CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

AN INVESTIGATION OF ANTIOXIDANT AND CYTOTOXIC PROPERTIES OF GREEN SYNTHESIZED SILVER NANOPARTICLES

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Abstract:

Silver nanoparticles are being used in numerous fields for their optical, conductive, antioxidant anticancer and antibacterial properties. Green synthesis of silver nanoparticles is a simple and reliable method over chemical, physical and mechanical methods. The leaf extract of Aegle marmelos was successfully used for the synthesis of silver nanoparticles. Use of the silver nanoparticles in the field of biomedicine is rapidly growing because of their high efficacy and low toxicity. With respect to biological and clinical applications, silver nanoparticles has ability to control and manipulate the accumulation of nanoparticles inside a cell can provide sensitivity towards diagnosis and therapeutic efficacy. Here the antioxidant property of silver nanoparticles studied by performing 1,1-diphenyl-2-picryl hydrazyl radical, nitric oxide and hydrogen peroxide scavenging assay. While cytotoxicity of the biogenic silver nanoparticles studied by performing the MTT assay. The result obtained for biogenic silver nanoparticles shows the dose dependent increase in antioxidant activity and it is comparable with standard ascorbic acid. Silver nanoparticles exhibits cytotoxic activity against A-431 squamous cell carcinoma cell lines. This potential to silver nanoparticles may impart form capping agent from leaf extract. Study of the anticancer mechanism of biogenic silver nanoparticles is the further way of research in coming future.

Keywords: *Biogenic silver nanoparticles, antioxidant, cytotoxicity, squamous cell carcinoma, A431 cell lines.*

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Please cite this article in press as Rajeshwari et al , **An Investigation of Antioxidant and Cytotoxic Properties of Green Synthesized Silver Nanoparticles,** *Indo Am. J. Pharm. Sci, 2015;2(10).*

INTRODUCTION

Nano sized materials in range of 1-100 nm are known as nanoparticles, because of their larger surface area to volume ratio, they possess unique and improved properties [1]. Nanotechnology is an emerging field of science concerned with synthesis of nanoparticles of variable sizes, shapes, chemical composition and controlled dispersity. Nanotechnology finds extensive applications in Nanomedicine [2]. Green synthesized nanoparticles have antimicrobial [3], antioxidant [4](Mohan et al., 2014), cytotoxic [5], and anti-inflammatory [6] property.

Nanoparticles can be synthesized by chemical and physical methods, but these methods are quite expensive and toxic [7]. Green synthesis of nanoparticles was proven better method of synthesis due to slower kinetics, better manipulation and control over crystal growth and their stabilization [8]. Synthesizing nanoparticles with desired size and composition are of great interest as they provide solutions to various environmental and technological challenges [9].

Green synthesis of silver nanoparticles from *Aegle marmelos* leaf extract is easy and quick method [10]. *Aegle marmelos* contains a number of phytoconstituents, which are the key factors in the medicinal value of this plant [11]. These phytoconstituents act as capping agents of silver nanoparticles that contribute to stabilization and medicinal properties of silver nanoparticles.

Free radicals are highly active substances due to the presence of unpaired electrons. Our antioxidant system have efficiency against oxidative stress condition which produces free radicals leads to number of human diseases including chronic complications like cardiovascular diseases, cancer, neurodegenerative diseases [12]. Antioxidants play very important role to prevent such type of diseases.

Cancer is an abnormal tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion which leads to a progressive increase in the number of dividing cell [13]. Skin cancer is the most common form of cancer, globally accounting for at least 40% of cases [14]. Squamous cell carcinoma represents the most common cancer capable of metastatic spread [15]. Many attempts have been made to use silver nanoparticles as an anti-cancer agent and all turned up positive [16].

In this present study green synthesis of silver nanoparticles was carried out by using *Aegle marmelos* leaf extract.

Synthesized silver nanoparticles have been investigated for antioxidant and cytotoxic activity. Antioxidant activity was studied by performing antioxidant assay while the cytotoxic potential of nano silver was determined using A - 431cell lines of SCC.

MATERIALS AND METHOD

Chemicals for synthesis, characterization and assays of silver nanoparticles were purchased from Merk Limited India. A-431 melanoma cell lines were purchased from NCCS Pune were maintained in Dulbecco's modified Eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°c in 5 % CO2 in a humidified atmosphere in a CO2 incubator.

