International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.10, pp 530-537, 2017

In vitro Free Radical Scavenging Activity of Zinc Oxide Nanoparticles Synthesized from the Brown Seaweed - *Turbinaria Conoides*

Khoushika Raajshree R and Brindha Durairaj*

Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India

Abstract : Free radicals are the atomic and molecular varieties of oxygen that are collectively known as reactive oxygen species that initiates oxidative stress which is an imbalance between the formation and neutralization of the pro-oxidants. The free radicals seek stability by stealing electrons from the biological macromolecules such as proteins, lipids and DNA in healthy human cells that in turn leads to the damage of proteins and DNA along with lipid peroxidation. 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 free radical scavenging activity of the zinc oxide nanoparticle synthesized from the crude extract of *Turbinaria conoides* was assessed against *in vitro* radicals like by DPPH, Superoxide, Nitric oxide, Hydroxyl and Hydrogen peroxide. The radical scavenging activity of zinc oxide nanoparticle synthesized from the crude extract of *Turbinaria conoides* shows potent scavenging activity and this may be mainly due to redox properties in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.
Keywords : Free radicals, scavenging, zinc oxide nanoparticles, *Turbinaria conoides.*

Introduction

Nanotechnology is a new area of science that involves working with materials and devices that are on the nano scale. Synthesis of 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 medicine, biotechnology, optics and others [1]. 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 [2].

Free radicals are molecules that contain an unpaired electron in an atomic orbital and are capable of independent existence that gives them the property of being highly reactive and unstable species. They either behave as oxidants by donating electrons or as reductants by accepting electrons from other molecules. The major free radicals that are shown to be implicated in various diseases are hydroxyl radical, superoxide anion radical, hydrogen peroxide, singlet oxygen, hypochlorite, nitric oxide radical and peroxynitrite radical. They attack the important macromolecules like the lipids, nucleic acids and proteins leading to cell death and homeostatic disruption [3]. Oxidative stress is resultant of the generation of free radicals that are constantly produced in the human body due to exogenous factors such as drugs, pollutants, deficiency of natural antioxidants, UV rays and tobacco. Mitochondria and peroxisomes play a role of an endogenous source that

leads to oxidative stress. Human system has the ability to neutralize the free radical generated, but an imbalance between the above two can lead to various problems and diseases such as cancer, diabetes, Alzheimer's disease, Parkinson's disease and aging [4].

Seaweeds are macrophytic marine algae that produce a great variety of secondary metabolites possessing broad-spectrum of biological activities. The present study was carried out to validate the free radical scavenging activity of ZnO-NPs synthesized from the crude extract of *Turbinaria conoides*, brown seaweed.

Materials and Methods

Collection and Preparation of Seaweed

The brown seaweed, *Turbinaria conoides*, was collected from Mandapam coastal region, Gulf of Mannar, Southeast coast of India. The algal samples were washed thoroughly with running tap water followed by distilled water to remove adhering salts and associated biota. The washed samples were dried under shade at room temperature for a week. The dried materials were ground to fine powder using mixer grinder and stored in an airtight container for further analysis.

Preparation of algal extract

The crude algal extract (CAE) was prepared by adding 10 g of algal powder into 100 ml of 50% ethanol and kept in rotatory shaker for 24 hours. Filtered, collected the solvent and was used for further analysis.

Green Synthesis of Zinc Oxide Nanoparticles from Crude Algal Extract

20 ml of the crude algal extract was heated at 50˚C for 10 min and 50 ml of 91 mM of zinc acetate solution (1 g of zinc acetate was dissolved in 50 ml of distilled water) was added drop wise. This was then placed in a magnetic stirrer for 2 hrs. Then the precipitate was collected by centrifugation at 16 000 rpm for 10 min at 4˚C. The pale white precipitate was then taken out and washed with distilled water followed by ethanol to get free of the impurities. The zinc oxide nanoparticles were obtained after drying at 60˚C in oven overnight and the sample was stored for further studies.

