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Original Article

Antioxidant activity of gold nanoparticles synthesized using *Lemna minor*

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ABSTRACT

The production of nanoparticles under nontoxic, green conditions is of vital importance to address the growing concerns on the overall toxicity of metallic nanoparticles for medical and technological applications. The present findings disclose *Lemna minor* synthesized gold nanoparticles antioxidant property. The free radical scavenging activity of the synthesized nanoparticles was determined by DPPH assay, Superoxide radical scavenging, Nitric oxide radical scavenging and Hydrogen Peroxide scavenging. The radical scavenging activity obtained in this study with *Lemna minor* synthesized by gold nanoparticles shows significant scavenging activity and as good antioxidant property. The antioxidant activity of natural compounds reported to be mainly due to redox properties in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.

Key words: *Lemna minor*, Gold Nanoparticles, Antioxidant activity, DPPH.

INTRODUCTION

Nanotechnology is a new area of science that involves working with materials and devices that are in the nano scale. Synthesis of noble metal nanoparticles attract an increasing interest due to their novel characteristics as compared with those of macroscopic phase and they find attractive applications in different fields such as antimicrobials [1] medicine, biotechnology, optics and others [2]. Currently the production of nanoparticles under environmentally benign conditions is of vital importance to address the growing concerns on the overall toxicity of nanoparticles for biological applications [3] [4] [5] [6]. The power of phytochemicals that initiate various chemical transformations within biological systems is well known [7] [8] [9]. Nano medicine is being applied at the molecular level to monitor, repair, construction and control of human biological systems using engineered nano devices and nanostructures [10]. Among metal nanoparticles, gold nanoparticles are of particular interest to applications that leverage on their strongly size and shape dependent particles. Hence, it is highly desirable to produce gold nanoparticles with different morphology, size and surface chemistry at high yields. The shape and size dependent optical properties of gold nanoparticles have been exploited in various surface coatings [11] and biomedical applications. They are nontoxic and are capable of delivering large biomolecules such as proteins, [12] DNA and RNA and expose a large surface area for immobilization of such biomolecules. The ability to modulate the surface chemistry of gold nanoparticles by binding suitable ligand has important applications in many areas such as novel organic reactions, [13] sensors, drug/DNA delivery [14] and imaging [15]. Various reports have proved that synthesis of nanoparticles under non-toxic green conditions is of vital importance to address the growing concerns on the overall toxicity of metallic nanoparticles for medical and technological applications [16]. Present findings disclose *Lemna minor* synthesized gold nanoparticles antioxidant property.

MATERIALS AND METHODS

Biological synthesis of Gold nanoparticles: The biological synthesis of gold nanoparticles using Lemna *minor has* been carried out as reported earlier [17]. Briefly the leaves of Lemna minor were collected, washed with deionized water and then shade dried, powdered using electronic blender. The coarse powder of *Lemna minor* was

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extracted using deionized water. Different reaction concentration of Lemna minor extract and $HAuCl_4$ solution (49:1, 48:2, 47:3, 46:4, and 45:5) was subjected respectively. The reduction of gold ions to gold particles was completed within 1 hour and forms ruby red color which indicates the formation of gold nanoparticles.

DPPH assay: The DPPH radical was assayed using the previously reported method [18]. Different concentrations(60µg-1mg/ml) of gold nanoparticles were added, in equal volume, to 0.1 mM ethanolic DPPH solution. The mixture was shaken vigorously and allowed to stand for 20 min in the dark at room temperature and the absorbance was monitored at 517 nm. DPPH solution without gold nanoparticles served as the control. BHT was used as the standard for the concentration range as considered in the sample.

Superoxide radical scavenging activity: Superoxide anion radical scavenging activity was estimated according to the previously reported method [19]. The purple formazan formed by nitrobluetetrazolium (NBT) by reacting with the superoxide radicals generated from phenazine meth sulfate–nicotinamide adenine dinucleotide (PMS/NADH) non-enzymatic system was measured spectrophotometric ally. In this assay, the 1 ml reaction mixture contained phosphate buffer (100 mM, pH 7.4), NADH (468 µM), NBT (156 µM), PMS (60 µM) and various concentrations (60µg-1mg/ml) of goldnanoparticles. After incubation for 5 min at room temperature the absorbance at 560nm was measured against appropriate blank to determine the quantity of formazan generated. Quercetin was used as positive control in this test.