Synthesis of Silver Nanoparticles:

Silver nanoparticles were synthesized by adding 25 ml of plant extract to 75 ml of silver nitrate to give the final concentration 0.1mM at room temperature. The colour change was observed from normal colorless solution to dark brown colour. Initially reduction of the silver ions was confirmed by the UV-Vis spectrum of the solution. The synthesized nanoparticles were separated out from the mixture by centrifugation. Then nanoparticles were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray Diffraction microscopy (XRD) and Energy Dispersive X-ray spectroscopy (EDX). The stability of synthesized nanoparticles was checked by measuring zetapotential [17].

DPPH Assay:

DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity was determined according to method described by Malterud et al., 2001[18] by taking different concentrations (50-1000 µg/l) of silver nanoparticles. To this 2.96 ml DPPH (0.1mM) solution was added. The reaction mixture was shaken vigorously and incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517 nm with a spectrophotometer. DPPH 0.1mM was taken as control. Lower absorbance indicates higher free radical scavenging activity. The formula used for calculating free radical scavenging activity is as follows.

$$\% \text{ scavenging activity} = \frac{control\ absorbance - test\ absorbance}{control\ absorbance} X100 \quad ---(1)$$

Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside was measured spectrophotometrically by Griess reaction using method of Navabi et al., 2009 [19]. Sodium nitroprusside (5 mM) in phosphate buffered saline of pH 7.4 was prepared and 100 µl of it was mixed with 100 µl different

concentration of the silver nanoparticles (50-1000µg/ ml) and incubated at 25°C for 30minutes. A control prepared without the test compound. After 30minutes, 1.5 ml of the incubated solution was removed and diluted with 1.5ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). The Absorbance of the chromophore formed was measured at 546 nm. Nitric oxide scavenging activity is calculated by using formula-1

Hydrogen Peroxide Assay

The hydrogen peroxide scavenging activity was determined according to method of Cetinkaya et al. 2012 [20] The different concentrations of the silver nanoparticles and ascorbic acid (50-1000 µg /ml) were mixed with hydrogen per oxide (0.6ml, 50 mM) prepared with phosphate buffer (pH 7.4). This reaction mixture was incubated for 10min. The absorbance was measured spectrophotometrically at 230nm using phosphatate buffer as blank. The percent hydrogen peroxide scavenging was calculated using equation 1.

Reducing Power Assay

The reducing power of green silver nanoparticles was determined by Oyaizu's method (1986) [21] with some modification. Different concentrations (50-1000 µg /ml) of AgNPs and ascorbic acid were mixed with 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The resulting mixture was incubated at 50˚C for 20 minutes and cooled it rapidly. Then, 2.5 ml of 10 % tri-chloro acetic acid was added and centrifuged at 3000 rpm for 10 minute. The supernatant was mixed with 2.5ml of deionised water and 1 ml of 0.1 % ferric chloride. Then the absorbance was measured at 700nm.The percentage of reducing power was calculated by using formula 2.

$$\% \text{ reducing power} = \frac{test\ absorbance - control\ absorbance}{test\ control\ absorbance} X100 \quad - - - (2)$$

MTT Assay

Cell viability was examined according to method developed by Denizot and Lang (1986) [22].The cells (1 × 105/well) were plated in 96-well plates. After 48 hours incubation the cell reaches the confluence. Then, silver nanoparticles were added in grown culture at concentrations of 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml, by maintaining control throughout the experiment. This plate was incubated for 24 hours at 37°c in 5 % CO2 in a humidified atmosphere. The cells were washed with PBS and then added 30 µl of the MTT solution to the culture (MTT -5 mg/ml dissolved in PBS). It was then incubated at 37^0C for 4 hours. The MTT was removed by washing with PBS and 200 µl of DMSO was added to the culture. Optical density was read at 540 nm using DMSO as blank. This determination was done in triplicate. The cell viability values were compared to the control to determine the effect of the silver nanoparticles on cells. The percent viability of cells was calculated by using the following formula.

$$\% \text{ viability} = \frac{OD\ of\ test}{OD\ of\ control} X100 \quad - - - (3)$$

A graph was plotted against the % viability Vs dilution of the sample. The results were given as the mean ± SD of three independent experiments.

