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ANTICANCER ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES OF *ABUTILON INDICUM L.* LEAF EXTRACT

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ABSTRACT

The present study was conducted to evaluate the *in-vitro* anticancer activity of green synthesized nanoparticles of *Abutilon indicum L.* leaf extract using MCF-7 breast cancer cell line. The shade dried leaves of *Abutilon indicum L.* was subjected to cold maceration with water and the extract utilized for green synthesis of silver nanoparticles. The UV-visible spectral analysis indicated the formation of nanoparticles, which were characterized by FTIR spectroscopy, SEM, TEM and EDX analysis. The *in-vitro* antioxidant activities of the synthesized nanoparticles were studied by DPPH radical scavenging activity and the anticancer activity by MTT assay in MCF-7 breast cancer cell line. The study indicated that the green synthesized silver nanoparticles of *Abutilon indicum L.* leaf extract possess antioxidant and anticancer activities.

KEYWORDS

Abutilon indicum L, Green synthesized nanoparticles, Antioxidant and Anticancer activity.

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INTRODUCTION

Nanotechnology is an active research area in the modern science field. Nanoparticles have dimensions upto 100nm and exhibits different properties based on their size and morphology[1,2]. The most studied nanoparticles are those made from the noble metals such as silver[3], gold[4] and platinum[5]. Silver (Ag) is the metal of choice for biological systems and medicine[6]. Cancer is characterized by uncontrolled multiplication and

spread of abnormal forms of the body's own cells[7]. There is a continuous search for new anticancer agents from natural products. Investigation by cytotoxicity screening contributed to identification of medicinal plant derived compounds with antitumor activity[8].

The plant *Abutilon indicum* L. belongs to the family Malvaceae commonly called as 'Country Mallow' (English). It is distributed in India, Srilanka, Tropical regions of America and Malaysia[9]. Almost all the parts have medicinal importance and used traditionally for the treatment of various ailments. Even though there are various methods for silver nanoparticle formation such as chemical precipitation, hydrothermal method and chemical vapour deposition, these methods are expensive and involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. Since noble metal nanoparticles are widely applied to areas of human contact, there is a need for 'green chemistry' with the possibility of rapid synthesis, economical, cost-efficient, nontoxic and environment-friendly method for nanoparticle synthesis[10]. In the present study, an attempt has been made to evaluate the anticancer activity of green synthesized silver nanoparticles of *Abutilon indicum* L. leaf extract (AiSNP) against MCF-7 breast cancer cell line.

MATERIALS AND METHODS

Plant collection and Preparation of the extract

The plant *Abutilon indicum* L. was collected from the Coimbatore area and authenticated (No.BSI/SRC/5/23/2012-13/Tech.455) at Botanical survey of India, Tamilnadu Agricultural University, Coimbatore. The leaves were shade dried and powdered. To 250gm of the powdered material, 500ml of distilled water was added in a conical flask and kept for cold maceration for 72 hours with intermittent shaking. The solution was then filtered using gauze cloth and the solvent present in the filtrate was dried by hot air oven. The extract yielded (16%w/w) was stored in a desiccator and dissolved in suitable volume of water to carry out the studies.

Green Synthesis and UV-Visible spectral analysis of AiSNP

For the synthesis of silver nanoparticles, 2 Mm $AgNO_3$ was reduced using 50 ml of 5% *Abutilon indicum* L. leaf extract at room temperature. A dark brown solution indicated the formation of silver nanoparticles. The reduction of Ag^+ ions was monitored by measuring the UV-visible spectrum (300-800 nm) of the solution on a spectrophotometer operating at a resolution of 1nm. Blank contained plant extract alone.

