cancer, antioxidant	 [26-29]
2	<i>Phellinus igniarius</i>	Willow bracket	Hymenochaetaceae	Fruiting body	Immunoregulatory Pulmonary inflammation, renal protective, Antioxidant	 [30-36]
3	<i>Parmelia sulcata</i>	Shield lichen	Parmeliaceae	Thallus	Anti-viral, anti-tumor, anti-inflammatory, analgesic, antipirethic, antiproliferative, antiprotosoal	 [37-40]
4	<i>Parmelia perlata</i>	Stone flowers	Parmeliaceae	Thallus	antimicrobial , Anti-viral, antioxidant activity analgesic, antipyretic antispasmodic potential	 [38,41-44]
5	<i>Agaricus bisporus</i>	Button mushroom	Agaricaceae	Fruiting body	antiinflammatory, hypoglycemic hypocholesterolemic	 [45-47]
6	<i>Polystichen acrostichoides</i>	Christmas fern	Dryopteridaceae	Leaf	blood purifier, emetic, febrifuge treatment for chills, fevers, pneumonia, stomach or bowel complaints rheumatism	 [48,49]
7	<i>Garcinia cambogia</i>	Brindal Berry	Clusiaceae	Leaf Fruit Water liquid	Anti-obesity	 [50-54]

8	<i>Santalum album</i>	Sandal wood	Santalaceae	Leaf Bark	heart, stomach, liver, anti-poison, fever, memory improvement blood purifier hemorrhage bleeding piles, vomiting, poisoning, hiccoughs initial phase of pox, urticaria, eye infections, inflammation of umbilicus	 [55-57]
9	<i>Courouptia guianensis</i>	Cannon ball	Lecythidaceae	Leaf Flower Fruit	antioxidant properties, protection against allergies, inflammation, platelet aggregation, microbes ,ulcers, heptatotoxins, viruses and tumors	 [58-60]
10	<i>Tridax procumbens</i>	Coat buttons	Asteraceae	Leaf	Immunomodulatory, Antidiabetic, Anti-hepatotoxic, Anti-oxidant, Anti-inflammatory, Analgesic and marked depressant action on Respiration	 [61-68]
11	<i>Myristica fragrans</i>	nutmug	Myristicaceae	Leaf Fruit Seed covering	anti-inflammatory, anti-cancer antifungal, antibacterial, larvicidal and antioxidant	 [69-73]
12	<i>Calotropis gignatea</i>	Gigantic Swallow wor	Apocynaceae	Flower Latex	Asthma,Abortifacient, Analgesic, Antinociceptive activity, Antifertility, emmenagogue , Anti-inflammatory activity, Anthelmintic activity, Anti cancer activity , Antidote, Anti tumor activity, Anti-diarrheal, anti dyssentery , Antimicrobial activity, Antiviral activity	 [73-84]

13	<i>Kigelia pinnata</i>	Sausage tree	Bignoniaceae	Flower Fruit Leaf	Treatment for sexually transmitted diseases, dysentery, leprosy, miscellaneous, microbial, parasitic infections, skin ulcers, and neoplastic Diseases	 [85-88]
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Unfortunately the chemicals used like sodium borohydrate, thiophenol, thiourea, macro capto acetate, etc. are toxic enough in is polluting the environment in the larger scale. The production of nanoparticles by bio source will not use any harmful chemicals. This provides a better and a toxic free environment²⁵.

Some specific plant parts or mushrooms have been taken for the studies (Table 1).

MATERIAL AND METHODS

Source of Silver Nitrate

Silver nitrate was purchased from sigma laboratories Pvt Ltd

Extraction and Processing of the Sample

Source of Latex

Crude latex was obtained by cutting the green stems of different plants. Milky white latex and watery latex both were stored at -20°C until use. All the aqueous solutions were prepared using triply distilled de-ionized water⁸⁹.

Source of Leaf, Flower, Seed and Fruit

Different parts of the plant were taken such as leaves, fruits, flowers, seeds and seed outer coating were washed several times with de-ionized water. The extract used for the synthesis of silver nanoparticles were prepared by taking 20 g of samples thoroughly washed, finely cut and boiled with 100ml of triply distilled de-ionized. The extract obtained was filtered through Whatman No 1 filter paper. The filtrate was collected and stored at 4°C for further use⁹⁰.

Source of Thallus and Fruiting Body

The mushrooms and lichens were shredded and mixed with triply de-ionized water in the proportion of 68gms in 250ml water. It was boiled for 2 minutes, cooled and filtered with Whatman No 1 filter paper. Then filtrate was stored at 4°C for further use⁹¹.

Synthesis of Ag Nanoparticles

Source of Latex

In a typical reaction procedure, 3 ml crude latex was diluted to 100 ml using triply distilled de-ionized water to make it 3% and 25 ml of this latex solution was taken and heated at 60°C with constant stirring for 15 min in water bath. Separately 25ml 2 milimolar aqueous silver nitrate solution was prepared and heated at 60°C with constant stirring for 15 min in water bath. Then both of it was mixed and heated at 80°C for 30 to 45 mins and silver nano particles were obtained gradually. This naturally occurs nanoparticles are generated by the erosion and chemical degradation of plant⁸⁹.

Source of Leaf, Flower, Seed and Fruit

1mM Silver Nitrate solution was prepared and to that 10ml extract solution was added with constant stirring. The formation of silver nanoparticles can be observed by the gradual change of colour from yellow to brown⁹⁰.