Statistical Analysis

The experiments were carried out in triplicates analysis of the data were performed by excel sheet. The results were expressed as mean ± standard error.

RESULTS AND DISCUSSION

DPPH Assay:

1,1-diphenyl-2-picryl hydrazyl is stable free radical which get reduced by accepting hydrogen or electron from donor [23]. Silver nanoparticles shows remarkable scavenging activity when compared with standard ascorbic acid. DPPH scavenging activity of silver nanoparticles was found to increase in dose dependent manner. The antioxidant potential of silver nanoparticles could be attributed by functional groups adhere to nanoparticles from leaf extract. As the *Aegle marmelos* leaves were good source of several antioxidant components such as, β-carotene, glutathione, α-tocopherol, ascorbic acid and total polyphenols and flavonoids [24] .

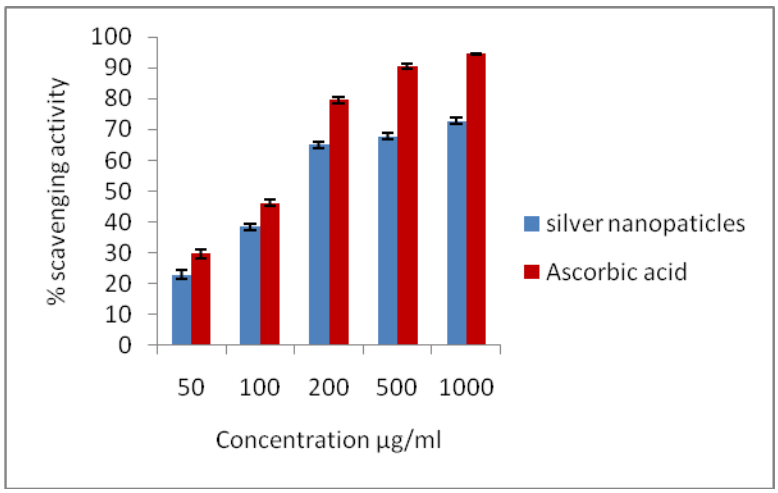

Fig 1: DPPH Scavenging Activity of Green Synthesized Silver Nanoparticles

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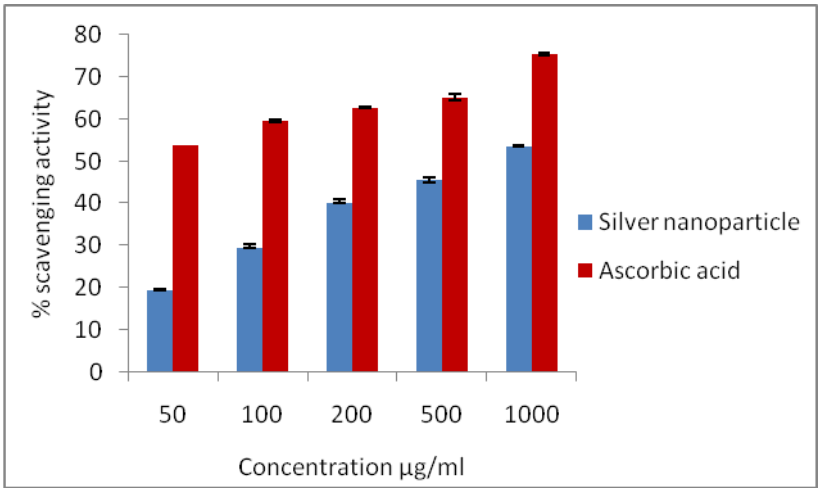

Fig 2: Nitric Oxide Scavenging Activity of Green Synthesized Silver Nanoparticles

Nitric Oxide Scavenging Activity:

The nitric oxide scavenging activity of the nanoparticles was detected by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides of nitrogen in reaction mixture [25]. Excess production of NO is associated with several diseases [26]. Present study shows the silver nanoparticles had nitric oxide scavenging activity. The scavenging activity of the nanoparticles was lesser as compared to standard ascorbic acid. A similar results were found with silver nanoparticles synthesized by using plant leaves *Excoecaria agallocha [27].*

Hydrogen Peroxide Assay

Hydroxyl radical scavenging activity of green synthesized silver nanoparticles is shown in fig. 3. In this present study hydroxyl radical scavenging effect of silver nanoparticles shows activity in dose dependent manner. The obtained results are compared with standard ascorbic acid. The hydroxyl radical induces severe damage to adjacent biomolecules such as lipids, proteins and DNA. Green synthesized silver nanoparticles are good scavenger of hydroxyl radical and may employed in such conditions. The total phenolic compounds are major compounds responsible for antioxidant activity [28].