FTIR spectroscopic analysis of AiSNP

The synthesized silver nanoparticles were lyophilized and mixed with KBr pellets and then subjected to FTIR spectral analyses. Silver nanoparticles synthesized after 24 hours of reaction of 2mM $AgNO_3$ solution with *Abutilon indicum* L. leaf extracts were centrifuged at 10,000rpm for 30 minutes at room temperature, after which the pellet was re-dispersed in sterile distilled water. The process of centrifugation and re-dispersion in sterile water was repeated thrice to remove any free biomass residue or compound that was not the capping lig and of the nanoparticles. The purified pellet was then dried and subjected to FTIR (Shimazdu IR prestige-21v) spectroscopy measurement in the spectral range of 4000-400cm^{-1} with resolution of 4cm^{-1}. The FTIR spectra of leaf extracts taken before and after the synthesis of silver nanoparticles were analyzed to study the possible functional groups responsible for the formation of silver nanoparticles.

SEM, TEM and EDX analysis of AiSNP

The morphological characterizations of the synthesized AiSNP were analyzed using SEM (S-2600 N; Hitachi, Tokyo, Japan). Thin films of the sample were prepared on a carbon coated copper grid using spin coater by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 mins. An electron beam was focused into sample and the interaction created various signals, which were detected. TEM images were obtained using JEOL

JEM 2100 analytical high resolution transmission electron microscope with an accelerated voltage of 120 kV. TEM and EDX samples were prepared by drop casting -5 µl of nanoparticle dispersion onto a carbon-coated copper TEM grid, followed by air drying at ambient conditions, stored in a desiccator and imaged shortly after collection. The size distributions were determined by image analysis using the Image J software package[11].

***In-vitro* antioxidant studies of AiSNP by DPPH radical scavenging assay**

The radical scavenging activity of AiSNP sample against DPPH was determined spectrophotometrically[12]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH reacts with an antioxidant compound that can donate hydrogen and gets reduced. The change in color from deep violet to blue was measured. The intensity of the color depends on the amount and nature of radical scavengers present in the given sample. The reaction mixture contained 1.0 ml of DPPH, 0.5 ml of sample and made up to 3.0 ml with water. The tubes were incubated for 10 minutes at 37ºC. The absorbance was measured at 517 nm against ascorbic acid as standard.

Determination of anticancer activity of the AiSNP by MTT assay

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The monolayer cells were detached with trypsin- EDTA to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with a medium containing 5% FBS to give a final density of $1x10^5$ cells/ml. 100µl/well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 hours, the cells were treated with serial concentrations (10.0, 5.0, 1.0 and 0.1 µg/ml) of AiSNP. The nanoparticles were initially dissolved in dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four dilutions were made to provide a total of five sample concentrations. Aliquots of 10 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 hours. The medium devoid of samples served as a control and triplicates were maintained for all the concentrations.

Statistical Analysis

Data are expressed as mean ± S.D. Statistical comparisons between the groups were done by one way analysis of variance (ANOVA) to analyze the differences. $P<0.05$ were considered as significant.

RESULTS AND DISCUSSION

Green synthesis of AiSNP

The synthesis of silver nanoparticles from *Abutilon indicum* L. was performed by treating the aqueous leaf extract with silver nitrate (2mM) and incubated for 24 hours in dark at room temperature. Color change observed from transparent to brown indicated the green synthesis of AiSNPs (Figure No.1). This characteristic color variation is due to the excitation of the surface Plasmon resonance in the metal nanoparticles[13].

UV- Visible spectral analysis AiSNP

UV-Visible spectroscopy could be used to examine the presence of silver nanoparticles in the aqueous suspension[14]. In order to verify the presence of silver nanoparticles, the test samples (leaf extract treated with 2mM $AgNO_3$) were confirmed by obtaining a spectrum in a visible range of 300-800 nm. This analysis showed the absorbance peak at 425 nm (Figure No.2) due to the surface Plasmon resonance of AiSNPs in which the broadening in peak indicated that the particles were polydispersed.