Source of Thallus and Fruiting Body

35mg AgNO_3 is dissolved in 250mL water. To obtain silver colloids 6ml of mushroom and

lichen extract was added in 30ml of AgNO₃ solution. The formation of Ag nanoparticles is indicated by light yellow colour and the reduction are complete in 30min⁹¹.

Characterization Techniques

UV-VIS spectroscopy

The characterization technique involves ultra-violet and visible spectroscopy UV-Vis absorption spectra were measured using a shimadzu UV-1601 spectrometer at the range 200-800nm. It shows the nature of the particle through different absorption peaks of various samples. The colour changes arise from the excitation of surface plasmon vibrations with the silver nanoparticles. The Surface Plasmon Resonance of silver nanoparticles produced a peak centred near 390-450 nm.

RESULTS AND DISCUSSION

Silver nanoparticles effective antimicrobial agents⁸⁹ are widely recognized and have been used in the various medical applications. There are many methods for synthesis, metal nanoparticles. In the present study, a biological method for synthesizing silver nanoparticles have been carried out by using various biological sources like Plants, Mushrooms and lichens variety (table 1).

The plant, mushroom and lichen extracts are reported to have lots of bioactive compounds and polysaccharides⁹², which display a wide role in biological activities including immune system development. The biological sources used in the study have only limited information available. The additional of biological source with silver nitrate solution under the agitating condition at room temperature, resulted in the gradual change of colour from pale yellow to golden yellow/dark brown after 30 minutes to 5 hours accordingly to source used. (Table 2 & 3) and in some other source there showed no colour change even after 24hours respectively.

The appearance golden yellow/ brown colour clearly indicates the formation of silver nanoparticles in the reaction mixture⁹³. The characteristics, colour change from pale yellow to brown of colloidal silver nanoparticles solution is due to the excitation of surface plasmon vibrations and provides an expedient spectroscopic moniker of their formation. There are several hydroquinones with excellent redox properties that could be act as an electron shuttle in metal reductions^{94,95}. Thus, it shows an evident electron shuttle or others reducing agents released by various biological sources are capable of reducing silver ions to silver nanoparticles.

Table 2: Physiochemical properties of silver nanoparticles synthesized

S. No	Binomial Name		Results	Colour form	Properties	UV-Vis spec Nm	specification
1	<i>Ganoderma lucidum</i>	F	+	Brown	Water soluble	420	Stable for almost 5 months
2	<i>Phellinus igniarius</i>	F	+	Brown		414	Stable for almost 3 months
3	<i>Parmelia sulcata</i>	T	+	Dark brown		432	Stable for almost 2 months
4	<i>Parmelia perlata</i>	T	+	Pale brown		436	Stable for almost 2 months

5	<i>Agaricus bisporous</i>	F	+	Yellow	Water soluble	496	Stable for almost 1 months
6	<i>Polystichen acrostichoides</i>	L	+	Golden yellow		398	Stable for almost 1 months
7	<i>Garcinia cambogia</i>	L	+	Golden yellow		401	Stable for almost 2 months
		F		Golden yellow		410	
		LI	-	Pale		367	
8	<i>Santalam album</i>	L	-	Pale		312	-
		B	-	Pale yellow		350	
9	<i>Courouptia guianesis</i>	L	+	Brown		435	Stable for almost 2 months
		FL	+	Brown		422	
		FR	+	yellow		389	
10	<i>Tridax procumbens</i>	L	-	Pale		282	-
11	<i>Myristica fragrans</i>	L	+	brown		422	Stable for almost 2 months
		S	+	Golden yellow		412	
		SC	+	Golden yellow	403		
12	<i>Calotropis gignatea</i>	LA	+	Dark brown	436	Stable for almost 1 months	
13	<i>Kigelia pinnata</i>	L	-	Pale yellowish	327	-	
		F	-	Pale yellowish	210		

F-FRUITING BODY, T-THALLUS, L-LEAF, FL-FLOWER, FR-FRUIT, S-SEED, SC-SEED COVERING, LA-LATEX, LI-WATERY LIQUID

Absorption peak of silver nano particles using the different extract under UV- Vis absorption spectroscopy

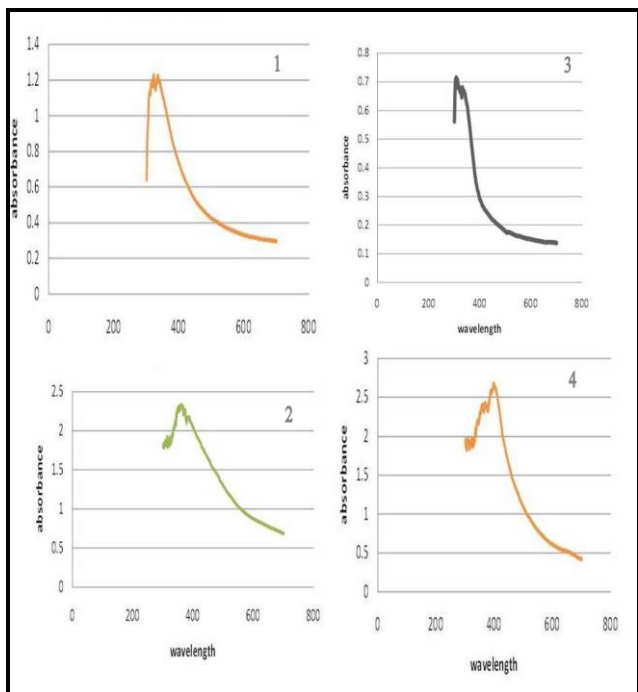


Figure: (1) *Ganoderma lucidum*-fruiting body (2), *Phellinus igniarius*- fruiting body (3), *Parmelia sulcata*-thallus (4), *Parmelia perlata*-thallus