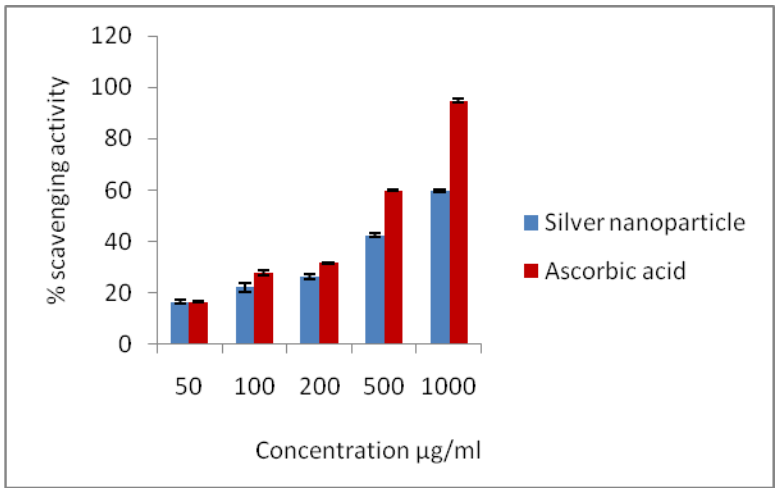

Fig 3: Hydrogen Peroxide Scavenging Activity of Green Synthesized Silver Nanoparticles

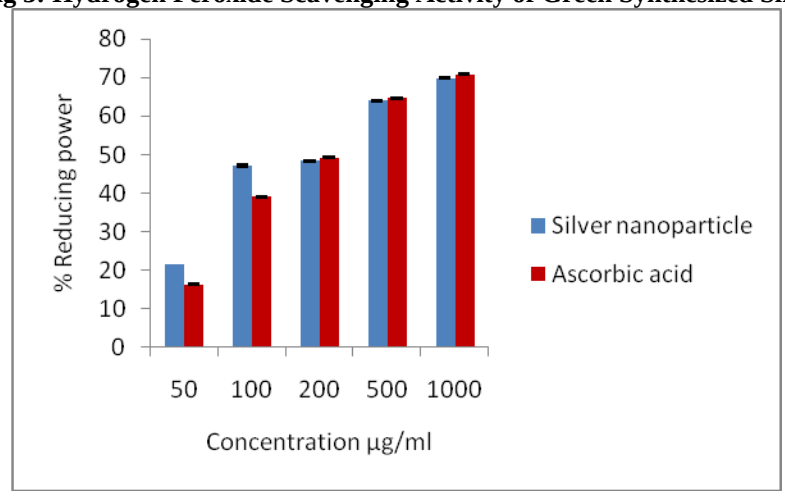

Fig 4: Reducing Power Assay of Green Synthesized Silver Nanoparticles

Reducing Power Assay

The electron donating capacity (reducing power) of compound is associated with antioxidant activity [29]. The reducing power of silver nanoparticles increases in dose dependent manner as shown in figure. The obtained results are shows nearly same efficacy as that of standard ascorbic acid surprisingly with lower concentration silver nanoparticles shown more reducing power than standard. Reducing power is evaluated by the transformation of Fe^{3+} to Fe^{2+} in presence of compound [30]. The reducing capacity of silver nanoparticles may serve as significant indicator of its potential antioxidant activity.

MTT Assay:

MTT assay was performed to determine the cytotoxic property of synthesized silver nanoparticles against A-431 cell lines. It is a colorimetric assay that measures the purple formazan product produced by the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The effect of green synthesized silver nanoparticles on the viability of cancerous cell was determined using 6.25, 12.5, 25, 50 and 100 µg/ml concentrations. The exposure time of silver nanoparticles in the experiment was 24 hours. The silver nanoparticles were reduced viability of the A431 cells in a dose dependent manner as shown in figure. The result obtained shown the IC50 at 47.5µg/ml.