FTIR spectroscopic analysis of AiSNP

FTIR measurements were carried out to characterize and identify the possible biomolecules responsible for the reduction of Ag^+ ions. FTIR absorption spectra of water soluble extract of *Abutilon indicum* L. before and after reduction of

Ag^+ ions were identified. Absorbance bands were obtained in the regions of 400-4000cm^{-1}. The total disappearance of 2929 cm^{-1} band after the bioreduction may be due to the fact that the presence of characteristic sugar is mainly responsible for the reduction of Ag^+ ions (Figure No.3). This phenomenon was also supported by a recent work which showed that sucrose and fructose can function as reducing agents for the synthesis of aqueous dispersions of silver nanoparticles[15] as well as stabilizing ligands for various metal nanoparticles (e.g., Au, Ag, Pd, and Pt) [16-18].

SEM and TEM observations of AiSNP

The SEM image showed the high density nanosilvers recorded from drop-coated films of the silver nanoparticles by treating aqueous silver nitrate solution with *Abutilon indicum* L. leaf extract. The formed AiSNPs were relatively spherical in shape with average of 70-95 nm in size. It is known that the shape of the metal nanoparticles considerably change their optical and electronic property[19]. The morphology of TEM image of AiSNP was spherical in nature. Further observations showed that the silver nanoparticles were surrounded by other materials, which are capping the organic material from *Abutilon indicum* L. leaf extract. The nanoparticles were in the range of 10-30 nm and few particles were agglomerated (Figure No.4).

EDX (Energy dispersive X-ray) spectrum analysis of AiSNP

The elemental analysis of the AiSNP was performed using the EDX on the TEM grid. The (Figure No.5) shows the EDX spectral plot of spherical shaped nanopaticles.These further confirm the presence of the signals characteristic of silver. The EDX reading showed the presence of copper (Cu^{2+}) and chloride (Cl^-) ions. The peaks for Cu^{2+} are from the grid used and the peak Cl^- ions corresponds to the capping material from the plant extract. These results indicated that the synthesised product composed of silver nanoparticles.

DPPH radical scavenging assay of AiSNP

The free radical scavenging activity of AiSNP was evaluated based on the ability to scavenge the synthetic DPPH. The standard ascorbic acid, AiSNP and the plant extract showed a concentration dependant free radical scavenging activity as shown in Table No.1. The maximum activity for scavenging of DPPH radicals was found to be 92.72%, 75.76% and 70.32% for ascorbate, AiSNP and the plant extract respectively at 250 µg/ml. There was a significant difference ($P<0.05$) in the radical scavenging activity, when the AiSNP and the plant extract alone were compared with the standard. Furthermore, the mean values of DPPH radical scavenging activity of AiSNP were found to be closer to the standard, when compared with the plant extract alone, which indicated that AiSNP had more radical scavenging activity than the plant extract alone. (* $^{=}P<0.05$).

Determination of anticancer activity of AiSNP against MCF-7 cell line by MTT assay

The *in-vitro* anticancer activity of AiSNP carried out against MCF-7 cell line by MTT assay showed 99.71 % cell death at 10µg/ml with IC_{50} value of 1.682 µg/ml, as shown in the (Table No.2).

From the results of MTT assay, it can be well predicted that the degree of cell mortality rate was directly proportional to the concentration (1.0, 5.0 and 10.0 µg/ml) of the AiSNP. The normal MCF-7 breast cancer cell lines were spherical in shape which on treatment with AiSNP, due to its anticancer activity, cell growth was inhibited and eventually the cell death occurred and aggregated to form round dead cells (Figure No.6).

The phytochemical screening of *Abutilon indicum* L. indicated the presence of alkaloids and flavonoids, which have been showed to possess cytotoxic activity[20]. In a previous study, Abdul *et al.,* 2010 also reported that the methanolic leaf extract of *Abutilon indicum* L. showed promising cytotoxic activity at 400µg/ml[21].