Characterisation of Zinc Oxide Nanoparticles

The obtained zinc oxide nanoparticles were measured for its maximum absorbance using UV-Vis spectrophotometry. The optical property of zinc oxide nanoparticles was determined via ultraviolet and visible absorption spectroscopy in the range of 280 – 420 nm. External morphology i.e. the shape of the nanoparticles was characterized by Scanning Electron Microscope (SEM). Elemental analysis was obtained from energy dispersive X-ray diffraction (EDX), which was attached with SEM.

Free Radical Scavenging Assay

The free radical scavenging activity of the synthesized zinc oxide nanoparticle and the crude algal extract was determined by employing the following methods.

DPPH Spectrophotometric Assay [5]

A methanolic solution of 0.5 ml of DPPH (0.4 mM) was added to 1 ml of the different concentrations (50 -250µg/ml) of samples and allowed to react at room temperature for 30 minutes. Methanol served as the blank. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$\text{Scavenging activity (\%)} = \frac{\text{A Control} - \text{A sample}}{\text{A Control}} \times 100$$

Super Oxide Radical Scavenging Activity [6]

Super oxide radical ($O2.^-$) was generated from the photo reduction of riboflavin and was detected by nitro blue tetrazolium dye (NBT) reduction method. The assay mixture contained samples of different concentration (50 -250µg/ml) with 0.1 ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M). The control tubes were also set up wherein DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound.

Nitric Oxide Radical Scavenging Activity [7]

The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) and 0.5ml of samples with different concentrations (50-250µg/ml) of various extracts were incubated at 25^0C for 5 hours. Control experiments without the test compounds, but with equivalent amounts of buffer were added conducted in an identical manner. After 5 hours, 0.5ml of Griess reagent. The absorbance of the chromophore formed during diazotization coupling with naphthyl ethylene diamine was read at 546nm.

Hydroxyl Radical Scavenging Activity [8]

The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe3+ -Ascorbate–EDTA –H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM), 0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, pH 7.4 (20mM) and various concentrations (50 -250µg/ml) of samples in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Hydrogen Peroxide Scavenging Activity [9]

Hydrogen peroxide scavenging activity of the samples was studied using slight modification [9]. In this test, H_2O_2 (100 mM) was prepared freshly in phosphate buffer saline (pH 7.4). 300 µl of test samples containing various concentrations (50µg-250µg/ml) was added to 600 µl of H_2O_2 (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.

Results and Discussions

Biosynthesis of ZnO-NPs using Brown seaweed

Zinc oxide nanoparticles (ZnO-NP's) were synthesized from crude algal extract of *Turbinaria conoides* by green synthesis, which is more reliable and less toxic when compared with other. The formation of pale white colour within 3 hours of preparation indicated the synthesis of ZnO nanoparticles.

UV-Visible Spectral Analysis

The optical absorption spectra of zinc oxide nanoparticles were recorded using UV/VIS 3000+ Double Beam UV-Visible Ratio-Recording Scanning Spectrophotometer from Lab India (SKU: 174-0020) with dimensions of (W × D × H)/Weight = 540 × 440 × 390 mm/36kg.

The spectral bandwidth of Spectrophotometer is 0.5, 1, 2, 5 nm and wavelength is in the range of 190 to 1100 nm. Figure 1 shows the UV-Vis absorption spectrum of zinc oxide nanoparticles. The absorption spectrum was recorded for the sample in the range of 280 - 420 nm. The spectrum showed the absorbance peak at 360 nm corresponding to the characteristic band of zinc oxide nanoparticles.