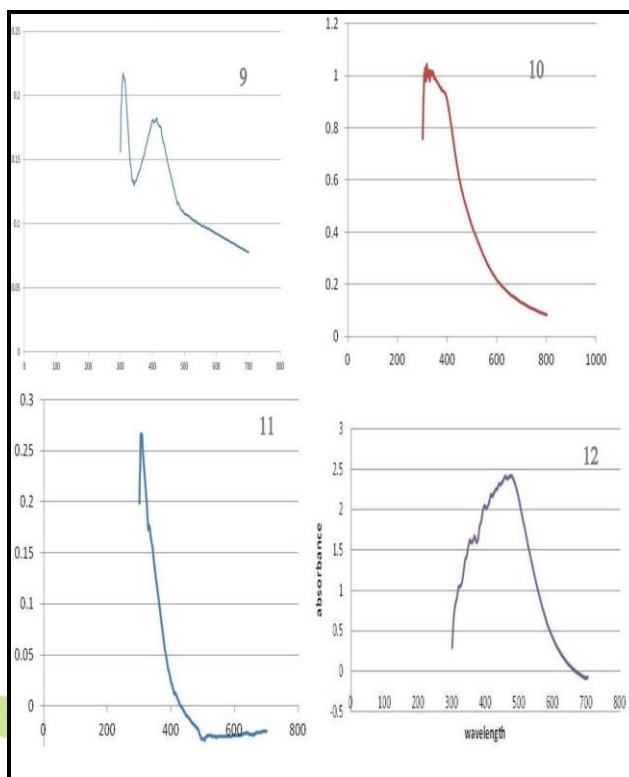


Figure: (9) *Garcinia cambogia*-fruit (10) *Santalum album*-leaf (11) *Santalum album*-bark (12) *Courouptia guianensis*-leaf

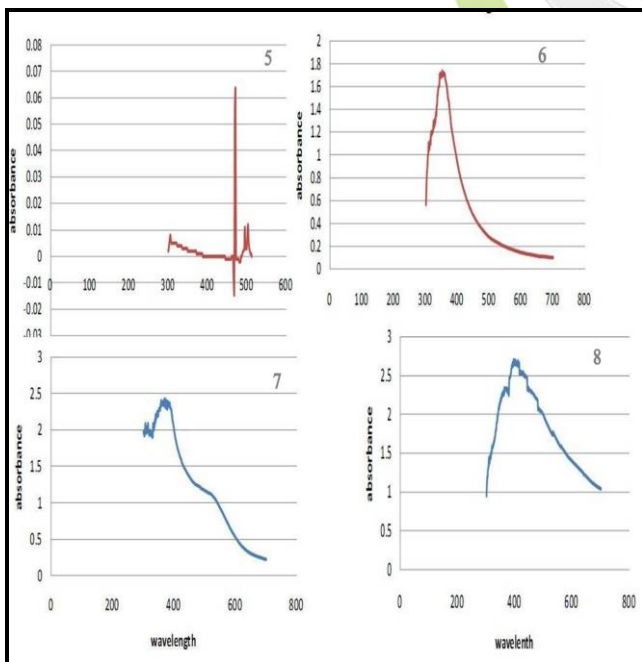


Figure: (5) *Agaricus bisporus*-fruiting body (6), *Polystichen aerostichoides*-leaf (7), *Garcinia cambogia*-leaf (8), *Garcinia cambogia*-flower

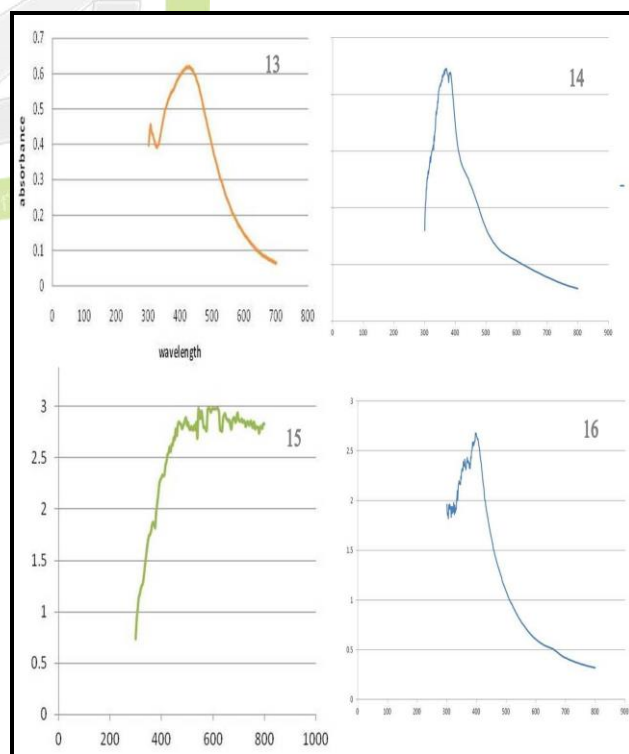


Figure: (13) *Courouptia guianensis*-flower (14) *Courouptia guianensis*-fruit (15) *Tridax procumbens*-leaf (16) *Myristica fragrans*-leaf