Table No.1: DPPH radical scavenging activity of AiSNP

S.No	Concentration (µg/ml)	Standard % inhibition	AiSNP % inhibition	Plant Extract % inhibition
1	50	63.96 ± 0.32	50.56 ± 0.56*	42.50 ± 0.56*
2	100	70.54 ± 0.79	57.10 ± 0.22*	50.04 ± 0.71*
3	150	77.78 ± 0.51	63.94 ± 0.66*	57.88 ± 0.54*
4	200	85.20 ± 0.67	69.04 ± 0.33*	64.66 ± 0.51*
5	250	92.72 ± 0.51	75.76 ± 0.55*	70.32 ± 0.89*

Table No.2: Determination anticancer activity of AiSNP by MTT assay

S.No	AiSNP	Conc. µg/ml	Absorbance	% inhibition	IC_{50} µg/ml	R^2
1	-	Control	0.466667	-	-	-
2	AiSNP 1	-	0.379667	18.64286	-	-
3	-	5	0.020667	95.57143	1.682 µg/ml	0.9998
4	-	10	0.001333	99.71429	-	-

Figure No.1: Aqueous solution of 2mm $AgNO_3$ before and after the addition of *Abutilon indicum L.* Leaf broth

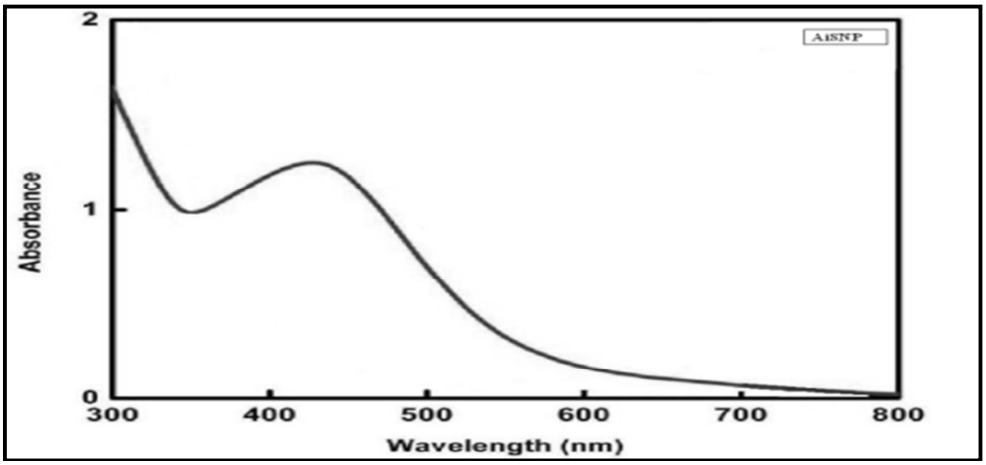


Figure No.2: UV-Visible absorption spectra for the reduction of silver ions to silver nanoparticles