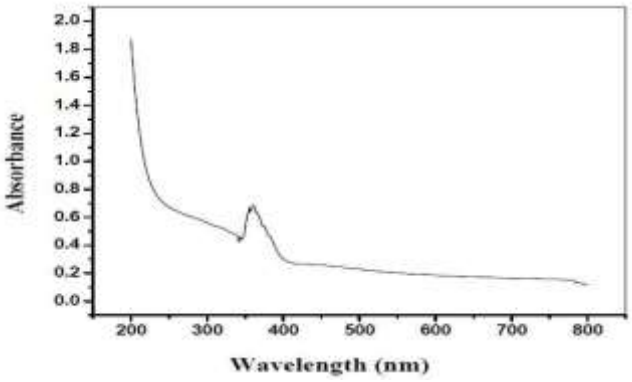

Figure 1: UV-Visible spectrum of synthesized ZnO-NPs

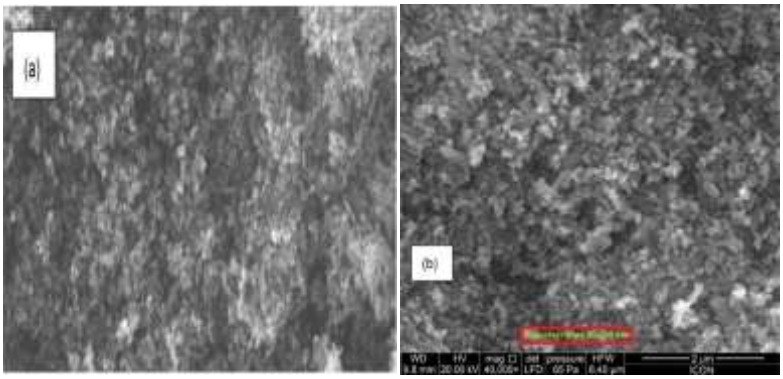

Figure 2[a] & [b]: SEM image of the synthesized zinc oxide nanoparticles

Scanning Electron Microscopy (SEM) Analysis

The morphology of the synthesized nanoparticles was examined using scanning electron microscopy. Figure 2(a) and Figure 2(b) show the surface morphology of the zinc oxide nanoparticles under different magnifications. The SEM image showed that most of the nanoparticles are spherical in shape formed within diameter range of 80 - 130 nm.

Energy Dispersive X-Ray Diffractive (EDX) Analysis

The Energy Dispersive X-ray Diffractive (EDX) study was carried out for the synthesized zinc oxide nanoparticles to elucidate the elemental composition. EDX confirms the presence of zinc and oxygen signals of zinc oxide nanoparticle as depicted in Figure 3.

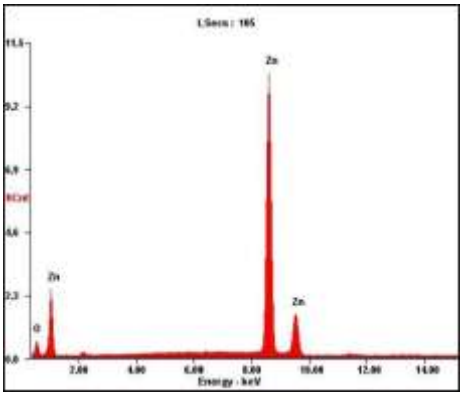

Figure 3: EDX spectrum of synthesized zinc oxide nanoparticles

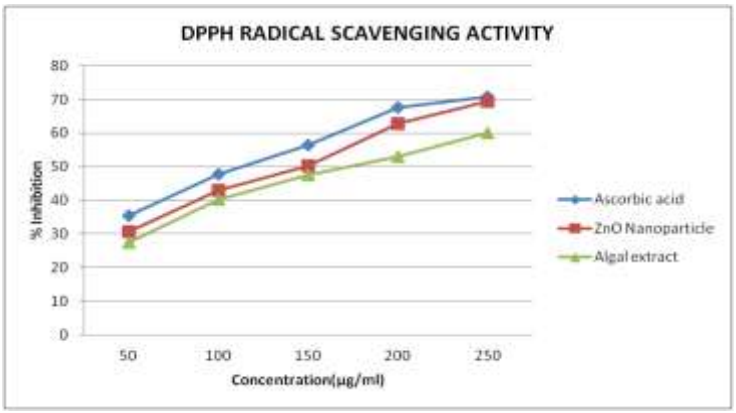

Figure 4: DPPH radical scavenging activity of ascorbic acid, ZnO-NP and algal extract

The results revealed that the peaks correspond to the optical absorption of the produced nanoparticle. The elemental analysis of the nanoparticle yielded 77.32% of zinc and 22.68% of oxygen which proves that the produced nanoparticle is in its highest purified form.

Free Radical Scavenging Assay

Greener approaches on synthesizing nanomaterials have gained enormous scientific and technological focus to get rid of the hazards and problems arising out of the use of chemical synthesis technology [10]. In the present study, free radical scavenging ability of zinc oxide nanoparticles (ZnO NP's) and crude algal extract was evaluated.