Nitric oxide radical scavenging: The NO scavenging activity of the *Lemna minor* synthesized gold nanoparticles was assessed according to the method described [20]. In this method, the nitric oxide generated from sodium nitroprusside interacts with oxygen and the resulting nitrite ions are quantified. In this test 3 ml of the reaction mixture contained sodium nitroprusside (10 mM), phosphate buffered saline (pH 7.4) and various concentrations of (60µg-1mg/ml) of gold nanoparticles and incubated at room temperature for 90 min. To 0.5 ml of this solution was added 1 ml of sulfanilamide (0.33% in 22% glacial acetic acid) followed by 1 ml of napthyl ethylenediamine hydrochloride (NED) (0.1% w/v) and the resulting pink chromophore was read at 540 nm spectrophotometric ally. BHT was used as positive control.

Hydrogen peroxide scavenging assay: Hydrogen peroxide scavenging activity of gold nanoparticles was studied using slightly modified method [21]. In this test, H2O2 (100 mM) was prepared freshly in phosphate buffer saline (pH 7.4). 300 µl of test samples containing various concentrations of gold nanoparticles (60µg-1mg/ml) was added to 600 µl of H2O2 (100 mM) and the final volume was made up to 1 ml with PBS. The absorbance was measured at 230 nm against the separate sample blanks. The percentage of inhibition was calculated and BHT used as positive control.

RESULTS AND DISCUSSION

DPPH free radical scavenging activity: The DPPH is considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid auto oxidation. Being a stable free radical, DPPH is regularly used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm, and its absorbance decreases upon reduction with an antioxidant [22]. Thus, the radical scavenging activity in the presence of a hydrogen-donating antioxidant can be monitored by a decrease in the absorbance of DPPH solution. **Fig. 1** presents the DPPH radical scavenging activity obtained in the studies with *Lemna minor*, the gold nanoparticles synthesized using *Lemna minor* and BHT. The *Lemna minor* and the gold nanoparticles had significant scavenging effects on the DPPH radical, with the gold nanoparticles showing higher activity. The positive DPPH test suggests that the samples are free radical scavengers. These results indicate that *Lemna minor* and the gold nanoparticles synthesized using *Lemna minor* exhibit the ability to quench the DPPH radical. The results also suggest that gold nanoparticles synthesized using *Lemna minor* are more active in *Lemna minor* alone, and are comparable to the reference drug, as good antioxidants with DPPH free radical scavenging activity.

Superoxide radical scavenging activity: **Fig.2** presents the superoxide radical scavenging activity obtained in the studies with *Lemna minor*, the gold nanoparticles and quercetin. The *Lemna minor* and the gold nanoparticles had significant superoxide radical scavenging activity. These results indicate that *Lemna minor* and the gold nanoparticles (Lm-GNP) exhibit the ability to scavenge superoxide radicals. The results also suggest that gold nanoparticles are more active than *Lemna minor* alone, and are comparable to the reference drug, as good antioxidants with superoxide radical scavenging activity. Superoxide anion is also very harmful to cellular components [23]. [24] Reported that flavonoids are effective antioxidants

mainly because they scavenge superoxide anions. As shown in Fig. 2, the superoxide radical scavenging activities of the test samples and the reference compound are increased markedly with increasing concentrations. The results suggest that *Lemna minor* and the gold nanoparticles are potent scavengers of superoxide radical.

Nitric oxide radical scavenging activity: Nitric oxide is well known to play a crucial role in the pathogenesis of various diseases caused by inflammation, especially when combined with superoxide radical to form peroxynitrite anion [25]. The scavenging activity of the nanoparticles against nitric oxide was detected by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides of nitrogen in the reacting mixture [26]. The significant decrease in the concentration of nitric oxide radical was comparable with the standard drug which is due to the scavenging ability of the nanoparticle. The percentage inhibition displayed by the extract showed a potent scavenger of nitric oxide. **Fig.3** presents the nitric oxide radical scavenging activity obtained in the studies with *Lemna minor*, gold nanoparticles and BHT. The *Lemna minor* and the gold nanoparticles had significant nitric oxide radical scavenging activity. These results indicate that *Lemna minor* and the gold nanoparticles synthesized using *Lemna minor* (Lm-GNP) exhibit the ability to scavenge nitric oxide radicals. The results also suggest that gold nanoparticles synthesized using *Lemna minor* are more active than *Lemna minor* alone, and are comparable to the reference drug, as good antioxidants with nitric oxide radical scavenging activity. Sustained levels of production of nitric oxide radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis [27]. The toxicity of NO° increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion (ONOO-) [28]. The extract inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The present study proved that the nanoparticles studied have more Potent nitric oxide scavenging activity than the standard BHT.