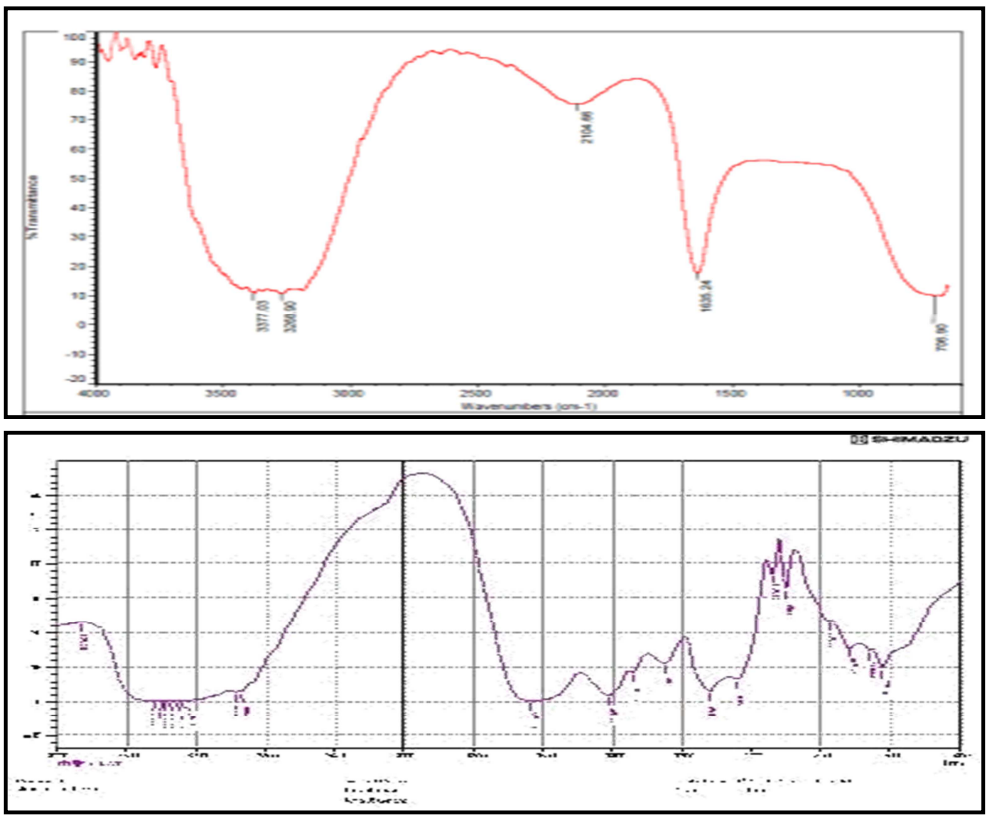


Figure No.3: FTIR spectra of dried powder of (a) *Abutilon indicum* L. extract and (b) AiSNP