DPPH radicals are scavenged by antioxidants through the donation of hydrogen, thus forming reduced DPPH-H, which changes the colour from purple to yellow following reduction and is quantified [10]. The percentage of DPPH radical inhibition by zinc oxide nanoparticle and crude algal extract was found to be 69.4% and 60% at 250µg/ml concentration which is comparable to the standard ascorbic acid (70.8%) at 250µg/ml. The IC_{50} value of zinc oxide nanoparticle, crude algal extract and ascorbic acid was found to be 157 µg/ml, 179µg/ml, and 148µg/ml respectively. The results suggested that the zinc oxide nanoparticles have a potent DPPH radical scavenging effect when compared with crude algal extract. The platinum nanoparticles exhibited DPPH radical scavenging effect [11].

Superoxide anions damage biomolecules directly or indirectly by forming hydrogen peroxide or singlet oxygen during aging and other pathological events. It has been showed to initiate lipid peroxidation [12]. Figure 5 depicts the superoxide radical scavenging effect of zinc oxide nanoparticle, crude algal extract and ascorbic acid in a dose-dependent manner with maximum scavenging activity of 70%, 65% and 77% at 250µg/ml respectively. The IC_{50} value of zinc oxide nanoparticle was found to be maximum (158µg/ml) when compared to crude algal extract and ascorbic acid. The results suggested that the crude algal extract is less effective in scavenging superoxide radical than zinc oxide nanoparticles. Silver nanoparticles synthesized from *Excoecaria agallocha* exhibited good superoxide scavenging activity [10].

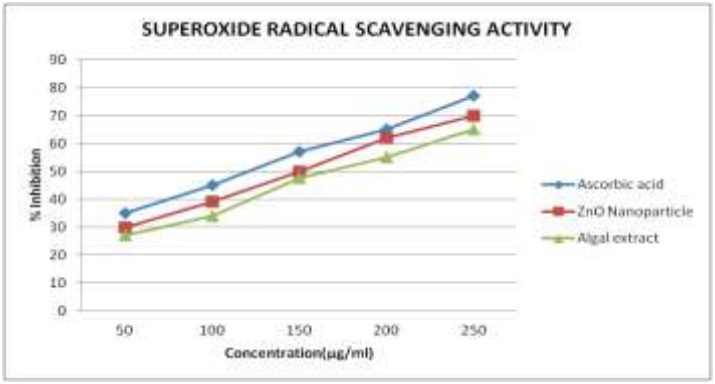

Figure 5: Superoxide radical scavenging activity of ascorbic acid, ZnO-NP and algal extract

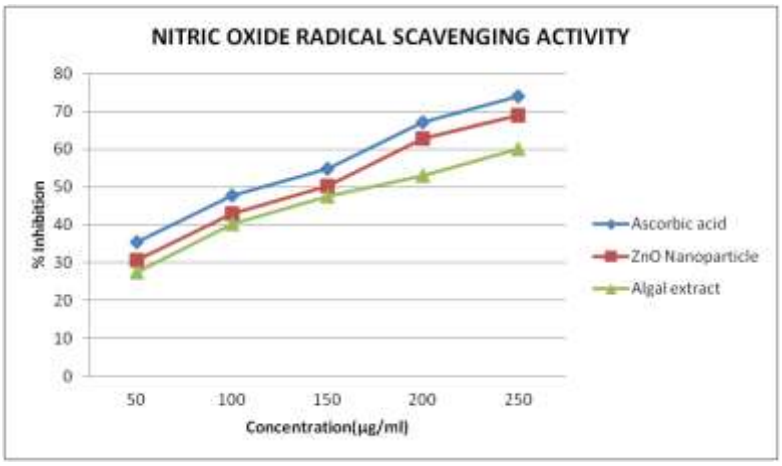

Figure 6: Nitric oxide radical scavenging activity of ascorbic acid, ZnO-NP and algalextract