Hydroxyl radical scavenging activity: The hydroxyl radical is the most reactive oxygen radical [29]. They were produced by incubating ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4, and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH [30]. When nanoparticles were added to the reaction mixture, they removed the hydroxyl radicals formed from deoxyribose and prevented the reaction. **Fig.4** presents the hydroxyl radical scavenging activity obtained in the studies with *Lemna minor*, the gold nanoparticles synthesized using *Lemna minor* and BHT. The *Lemna minor* and the gold nanoparticles had significant hydroxyl radical scavenging activity. These results indicate that *Lemna minor* and the gold nanoparticles synthesized using *Lemna minor* (Lm-GNP) exhibit the ability to scavenge hydroxyl radicals. The results also suggest that the gold nanoparticles of synthesized using *Lemna minor* are more active than *Lemna minor* alone, and are comparable to the reference drug, as good antioxidants with hydroxyl radical scavenging activity. Hydroxyl radicals are major active oxygen species causing lipid peroxidation and enormous biological damage [31]. It is formed via Fenton's reaction in the living systems [32]. In the oxidative metabolism, the detrimental byproduct, hydroxyl radical, causes the molecular damage of nerve in the living organism. These radicals have major direct or indirect role in several pathological conditions such as brain ischemia, Parkinson's disease, hepatitis and carcinogenesis. Thus, *Lemna minor* and the newly synthesized gold nanoparticles (Lm-GNP) demonstrated antioxidant activity in a concentration-dependent manner on hydroxyl radical scavenging activity. The IC50 values of the samples in all the assays are presented in **Fig.5**. The values obtained for the gold nanoparticles of *Lemna minor* are almost equal to the values obtained for the standards, suggesting that the gold nanoparticles synthesized using *Lemna minor* are very good antioxidants comparable to that of standard. The higher values obtained for *Lemna minor* indicate that *Lemna minor* also has some antioxidant activity. The activity increases when they are in the form of gold nanoparticles. These results suggest that the gold nanoparticles are better antioxidants than the compounds alone, with which they are coupled to form nanoparticles.

All these tests reveal that the gold nanoparticles synthesized using *Lemna minor* are showing good antioxidant activity. The growing interest in the substitution of synthetic food antioxidants with natural ones has fostered research on plant sources and screening of raw materials to identify new antioxidants. In this view, some biological properties such as anticarcinogenicity, antimutagenicity, antiallergencity and antiaging activity have been reported for natural and synthetic antioxidants [33]. The antioxidant activity of natural compounds is reported to be mainly due to their redox properties [34], which can play an

important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

Conclusion: In conclusion gold nanoparticles were synthesized using *Lemna minor*. Bio reduction property of gold ions into gold particles was studied by UV- Vis spectra, FTIR, TEM and SEM. The important application of *Lemna minor* synthesized nanoparticles got good antioxidant activity of nature compounds reported to be mainly due to redox properties in absorbing and neutralizing free radicles, quenching singlet and tripled oxygen or decomposing peroxides.