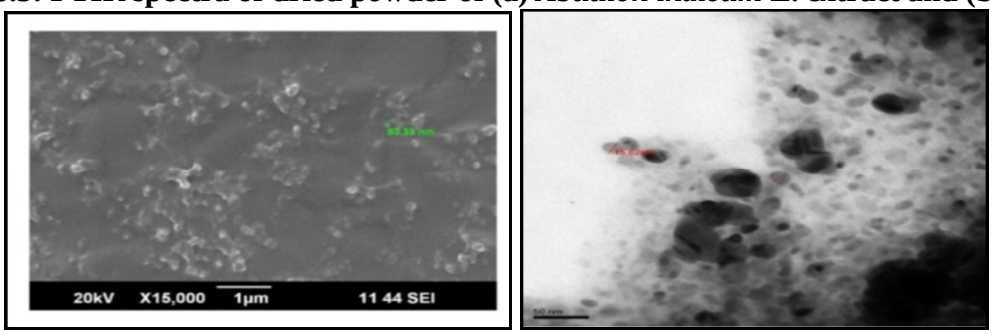


Figure No.4: SEM and TEM image of AiSNP

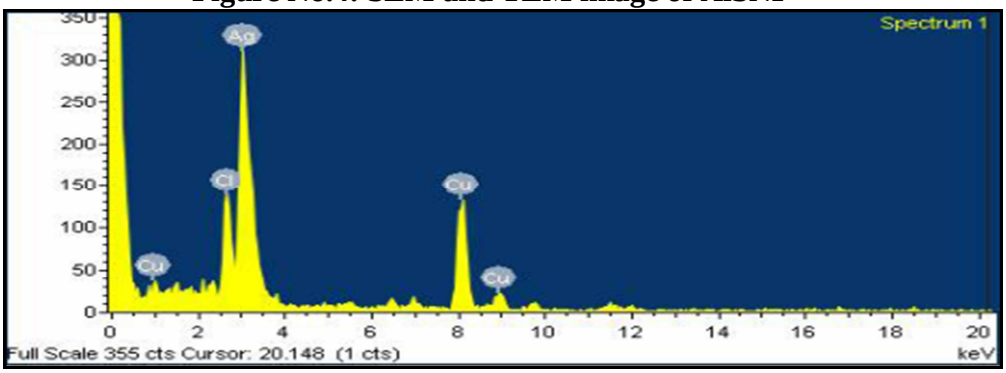
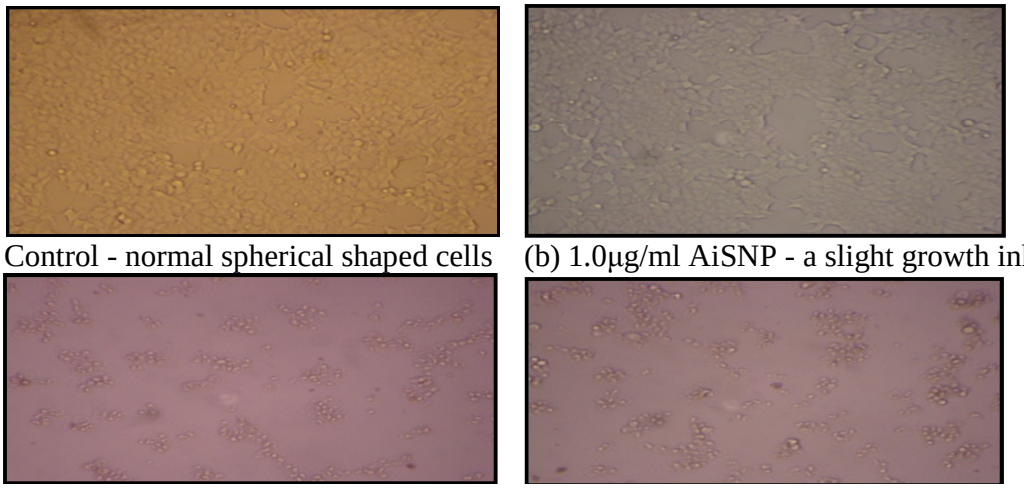


Figure No.5: EDX for AiSNP



(a) Control - normal spherical shaped cells (b) 1.0µg/ml AiSNP - a slight growth inhibition

(c) 5.0µg/ml AiSNP - nearly maximum cell death occurred. (d) 10.0µg/ml AiSNP - almost all cells were died by showing round cells in purple color layer.

Figure No.6: Effect of AiSNP on MCF-7 cell line (a) control, (b) 1µg/ml, (c) 5µg/ml, and (d) 10µg/ml

CONCLUSION

The present study concerns with cost effective and environment friendly synthesis of silver nanoparticles using the aqueous leaf extract of *Abutilon indicum* L. The synthesized nanoparticles showed dose dependent free radical scavenging activity and anticancer activity against MCF-7 breast cancer cell line.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BLBLIOGRAPHY

1. Murphy C J. Sustainability as a Design Criterion in Nanoparticle Synthesis and applications, *J. Mater Chem,* 18(19), 2008, 2173-2176.
2. Naiwa H S. Hand Book of Nanostructural Materials and Nanotechnology, *Academic press*, 23(2), 2000, 1-5.
3. Nelson Duran, Priscyla D Marcato, Oswaldo L Alves, Gabriel I H De Souza and Elisa Esposito. Sustainability as a Design Criterion in nanoparticle Synthesis and applications, *Journal of Nanobiotechnology*, 3(8), 2005, 1-7.
4. Ankamwar B. Biosynthesis of glod nanoparticles (green gold) using leaf extract of Terminalia catappa, *E-Journal of Chemistry*, 7(4), 2010, 1334-1339.
5. Deng Q Y, Yang B, Wang J F, Whiteley C G and Wang X N. Biological synthesis of platinum nanoparticles with apoferritin, *Biotechnol. Lett*, 31(10), 2009, 1505-1509.
6. Jain D, Daima H K, Kachhwaha S and Kothari S L. Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities, *Dig. J. Nanomater. Biostruct*, 4(3), 2009, 723-727.
7. Akdan F and Khosro P. *In vitro* Cytotoxic Activity of Aqueous Root Extract of *Althea kurdica* against Endothelial Human Bone Marrow Cells (line k562) and Human Lymphocytes, *Bull. Env. Pharmacol. Life Sci*, 2(6), 2013, 23-29.
8. Cardellina J H, Fuller R W, Gamble W R, Westergaard C, Boswell J, Munro M H G, Currens M, Boyd M P. Evolving strategies for the selection dereplication and prioritization of antitumor and HIV inhibitory natural products extracts, (Eds. Bohlin, L. and Bruhn, J.G.) Bioassay Methods in Natural Product Research