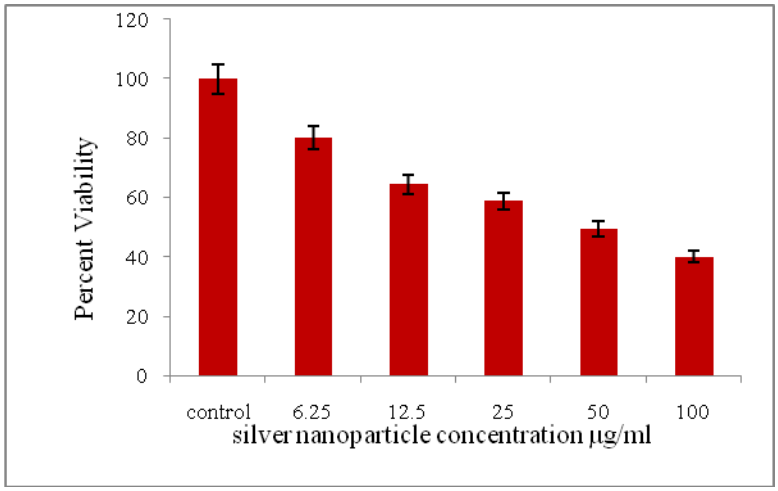

Fig 5: Dose Dependent Effect of Silver Nanoparticles on Cell Viability Studied By Mtt Assay

CONCLUSION

The present study suggest that silver nanoparticles have free radical scavenging activity against DPPH, nitric oxide and hydrogen per oxide radicals also it have reducing power, so silver nanoparticles from *Aegle marmelos* leaf extract is explored for its applications in the prevention of free radical related diseases. Also the green synthesized silver nanoparticles exhibit cytotoxicity against A-431 cell lines, further it may used effectively in treatment of squamous skin cell carcinomas. In coming future an investigation of mechanism of cytotoxic activity will required to be done for efficient use.

REFERENCES:

1. Bunghez IR, Barbinta Patrascu ME, Badea N, Doncea SM, Popescu A, Ion RM. Antioxidant silver nanoparticles green synthesized using ornamental plants. Journal of optoelectronics and advanced materials, 2012 14(11): 1016-1022.
2. Mani A, Lakshmi Gopal V. Bio-mimetic synthesis of silver nanoparticles and evaluation of its free radical scavenging activity. International Journal of Biological & Pharmaceutical Research, 2012; 3(4): 631-633.
3. Rajendran R, Ganesan N, Balu S, Alagar S, Thandavamoorthy P, Thiruvengadam D. Green synthesis, characterization, antimicrobial and cytotoxic effects of silver nanoparticles using *origanum heracleoticum* L leaf extract. Int J Pharm Pharm Sci, 2015; 7(4): 288-293.
4. Mohan CS, Sasikala K, Anand T, Vengaiahand PC, Krishnaraj. Green Synthesis, Antimicrobial and Antioxidant Effects of Silver Nanoparticles using*Canthium coromandelicum* Leaves Extract. Res. J. Microbiol, 2014; 9(3): 142-150.
5. Shawkey AM, Mohamed AR, Abdulall AK and Abdellatif AO. Green nanotechnology: Anticancer Activity of Silver Nanoparticles using Citrullus colocynthis aqueous extracts. Advances in Life Science and Technology, 2013; 13: 60-70.
6. Mani KM, Seethalakshmi S, Gopal V. Evaluation of In-vitro Anti-Inflammatory Activity of Silver Nanoparticles Synthesised using Piper Nigrum Extract, 2015; 6(2): 1-5.
7. Putheti RR, Okigbo RN, Saiadvanapu M, Chavanpatil S. Nanotechnology importance in the pharmaceutical industry. Afr J Pure Applied Chem, 2008; 2: 27-31.
8. Johnsona AS, Obota IB, Ukponga US. Green synthesis of silver nanoparticles using Artemisia annua and Sida acuta leaves extract and their antimicrobial, antioxidant and corrosion inhibition potentials. J. Mater. Environ. Sci, 2014; 5(3) : 899-906.
9. Priyaragini S, Sathishkumar SR Bhaskararao KV. Biosynthesis of silver nanoparticles using actinobacteria and evaluating its antimicrobial and cytotoxicity activity. Int J Pharm Pharm Sci, 2013; 5(2): 709-712.
10. Patil S, Rajeshwari S, Rajiv P, Rajendran V, Seenivasan R. Green synthesis of silver nanoparticle from leaf extract of *Aegle marmelos* and evaluation of its antibacterial activity. Int J Pharm Pharm Sci, Vol, 2014; 7(6): 169-173.
11. Dinesh KS, Gaurav K, Karthik L, Bhaskara Rao KV. A review on pharmacological and phytochemical properties of Aegle