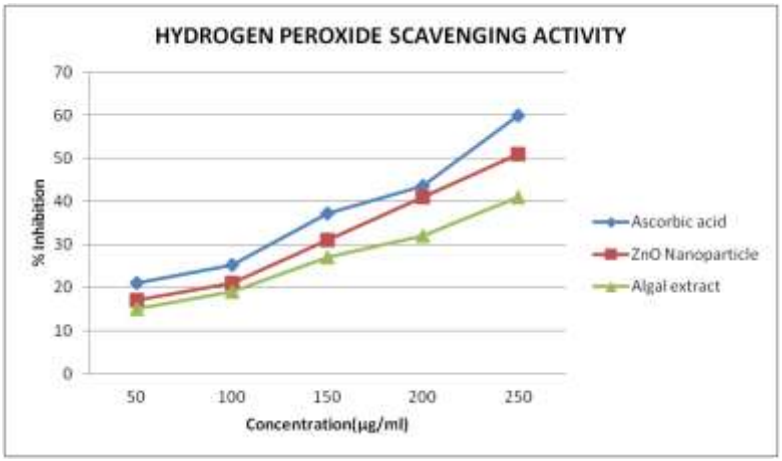

Figure 7: Hydrogen peroxide radical scavenging activity of ascorbic acid, ZnO-NP and algal extract

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological process including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. However, excess production of NO is associated with several diseases [13]. The percentage of nitric oxide radical inhibition by zinc oxide nanoparticle and crude algal extract was found to be 69% and 60% at 250µg/ml concentration which is comparable to the standard ascorbic acid (73.9%) at 250µg/ml. The IC_{50} value of zinc oxide nanoparticle, crude algal extract and ascorbic acid was found to be 158µg/ml, 179µg/ml and 146µg/ml respectively. The results suggested that the zinc oxide nanoparticles have a potent nitric oxide radical scavenging effect when compared with crude algal extract. Cerium nanoparticles showed good nitric oxide scavenging activity by an internal electron transfer mechanism [14].

Hydrogen peroxide is an important reactive oxygen species due to its ability to penetrate into the biological membrane and produces many toxic effects [15]. Figure 7 depicts the hydrogen peroxide scavenging effect of zinc oxide nanoparticle, crude algal extract and ascorbic acid in a dose-dependent manner with maximum scavenging activity of 51%, 41% and 59.9% at 250µg/ml respectively. The IC_{50} value of zinc oxide nanoparticle, crude algal extract and ascorbic acid was found to be 236µg/ml, 289µg/ml, and 206µg/ml respectively. The results suggested that the crude algal extract is less effective in scavenging hydrogen peroxide than zinc oxide nanoparticles. The platinum nanoparticles also exhibited hydrogen peroxide scavenging activity [11].

Hydroxyl radical has a short half-life and is the most reactive, known to be capable of abstracting hydrogen atoms from cell membranes and bring about peroxidic reactions of lipids [16]. The percentage of hydroxyl radical inhibition by zinc oxide nanoparticle and crude algal extract was found to be 63.2% and 60% at 250µg/ml concentration which is comparable to the standard ascorbic acid (78%) at 250µg/ml. The IC_{50} value of zinc oxide nanoparticle was found to be maximum (181 µg/ml) when compared with crude algal extract and

ascorbic acid. The results suggested that the zinc oxide nanoparticles have potent hydroxyl radical scavenging effect when compared with crude algal extract. Silver nanoparticles synthesized from *Excoecaria agallocha* exhibited good Hydroxyl radical scavenging activity [10].