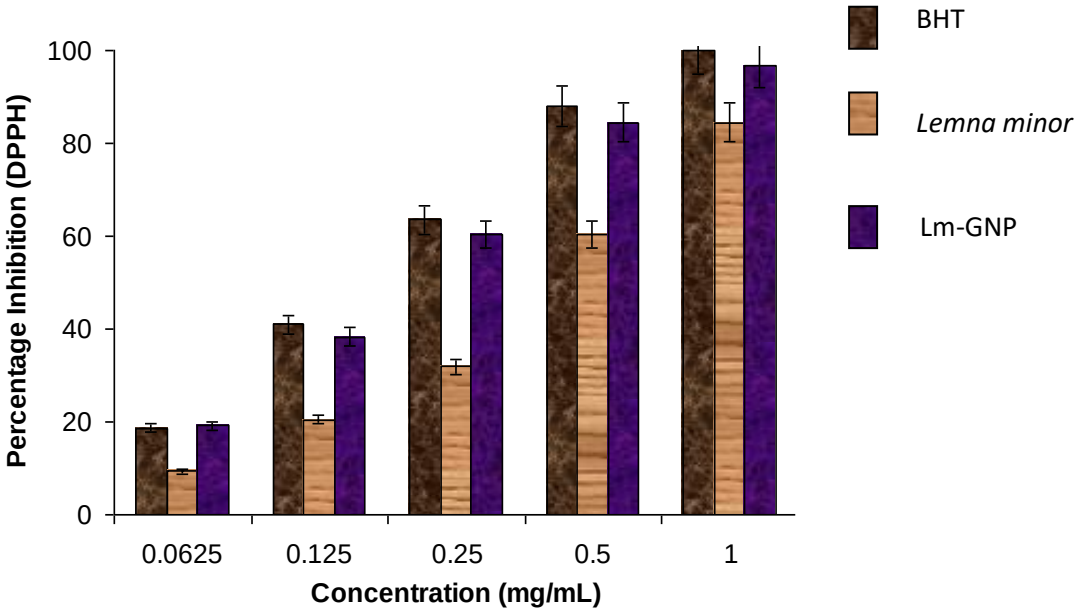

Fig.1.DPPH Radical Scavenging Activity of BHT, *Lemna minor* and Lm-GNP.

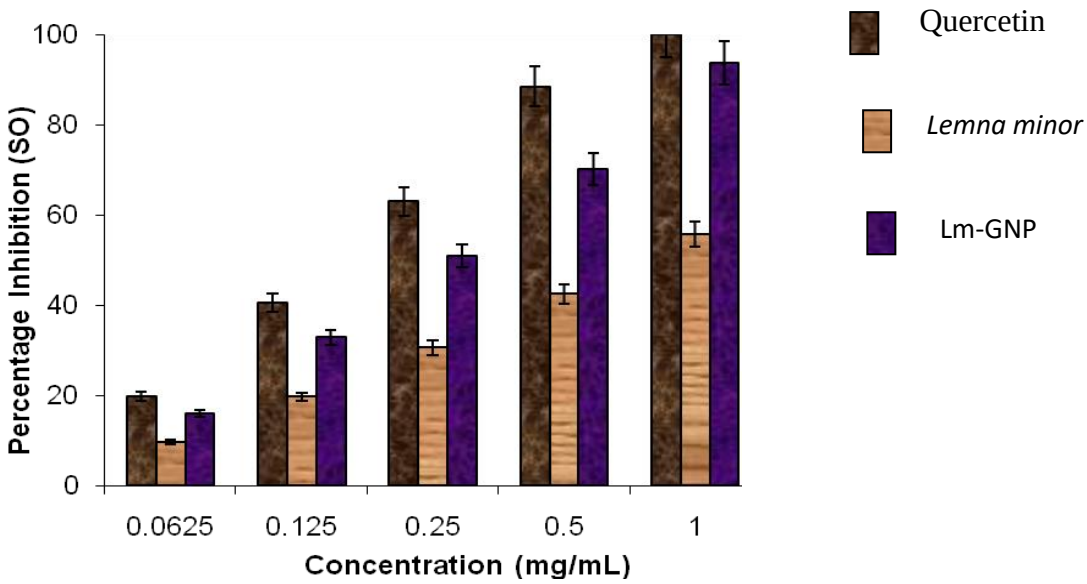

Fig.2. Superoxide Radical Scavenging Activity of Quercetin, *Lemna minor* and the newly synthesized gold nanoparticles (Lm-GNP).