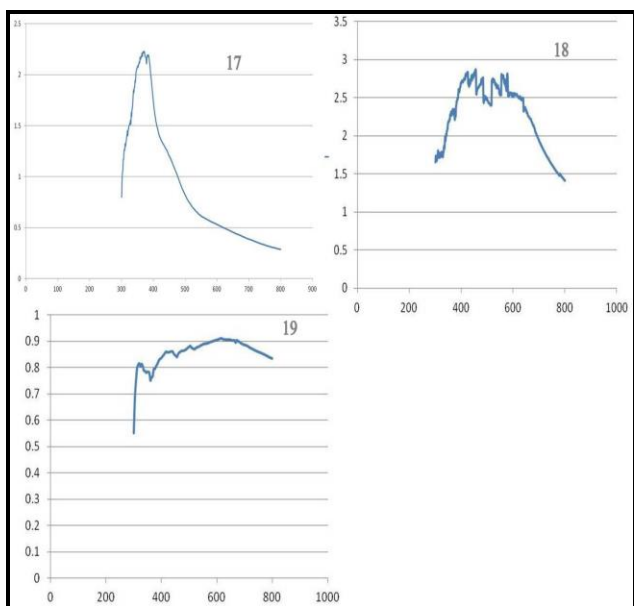


Figure: (17) *Calotropis-latex*, (18) *Kigelia pinnata-leaf* 19, *Kigelia pinnata-flower*

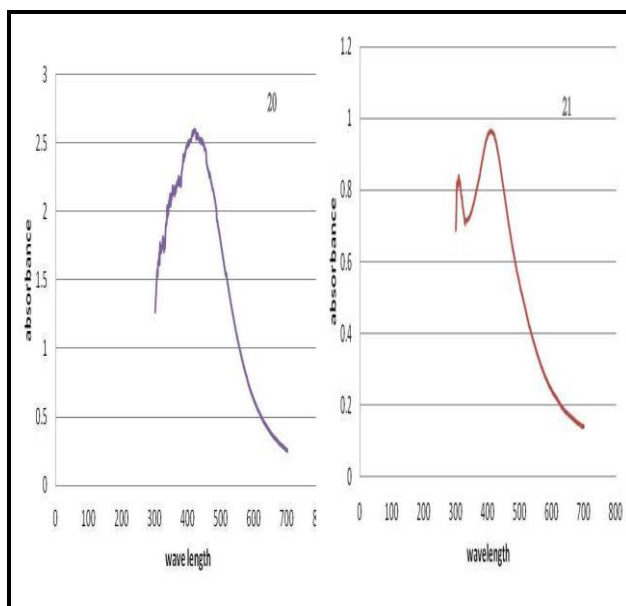


Figure: (20) *Myristica fragrans-seed* (21) *Myristica fragrans-seed covering*

Table 3: Ranking of biological source according to the time taken for silver nanoparticles reduction

S. No	Biological source	Parts	Reduction Time	Rank
1	<i>Ganoderma lucidum</i>	F	15 minutes	3
2	<i>Phellinus igniarius</i>	F	20 minutes	4
3	<i>Parmelia sulcata</i>	T	10 minutes	2
4	<i>Parmelia perlata</i>	T	5 minutes	1
5	<i>Agaricus bisporous</i>	F	3 hours	9
6	<i>Polystichen acrostichoides</i>	L	30 minutes	5
7	<i>Garcinia cambogia</i>	L	30minutes-1 hour	7
		F		
		LI	Nil	Nil
8	<i>Santalum album</i>	L	Nil	Nil
		B	Nil	Nil
9	<i>Courouptia guianensis</i>	L	1.5 hour	8
		FL	20 minutes	4
		FR	1 hour	8
10	<i>Tridax procumbens</i>	L	Nil	Nil
11	<i>Myristica fragrans</i>	L	1.5 hours	8
		S	45 minutes	6
		SC	45 minutes	6
12	<i>Calotropis gignatea</i>	LA	3-4.5 hours	10
13	<i>Kigelia pinnata</i>	L	Nil	Nil
		F	Nil	Nil

It clearly states the reduction of silver nanoparticles happened due to their polyphenolic compounds present in the sample. The ranking of biological source in the reduction can be observed in the table 3.

CONCLUSION

Nano science is the study of phenomena and manipulation of materials at “nanoscale” size. In the present scenario the nanotechnology field has risen and one of the most competitive fields currently. It just the preliminary screening analysis of biological sources for synthesis of silver nanoparticles, for the detail study and confirmation of silver nanoparticles the following method has to carried out: X-ray diffraction spectroscopy (X R D) , Fourier transform infrared (FTIR), Transmission electron microscopy(TEM) & scanning electron microscopy(S E M). In future the following parameter and kinetic study to be practiced to control the size of the nanoparticles, and the various application oriented study can be also opted. Some of the biological sources that have been selected for the study are first to be reported for synthesis of silver nanoparticles.