marmelos (L.) Corr. Serr. (Rutaceae). Asian Journal of Plant Science and Research, 2011; 1 (2): 8-17.
12. Jaouad B, Torsten B. Exogenous antioxidants—Double-edged swords in cellular redox state Health beneficial effects at physiologic doses versus deleterious effects at high doses. Landes Bioscience, 2010 3(4): 228-237.
13. Kanchana A, Balakrishna M. Anti-cancer effect of saponins isolated from solanum trilobatum leaf extract and induction of apoptosis in human larynx cancer cell lines. International journal of pharmacy and pharmaceutical sciences, 2011; 3, 356-364.
14. Cakir BO, Adamson P Cingi C. Epidemiology and economic burden of nonmelanoma skin cancer. Facial plastic surgery clinics of North America, 2012; 20 (4): 419-422.
15. Marinkovich MP. Tumour microenvironment: laminin 332 in squamous-cell carcinoma. Nat Rev Cancer, 2007; 7:370-380.
16. Vaidyanathan R, Kalishwaralal K, Gopalram S, Gurunathan S. Nanosilver- the burgeoning therapeutic molecule and its green synthesis. Biotechnol Adv, 2009; 27(6), 924-937.
17. Patil S, Rajeshwari S, Rajiv P, Rajendran V, Seenivasan R. Green synthesis of silver nanoparticle from leaf extract of *Aegle marmelos* and evaluation of its antibacterial activity. Int J Pharm Pharm Sci, Vol, 2015; 7(6): 169-173.
18. Malterud KE, Farbrot TL, Huse AE, Sund RB. Antioxidant and radical scavenging effects of anthraquinones and anthrones, Pharmacology, 1993; 47, 77-85.
19. Nabavi SM, Ebrahimzedh MA, Navabi SF, Bahmaneslami MF. In vitro Antioxidant and Free Radical Scavenging Activity of *Diospyros lotus* and *Pyrus boissieriana* growing in IranPharmacognosy magazine, 2009; 4 (18): 122-126.
20. Gocer HY, Menzek A Gülçin I. Synthesis and antioxidant properties of (3,4-dihydroxyphenyl)(2,3,4-trihydroxyphenyl) methanone and its derivatives. Arch Pharm (Weinheim), 2012; 345:323–334.
21. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr, 1986; 44:307–315.
22. Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal oflmmunologicalMethods, 1986; 89: 271-277.
23. Bhakya S, Muthukrishnan S, Sukumaran M, Muthukumar M. Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. Appl Nanosci, 2015; 10:1-12.
24. Vanitha P, Reddy, Asna Urooj. Antioxidant properties and stability of aegle marmelos leaves extracts. J Food Sci Technol, 2013; 50(1):135–140.
25. 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.
26. Sun T, Ho CT. Antioxidant activities of buckwheat extracts. Food Chemistry, 2005; 90; 743-749.
27. Arun S, Saraswathi U, Singaravelu. Investigation on the free radical scavenging activity of biogenic silver nanoparticle. Int J Pharm Bio Sci, 2015; 6(2):439 – 445.
28. Sathya K, Tamil Selvi S., Bharathidasan R, Rajkumar K, Ilakkiya R, Prabakaran M. Evaluation of phenolic content and antioxidant activity of Aegle marmelos (L.) Corr. Serr. J. Nat. Prod. Plant Resour. 2013; 3 (4):24-28.
29. Yen GC, Duh, PD, Tsai CL. Relationship between antioxidant activity and maturity of peanut hulls. J Agric Food Chem, 1993; 41:67–70.
30. Gulcin I, Oktay M, Kirecci E, Kufrevioglu I , Screening of antioxidant and antimicrobial activities of anise (Pimpella anisum L.) Seed extracts. Food Chem, 2003;83:371-382