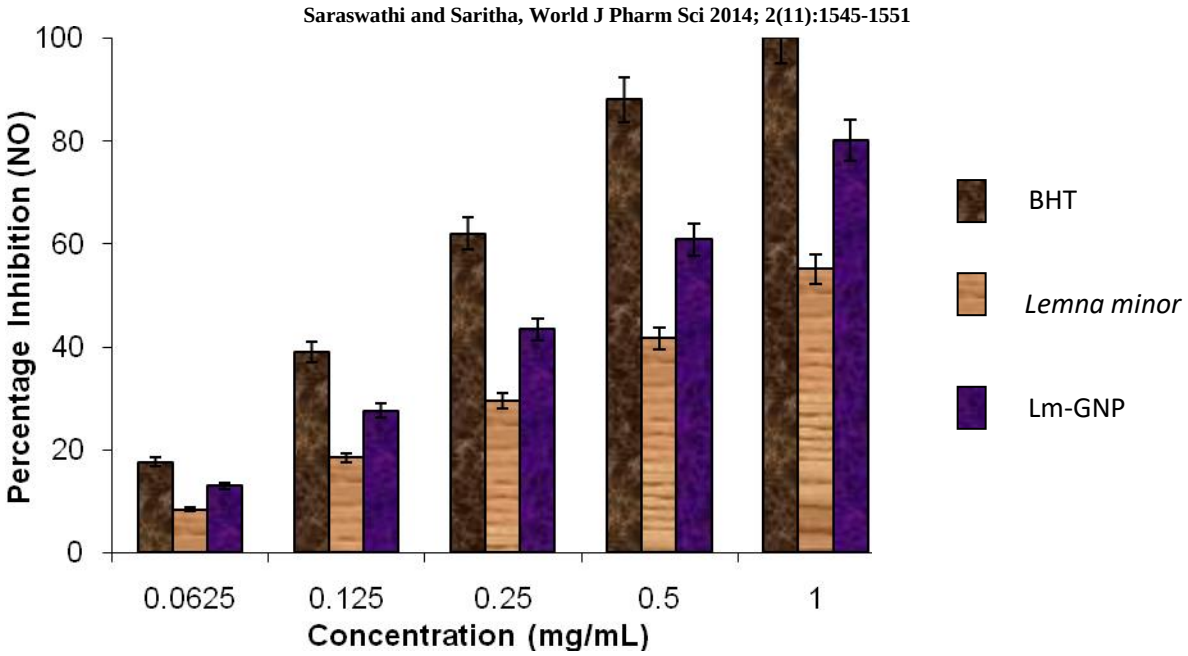

Fig .3 The Nitric Oxide radical scavenging activity obtained in the studies with *Lemna minor*, gold nanoparticles and BHT.

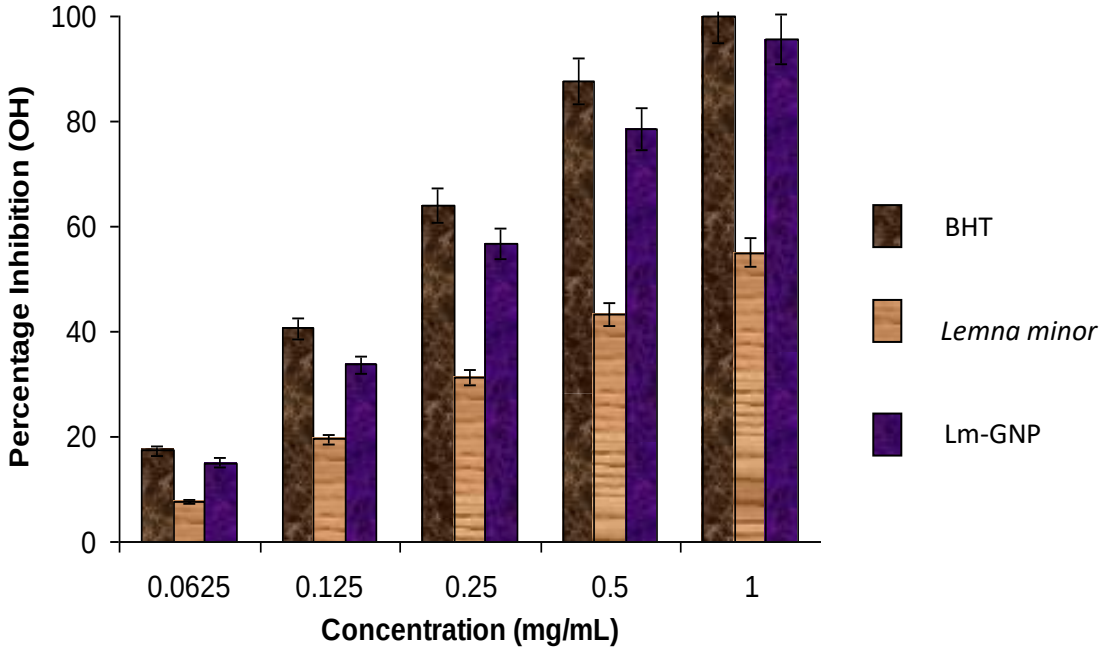

Fig. 4. Hydroxyl Radical Scavenging Activity of BHT, *Lemna minor* and Lm-GNP.