REFERENCES

1. Nowack, B., & Bucheli, T. D. (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Environmental Pollution*, 150(1), 5-22.
2. Dunphy Guzman, K. A., Taylor, M. R., & Banfield, J. F. (2006). Environmental risks of nanotechnology: National nanotechnology initiative funding, 2000-2004. *Environmental Science & Technology*, 40(5), 1401-1407.
3. Mandal, D., Bolander, M. E., Mukhopadhyay, D., Sarkar, G., & Mukherjee, P. (2006). The use of microorganisms for the formation of metal nanoparticles and their application. *Applied Microbiology and Biotechnology*, 69(5), 485-492.
4. Basavaraja, S., Balaji, S. D., Lagashetty, A., Rajasab, A. H., & Venkataraman, A. (2008). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. *Materials Research Bulletin*, 43(5), 1164-1170.
5. Vigneshwaran, N., Ashtaputre, N. M., Varadarajan, P. V., Nachane, R. P., Paralikar, K. M., & Balasubramanya, R. H. (2007). Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. *Materials Letters*, 61(6), 1413-1418.
6. Vigneshwaran, N., Ashtaputre, N. M., Varadarajan, P. V., Nachane, R. P., Paralikar, K. M., & Balasubramanya, R. H. (2006). Biomimetics of silver nanoparticles by white rot fungus, *Phaenerochaete chrysosporiurit*. *Colloids Interf. B* 53, 55-59.
7. Shahverdi, A. R., Minaeian, S., Shahverdi, H. R., Jamalifar, H., & Nohi, A. A. (2007). Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: a novel biological approach. *Process Biochemistry*, 42(5), 919-923.
8. Shahverdi, A. R., Fakhimi, A., Shahverdi, H. R., & Minaian, S. (2007). Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomedicine: Nanotechnology, Biology and Medicine*, 3(2), 168-171.
9. P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati. S.R. Sainkar, M.I. Khan, R. Ramani, R. Parischa, P.V. Ajaykumar, M. Alam, M. Sastry, R. Kumar., (2001). Bioreduction of AUC14- ions by the fungus, *Verticillium sp*, and surface trapping of the gold nanoparticles formed. *Angewandte Chemie*, Int. Ed. 40, 3585-3588.
10. Roh, Y., Lauf, R. J., McMillan, A. D., Zhang, C., Rawn, C. J., Bai, J., & Phelps, T. J. (2001). Microbial synthesis and the characterization of metal-substituted magnetites. *Solid State Communications*, 118(10), 529-534.
11. Bhattacharya, D., & Gupta, R. K. (2005). Nanotechnology and potential of

- microorganisms. *Critical Reviews in Biotechnology*, 25(4), 199-204.
12. Philip, D. (2009). Biosynthesis of Au, Ag and Au–Ag nanoparticles using edible mushroom extract. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 73(2), 374-381.
 13. Nithya, R., & Ragunathan, R. (2009). Synthesis of silver nanoparticle using *Pleurotus sajor caju* and its antimicrobial study. *Digest Journal of Nanomaterials and Biostructures*, 4(4), 623-629.
 14. Gardea-Torresdey, J. L., Gomez, E., Peralta-Videa, J. R., Parsons, J. G., Troiani, H., & Jose-Yacaman, M. (2003). Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. *Langmuir*, 19(4), 1357-1361.
 15. Vilchis-Nestor, A. R., Sánchez-Mendieta, V., Camacho-López, M. A., Gómez-Espinosa, R. M., Camacho-López, M. A., & Arenas-Alatorre, J. A. (2008). Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract. *Materials Letters*, 62(17), 3103-3105.
 16. S. Shiv Shankar, A. Rai. A. Ahmad, M. Sastry. (2004). Rapid synthesis of Act. Ag, and bimetallic Au core-Ag shell nanoparticles using neem (*Azadirachta indica*) leaf S, broth. *Journal of Colloid and Interface Science*, 275, 496-502.
 17. Bakar, N. A., Ismail, J., & Bakar, M. A. (2007). Synthesis and characterization of silver nanoparticles in natural rubber. *Materials Chemistry and Physics*, 104(2), 276-283.
 18. Yu, D. G. (2007). Formation of colloidal silver nanoparticles stabilized by Na⁺–poly (γ -glutamic acid)–silver nitrate complex via chemical reduction process. *Colloids and Surfaces B: Biointerfaces*, 59(2), 171-178.
 19. Tan, Y., Wang, Y., Jiang, L., & Zhu, D. (2002). Thiosalicylic acid-functionalized silver nanoparticles synthesized in one-phase system. *Journal of Colloid and Interface Science*, 249(2), 336-345.
 20. Petit, C., Lixon, P., & Pileni, M. P. (1993). In situ synthesis of silver nanocluster in AOT reverse micelles. *The Journal of Physical Chemistry*, 97(49), 12974-12983.
 21. Vorobyova, S. A., Lesnikovich, A. I., & Sobal, N. S. (1999). Preparation of silver nanoparticles by interphase reduction. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 152(3), 375-379.
 22. Gericke, M., & Pinches, A. (2006). Biological synthesis of metal nanoparticles. *Hydrometallurgy*, 83(1), 132-140.
 23. Ravindran, T. R., Arora, A. K., Balamurugan, B., & Mehta, B. R. (1999). Inhomogeneous broadening in the photoluminescence spectrum of CdS nanoparticles. *Nanostructured Materials*, 11(5), 603-609.
 24. Pattabi, M., & Uchil, J. (2000). Synthesis of cadmium sulphide nanoparticles. *Solar Energy Materials and Solar Cells*, 63(4), 309-314.
 25. Song, J. Y., & Kim, B. S. (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess and Biosystems Engineering*, 32(1), 79-84.
 26. Chiu, S. W., Wang, Z. M., Leung, T. M., & Moore, D. (2000). Nutritional value of Ganoderma extract and assessment of its genotoxicity and anti-genotoxicity using comet assays of mouse lymphocytes. *Food and Chemical Toxicology*, 38(2), 173-178.
 27. Fang, Q. H., Tang, Y. J., & Zhong, J. J. (2002). Significance of inoculation density control in production of polysaccharide and ganoderic acid by submerged culture of *Ganoderma lucidum*. *Process Biochemistry*, 37(12), 1375-1379.
 28. Liu, X., Yuan, J. P., Chung, C. K., & Chen, X. J. (2002). Antitumor activity of the sporoderm-broken germinating spores of