and Development, *Kluwer Academic Publishers, Dordrecht*, 24(2), 1999, 25-36.
9. Giri K R, Kanungo S K, Patro V J, Dash S and Sahoo D C. Lipid lowering activity of *Abutilon indicum* (L.) leaf extracts in rats, *Journal of Pharmacy Research*, 2(11), 2009, 1725-1727.
10. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar S R, Khan M I, Parischa R, Ajayakumar P V, Alam M, Kumar R and Sastry M. Biological approach to Nanoparticle synthesis, *Nano Lett*, 1(1), 2001, 515-519.
11. Abramoff M D, Magalhaes P J and Ram S. Image processing with image, *J. Biophotonics International*, 11(7), 2004, 36-41.
12. Brand-Williams W, Cuvelier M E and Berset C. Use of a free radical method to evaluate antioxidant activity, *LWT Food Sci Technol*, 28(1), 1995, 25-30.
13. Mallikarjuna K, Narasimha G, Dilip G R, Praveen B, Shreedhar B, Sree Lakshmi C, Reddy B V S, Deva Prasad Raju B. Green synthesis of Silver nanoparticles using *Ocimum* leaf extract and their characterization, *Digest Journal of nanomaterials and Biostructures*, 6(1), 2011, 181-186.
14. Wiley B J, Im S H, Li Z Y, McLellan J, Siekkinen A, and Xia Y. Maneuvering the Surface Plasmon Resonance of Silver Nanostructures through Shape-Controlled Synthesis, *Journal of Physical Chemistry B*, 110(32), 2006, 15666-15675.
15. Mehta S K, Chaudhary S, Gradzielski M. Time dependence of nucleation and growth of silver nanoparticles generated by sugar reduction in micellar media, *Journal of Colloid and interface science*, 343(2), 2010, 447-453.
16. Liu J, He F, Gunn T M, Zhao D and Roberts C B. Precise seed-mediated growth and size-controlled synthesis of palladium nanoparticles using a green chemistry approach, *Langmuir*, 25(12), 2009, 7116-7128.
17. Liu J, Sutton J and Roberts C B. Synthesis and extraction of monodisperse sodium carboxymethylcellulose-stabilized platinum nanoparticles for the self-assembly of ordered arrays, *Journal of physical chemistry*, 111(31), 2007, 11566-11576.
18. Jana N R, Gear heart L, and Murphy C J. Wet chemical synthesis of high aspects ratio cylindrical gold nanorods, *Journal of Physical Chemistry B*, 105(19), 2001, 4065-4067.
19. Xu H and Kall M. Morphology effects on the optical properties of silver nanoparticles, *Journal of Nanoscience and Nanotechnology*, 4(1), 2002, 254-259.
20. Brown J P. A review of genetic effects of naturally occurring flavonoids, anthraquinones and related compounds, *Mutation research*, 75(3), 1980, 243-277.
21. Abdul M, Sarker A, Saiful I and Muniruddin A. Cytotoxic and antimicrobial activity of the crude extract of *Abutilon indicum*, *International Journal of pharmacognosy and phytochemical research*, 2(1), 2010, 1-4.

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