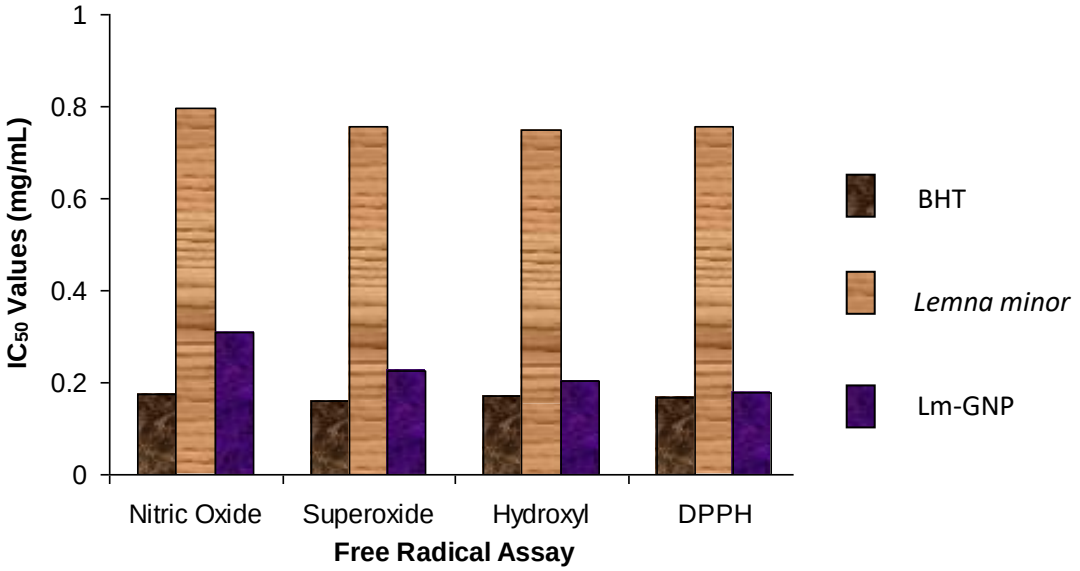

Fig.5. IC50 values of BHT, *Lemna minor* and LM-GNP.