- Ganoderma lucidum. *Cancer Letters*, 182(2), 155-161.
29. Shao, B. M., Dai, H., Xu, W., Lin, Z. B., & Gao, X. M. (2004). Immune receptors for polysaccharides from Ganoderma lucidum. *Biochemical and Biophysical Research Communications*, 323(1), 133-141.
30. Dai, H. Y., Wang, P., Feng, L. Y., Liu, L. M., Meng, Z. Q., Zhu, X. Y., & Chen, Z. (2010). The molecular mechanisms of traditional Chinese medicine ZHENG syndromes on pancreatic tumor growth. *Integrative Cancer Therapies*.
31. Zhu, T., Kim, S. H., & Chen, C. Y. (2008). A medicinal mushroom: Phellinus linteus. *Current Medicinal Chemistry*, 15(13), 1330-1335.
32. Chang, Z. Q., Hwang, M. H., Rhee, M. H., Kim, K. S., Kim, J. C., Lee, S. P., ... & Park, S. C. (2008). The in vitro anti-platelet, antioxidant and cellular immunity activity of Phellinus gilvus fractional extracts. *World Journal of Microbiology and Biotechnology*, 24(2), 181-187.
33. Shon, Y. H., & Nam, K. S. (2001). Antimutagenicity and induction of anticarcinogenic phase II enzymes by basidiomycetes. *Journal of Ethnopharmacology*, 77(1), 103-109.
34. Shon, Y. H., & Nam, K. S. (2002). Cancer chemoprevention: inhibitory effect of soybeans fermented with basidiomycetes on 7, 12-dimethylbenz [a] anthracene/12-O-tetradecanoylphorbol-13-acetate-induced mouse skin carcinogenesis. *Biotechnology Letters*, 24(12), 1005-1010.
35. Shon, Y. H., & Nam, K. S. (2004). Inhibition of cytochrome P450 isozymes and ornithine decarboxylase activities by polysaccharides from soybeans fermented with Phellinus igniarius or Agrocybe cylindracea. *Biotechnology Letters*, 26(2), 159-163.
36. Han, S. B., Lee, C. W., Jeon, Y. J., Hong, N. D., Yoo, I. D., Yang, K. H., & Kim, H. M. (1999). The inhibitory effect of polysaccharides isolated from Phellinus linteus on tumor growth and metastasis. *Immunopharmacology*, 41(2), 157-164.
37. Davies, J. E., Waters, B., & Saxena, G. (2001). *U.S. Patent No. 6,319,898*. Washington, DC: U.S. Patent and Trademark Office.
38. Halama, P., & Van Haluwin, C. (2004). Antifungal activity of lichen extracts and lichenic acids. *Bio. Control*, 49(1), 95-107.
39. Huneck, S. (1999). The significance of lichens and their metabolites. *Naturwissenschaften*, 86(12), 559-570.
40. Lawrey, J. D. (1986). Biological role of lichen substances. *Bryologist*, 111-122.
41. Thippeswamy, B., Sushma, N. R., & Naveenkumar, K. J. (2012). Antimicrobial property of bioactive factor isolated from Parmelia perlata. *International Multi-disciplinary Research Journal*, 2(2). 01-05.
42. Esimone, C. O., Ofokansi, K. C., Adikwu, M. U., Ibezim, E. C., Abonyi, D. O., Odaibo, G. N., & Olaleye, D. O. (2007). In vitro evaluation of the antiviral activity of extracts from the lichen Parmelia perlata (L.) Ach. against three RNA viruses. *The Journal of Infection in Developing Countries*, 1(03), 315-320.
43. Patil, S. B., Ghadyale, V. A., Taklikar, S. S., Kulkarni, C. R., & Arvindekar, A. U. (2011). Insulin secretagogue, alpha-glucosidase and antioxidant activity of some selected spices in streptozotocin-induced diabetic rats. *Plant Foods for Human Nutrition*, 66(1), 85-90.
44. Robbins, S. L., & Cotran, R. S. (1979). *Pathologic Basis of Disease*. Saunders.
45. Mathe, D. (1995). Dyslipidemia and diabetes: animal models. *Diabete & Metabolisme*, 21(2), 106-111.
46. Kuller, L. H. (2006). Nutrition, lipids, and cardiovascular disease. *Nutrition Reviews*, 64(suppl 1), S15-S26.