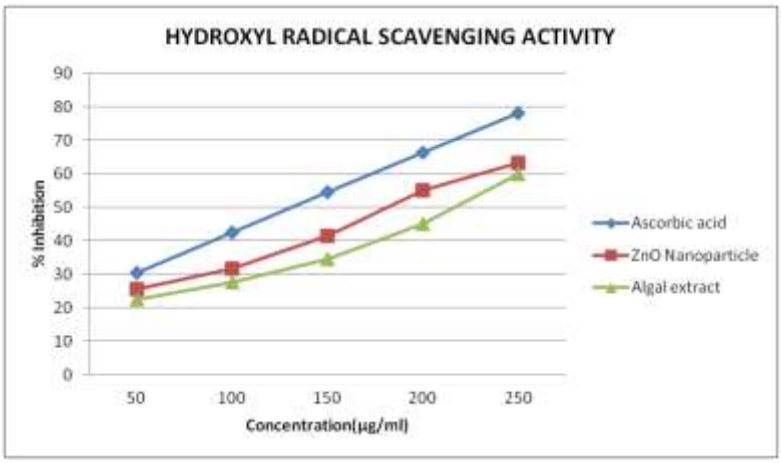

Figure 8: Hydroxyl radical scavenging activity of ascorbic acid, ZnO-NP and algal extract

Conclusion

In the present study, the free radical scavenging potential of Zinc oxide nanoparticles and the crude algal extract was evaluated and it can be concluded that the synthesized Zinc oxide nanoparticles are an efficient free radical quencher *in vitro*. With this kind of investigation, it would be easier to treat and prevent the damages occurring due to the free radicals. The present study suggests that ethanopharmacological approach on the synthesis of Zinc oxide nanoparticles will greatly facilitate the creation of nanomedicine.

Acknowledgment

Authors wish to acknowledge the management of PSG College of Arts and Science, Tamil Nadu, India, for providing the necessary laboratory facilities.

Conflict of Interests

Declared none.

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. Zhang XQ and Salem JAK. Conjugation of polyamidoamine dendrimers on biodegradable micro particles for nonviral gene delivery. *Bioconjug. Chem.* 2007; 18(6):2068-2076.
3. Ahmed M., Khan MI, Khan MR, Muhammad N, Khan AU, Khan RA. Role of medicinal plants in oxidative stress and cancer. 2013; 2:641, doi:10.4172/scientificreports.641.
4. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn rev. 2010; 4:118-126.
5. Khalaf NA, Shakya AK, Al-othman A, El-agbar Z, Farah H. Antioxidant Activity of Some Common Plant, Turkish Journal of Biology. 2008; 32:51-55.
6. Winterbourne CC, Hawkins RE, Brain M, Carrel RW. The estimation of red cell superoxide dismutase activity. J Lab chem Med. 1975; 85:337-341.
7. Garrat DC. The quantitative analysis of drugs. Chapman and hall, Japan 1964; 3:456-458.
8. Elizabeth K and Rao MNA. Oxygen radical scavenging activity of curcumin. Int.J.Pharm 1990; 58:237-240.

9. Patel A, Patel A, Patel NM, Determination of polyphenols and free radical scavenging activity of *Tephrosia purpure* linn leaves (Leguminosae), *Pharmacogn. Res.*2010; 2:152-158.
10. Arun S, Saraswathi U and Singaravelu. Investigation on the Free Radical Scavenging Activity Of Biogenic Silver Nanoparticle. Int J Pharm Bio Sci. 2015; 6(2):439-445.
11. Watanabe A, Kajita M, Kim J, Kanayama A, Takahashi K, Mashino T, Miyamoto Y. *Invitro* free radical scavenging activity of platinum nanoparticles. Nanotechnology. 2009; 20(45):455105.
12. Yen M., Schlesier K and Harwart M. Assessment of antioxidant of *Coscinium fenestratum*. Natural products Radiance. 1994; 33:98-104.
13. Revathi S, Saraswathi U, Poorni K.E. Free Radical Scavenging Potential of A Poly Herbal Formulation. Int. J. Pharm. Sci. Rev. Research. 2009; 23(2): 47-51.
14. Dowding JM., Dosani T., Kumar A., Seal S., Self WT. Cerium oxide nanoparticles scavenge nitric oxide radical (NO). Chemical Communications. 2012; 48:4896-4898.
15. Chen Y, Miao Y, Huang L, Li J, Sun H, Zhao Y. Antioxidant activities of saponins extracted from Radix trichosanthis: an *in vivo* and *in vitro* evaluation. BMC Complementary Altern Med. 2014; 14:86-93.
16. Mohana M and Padma PR. Free Radical Scavenging Activity of the Bacoside Fraction from *Bacopa Monnieri*. Int J Curr Pharm Res. 2016; 8(2): 61-64.

*****