REFERENCES

1. Priyadarshini S, Gopinath V, Priyadarshini NM, MabarokAli D, Velusamy P. Synthesis of anisotropic Silver nanoparticles using novel strain, *Bacillus flexus* and its biomedical application. *Collides surf. B* .2013; 102: 232-237.
2. Yeo SY, Lee HJ, Jeong SH. Preparation of nano composite fishers for permanent antibacterial effect. *J. Mater. sci*. 2003; 38: 2143-2147.
3. Zhang XQ, Salem JAK.Conjugation of polyamidoamine dendrimers on biodegradable micro particles for nonviral gene delivery. *Bioconjug. Chem*. 2007; 18(6):2068-2076.
4. Jin Y, Wang P, Yin D, Liu J, Qin L, Yu N, Xie G, Li B. Gold nanoparticles prepared by sonochemical method in thiol-functionalized ionic liquid. *Collids Surf. A.* 2007; 20:1126-1133.
5. Mohanpuria P, Rana NK, Yadav SK. Biosynthesis of nanoparticles: Technological concepts and future applications. *J. Nanoparticle Res*. 2008; 10: 507-517.
6. Lewinski N, Colvin V, Drezek R. Cytotoxicity of nanoparticles. *Small*. 2008; 4: 26-49.
7. Kwan KH, Barve A, Yu S, Huang MT, Kong ANT. Cancer chemoprevention by Phytochemicals: Potential molecular targets, biomarkers and animal models. *Acta. Phamacol. Sin*. 2007; 26: 1409 – 1421.
8. Nishino H, Satomi Y, Tokuda H. Masuda M. Cancer control by phytochemicals. *Curr. Pharma. Design* .2007; 13: 3394 -3399.
9. Deorukhkar A, Kwshnan S, Sethi G, Aggarwal BB. Back to Basic: how natural products can provide the basis for new therapeutics. *Exp. Opin. Inves . Drugs.* 2007; 16: 1753-1773.
10. Emerich DF, Thanos CG. Nanotechnology and medicine. *Expert Opin. Biol. Ther* .2003; 3(4): 655-663.
11. Shankar SS, Rai A, Ankamwar B, Singh A, Ahmad A. Sastry M. Controlling the optical properties of lemongrass extract synthesized gold Nano triangles and potential application in infrared- absorbing optical coatings. *Chem. Mater.*2005;17:566-572.
12. Bhumkar DR, Joshi HM, Sastry M, Pokharkar VB. Chitosan Reduced .Gold nanoparticles as novel carriers for trans mucosal delivery of insulin. *Pharm. Res*. 2007; 24 (8): 1415-1426.
13. Ingram RS, Hostetler MJ, Murray RW. Poly-hetero--functionalized alkanethiolate-stabilized gold cluster compound. *J. Am. Chem. Soc.* 1997; 119:9175-9178.
14. Niemeyer CM. Nanoparticles, Proteins, and Nucleic Acids: Biotechnology Meets Materials Science. *Angew. Chem. Int. Ed*., 2001; 40: 4128–4158.
15. Bielinska A, Jonathan D, Lee EI, James R, Baker JR and Balogh L.Imaging fAu 0-PAMAMg gold-dendrimer Nano composites in cells. *Nanopart. Res.*2002;4:395-403.
16. Kumar V and Yadav SK.Plant-mediated synthesis of silver and gold nanoparticles and their applications. *J. Chem. Technol. Biotechnol.*2009; 84: 151–157.
17. Saritha K ,Saraswathi U, Singaravelu G, Revathi S, Jayanthi V. Biological Synthesis of gold nanoparticles using *Lemna minor*. *Asian J. Pharm. Clin. Res.* 2014; 7: (2) 165-167.
18. Mensor LL, Meneze FS, Leitao GG, Reis AS, Dos santor JC, Coube CS, Leitao SG. Screening of Brazilian plant extract for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.* 2001; 15: 127 – 130.
19. Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and Molecular oxygen. *Biochem. Biophys. Res. Comm*. 1972; 46: 849-853.
20. Garratt DC. The quantitative analysis of drugs, Second ed. *Chapman and Hall Ltd.,Japan.*1964.
21. Avani Patel, Amit Patel, Patel NM, Determination of polyphenols and free radical scavenging activity of *Tephrosia purpure* linn leaves (Leguminosae), *Pharmacogn. Res.*2010;2:152-158.
22. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958; 181: 1199-1200.
23. Robak J, Gryglewsk RJ. Flavonoids are scavengers of superoxide Anions. *Biochem. Pharmacol*. 1988; 37(5): 837-841.
24. Michaels HB, Hunt JW. Reactions of the hydroxyl radical with polynucleotides. *Radiat. Res.*1973; 56(1): 57-70.
25. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. Peroxyl radical scavenging activity of *Ginkgo biloba* extracts EGb 761. *Biochem. Biophys. Res. Commun.*1991; 201: 748-748.

26. Tylor BS, Kion YM, Wang QI, Sharpio RA, Billiar TR, Geller DA. Nitric oxide down regulates hepatocyte-inducible nitric oxide synthase gene expression. *Arch. Surg*. 1997; 132:1177-1183.
27. Hui RE, Padmaja S. The reaction of NO with superoxide.*Free Rad. Res.Commun*. 1993; 18:195.
28. Korycka-dahl, Richardson T.Photogeneration of Superoxide anion in Serum of Bovine milk& in Model Systems containing riboflavin &amino acids. *J. Dairy Sci*.1978; 61: 400-407.
29. Halliwell B, Guttridge JMC. The deoxyribose method: A simple "test tube" assay for determination of rate constants for reactions of hydroxyl radical. *Anal. Biochem.*1987; 165: 215- 219.
30. Aurand LW, Boone NH, Gidding GG . Superoxide and singlet Oxygen in milk lipid peroxidation. *J. Dairy Sci*. 1977; 60: 363-369.
31. Repine JE, Fox RB, Berger FM. Hydrogen peroxide kills Staphylococcus aureus by reacting with staphylococcal iron to form hydroxyl radical. *J.Biol.Chem.*1981; 256:7094-7096.
32. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev*. 1991; 43:109–142.
33. Moure A, Cruz J M, Franco D , Domingue J M, Sineiro J, Dominguezt H, Nunez MJ, Carlos Paraju Natural antioxidants from residual sources. *Food Chem*.2001; 72: 145–171.
34. Zhang W, Wang SY.Antioxidant Activity and Phenolic Compounds in Selected Herbs. *Agric. Food Chem.*2001; 49(11):5165-5170.