47. Layman, D. K., Clifton, P., Gannon, M. C., Krauss, R. M., & Nuttall, F. Q. (2008). Protein in optimal health: heart disease and type 2 diabetes. *The American Journal of Clinical Nutrition*, 87(5), 1571S-1575S.
48. Duke, J. A., Rose, J. B., & Peterson, L. A. (1990). *A field guide to medicinal plants: eastern and central North America* (pp. 225-227). Chicago: Houghton Mifflin.
49. Moerman, D. E. (1998). *Native American Ethnobotany* (Vol. 879). Portland: Timber Press.
50. Conte, A. A. (1993). A non-prescription alternative in weight reduction therapy. *Am J Bariatric Med Summer*, 17-19.
51. Thom, E. (1996). Hydroxycitrate (HCA) in the treatment of obesity. *International Journal of Obesity*, 20 (Suppl 4), 48.
52. Rothacker, D. Q., & Waitman, B. E. (1997). Effectiveness of a Garcinia cambogia and natural caffeine combination in weight loss: A double-blind, placebo-controlled pilot study. *International Journal of Obesity*, 21(Suppl 2), 53.
53. Sawada, H., Tomi, H., Tamura, K., & Anno, T. (1997). Effects of liquid Garcinia extract and soluble Garcinia powder on body weight change: a possible material for suppressing fat accumulation. *Nihon Yukagaku Kaishi*, 46(12), 1467-1474.
54. Heymsfield, S. B., Allison, D. B., Vasselli, J. R., Pietrobelli, A., Greenfield, D., & Nunez, C. (1998). Garcinia cambogia (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial. *Jama*, 280(18), 1596-1600.
55. Benencia, F., & Courreges, M. C. (1999). Antiviral activity of sandalwood oil against herpes simplex viruses-1 and-2. *Phytomedicine*, 6(2), 119-123.
56. Rohadi, D., Maryani, R., Belcher, B., Perez, M. R., & Widnyana, M. (2000). Can sandalwood in East Nusa Tenggara survive? Lessons from the policy impact on resource sustainability. *Sandalwood Research Newsletter*, (10), 3-6.
57. Desai V. B. (1991). Pharmacological Screening of HESP and Sandalwood oil, *Indian Perfumer*, 35(2), 69-70.
58. Elumalai, A., Naresh, V., Eswaraiah, M. C., Narendar, P., & Kumar, R. (2012). Evaluation of Antiulcer Activity of Couroupita guianensis Aubl Leaves. *Asian Journal of Pharmaceutical Sciences*, 2(2), 64-66.
59. Pinheiro, M. M., Fernandes, S. B., Fingolo, C. E., Boylan, F., & Fernandes, P. D. (2013). Anti-inflammatory activity of ethanol extract and fractions from Couroupita guianensis Aublet leaves. *Journal of Ethnopharmacology*, 146(1), 324-330.
60. Manimegalai, S., & Rakkimuthu, G. (2012). Phytochemical screening of stem of couroupita guianensis. *International Journal of Pharmaceutical Sciences & Research*, 3(11).
61. Ravikumar, V., Shivashangari, K. S., & Devaki, T. (2005). Effect of Tridax procumbens on liver antioxidant defense system during lipopolysaccharide-induced hepatitis in D-galactosamine sensitised rats. *Molecular and Cellular Biochemistry*, 269(1), 131-136.
62. Tiwari, U., Rastogi, B., Singh, P., Saraf, D. K., & Vyas, S. P. (2004). Immunomodulatory effects of aqueous extract of Tridax procumbens in experimental animals. *Journal of Ethnopharmacology*, 92(1), 113-119.
63. Bhagwat, D. A., Killedar, S. G., & Adnaik, R. S. (2008). Anti-diabetic activity of leaf extract of Tridax procumbens. *International Journal of Green Pharmacy*, 2(2), 126.
64. Hemalatha, R. (2008). Anti-hepatotoxic and anti-oxidant defense potential of Tridax procumbens. *International Journal of Green Pharmacy*, 2(3), 164.
65. Diwan, P. V., Iravati, K., Margaret, I., & Sattur, P. B. (1989). Pharmacology and

- biochemical evaluation of *Tridax procumbens* on inflammation. *Indian Journal of Pharmacology*, 21(2), 1.
66. Hitesh, J. (2006). Formulation and evaluation of analgesic activity of *Tridax procumbens* Gel. *Indian Journal of Natural Products*, 23(1), 31-33.
67. Cai, X., Zhou, H., Wong, Y. F., Xie, Y., Liu, Z. Q., Jiang, Z. H., & Liu, L. (2005). Suppressive effects of QFGJS, a preparation from an anti-arthritis herbal formula, on rat experimental adjuvant-induced arthritis. *Biochemical and Biophysical Research Communications*, 337(2), 586-594.
68. Nakamura, N., Kiuchi, F., Tsuda, Y., and Kondo, K. (1988). Studies on crude drugs effective on visceral larva migrans. V. The larvicidal principle in mace (aril of *Myristica fragrans*). *Chemical & Pharmaceutical Bulletin*, 36(7), 2685-2688.
69. Ozaki, Y., Soedigdo, S., Wattimena, Y. R., & Suganda, A. G. (1989). Antiinflammatory effect of mace, aril of *Myristica fragrans* Houtt., and its active principles. *The Japanese Journal of Pharmacology*, 49(2), 155-163.
70. Saeki, Y., Ito, Y., Shibata, M., Sato, Y., Okuda, K., & Takazoe, I. (1989). Antimicrobial action of natural substances on oral bacteria. *The Bulletin of Tokyo Dental College*, 30(3), 129-135.
71. Singh, G., Marimuthu, P., Heluani, C. S. D., & Catalan, C. (2005). Antimicrobial and antioxidant potentials of essential oil and acetone extract of *Myristica fragrans* Houtt.(aril part). *Journal of Food Science*, 70(2), M141-M148.
72. Roy, S., Uddin, M. Z., Hassan, M. A., & Rahman, M. M. (2008). Medico-botanical report on the Chakma community of Bangladesh. *Bangladesh Journal of Plant Taxonomy*, 15(1), 67-72.
73. Saxena, H. O. (1986). Observations of the ethnobotany of Madhya Pradesh. *Bull. Bot. Surv. India*, 28, 149-156.
74. Saha, J. C., Savini, E. C., & Kasinathan, S. (1961). Ecobolic properties of Indian medicinal plants. Part 1, *Indian Journal of Medical Research*, 49 (1961). 130 - 151.
75. Patel, N. K., & Patel, K. B. (2004). Study of abortifacient plants used by Adivasi of Ambaji area in Danta taluka in Gujarat. *Advances in Plant Sciences*, 17(1), 37 - 40.
76. Nadkarni, K. M. (1976). Indian materia medica. *Popular Book Dept, Bombay, India*. Mumbai, (1976). 237- 246.
77. Saumya, D., Sanjita, D., Manas, K. D., & Saumya, P. B. (2009). Evaluation of anti-inflammatory effect of *Calotropis gigantea* and *Tridax procumbens* on Wistar albino rats. *Journal of Pharmaceutical Sciences and Research*, 4, 123-126.
78. Adak, M., & Gupta, J. K. (2006). Evaluation of anti-inflammatory activity of *Calotropis gigantea* (AKANDA) in various biological system. *Nepal Medical College journal: NMCCJ*, 8(3), 156-161.
79. Iqbal, Z., Lateef, M., Jabbar, A., Muhammad, G., & Khan, M. N. (2005). Anthelmintic activity of *Calotropis procera* (Ait.) Ait. F. flowers in sheep. *Journal of Ethnopharmacology*, 102(2), 256-261.
80. Sureshkumar, P., Senthilraja, P., & Kalavathy, S. (2012). In-Silico Docking Analysis of *Calotropis gigantea* (L.) R. Br Derived Compound against Anti-Cervical Cancer Activity. *World Research Journal of Computer-Aided Drug Design*, 1(1), 9-12.
81. Choedon, T., Mathan, G., Arya, S., Kumar, V. L., & Kumar, V. (2006). Anticancer and cytotoxic properties of the latex of *Calotropis procera* in a transgenic mouse model of hepatocellular carcinoma. *World Journal of Gastroenterology: WJG*, 12(16), 2517-2522.
82. Hutt, M. J., & Houghton, P. J. (1998). A survey from the literature of plants used to treat scorpion stings. *Journal of Ethnopharmacology*, 60(2), 97-110.

83. Jayaweera, D. M. A. (1980). Medicinal plants (Indigenous and exotic) used in Ceylon: part II." Vol. I-V, National Science Council of Sri Lanka, Colombo, 1980–1982.
84. Alam, M. A., Habib, M. R., Nikkon, R., Rahman, M., & Karim, M. R. (2008). Antimicrobial Activity of Akanda (*Calotropis gigantea* L.) on Some Pathogenic Bacteria. *Bangladesh Journal of Scientific and Industrial Research*, 43(3), 397-404.
85. Houghton, P. J. (2002). The sausage tree (*Kigelia pinnata*): ethnobotany and recent scientific work. *South African Journal of Botany*, 68(1), 14-20.
86. Gabriel, O. A., & Olubunmi, A. (2009). Comprehensive scientific demystification of *Kigelia africana*: A review. *African Journal of Pure and Applied Chemistry*, 3(9), 158-64.
87. Harris, B. J., & Baker, H. G. (1958). Pollination in *Kigelia africana* Benth. *J. West Afr. Sci. Assoc*, 4, 25-30.
88. Akunyili, D. N, Houghton, P. J, Raman, A. (1991). Antimicrobial activities of the stem bark of *Kigelia pinnata*. *Journal of Ethnopharmacology*. 35(2), 173-7.
89. Mondal, A. K., Mondal, S., Samanta, S., & Mallick, S. (2011). Synthesis of Ecofriendly Silver Nanoparticle from Plant Latex used as an Important Taxonomic Tool for Phylogenetic Interrelationship Advances in Bioresearch Vol. 2. *Synthesis*, 31, 33.
90. Rastogi, L. and Arunachalam, J. (2001). *Materials Chemistry and Physics*, 129(1-2), 58- 563.
91. X. Chen, G. Zou, Y. Deng and Q. J. Zhang. (2008). *Nanotechnology*, 19, 195703.
92. J.Cui, Y.Chisti. (2003). *Biotechnology Advances*, 21, 109.
93. Durán N, Marcato P D, Alves O L, Gabriel I H, Souza D E and Esposito E, (2005). *Journal of Nanobiotechnology*, 3, 1-7.
94. Newman, D, K. & Kolter, R. (2000). *Nature*, 405, 94-97.
95. Baker, R. A. & Tatum, J. H. (1998). *Journal of Fermentation and Bioengineering*, 85, 359-361.