Middle-East Journal of Scientific Research 21 (12): 2257-2263, 2014
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2014.21.12.21876

Green Synthesis of Gold Nano Particles VII: Green Synthesis and Characterization of Gold Nano Particles Using the Extract of Dhania (*Coriandrum sativum*) and Study of its Cytotoxicity Properties

[1]Saber E. Mansou, [2]Monalisa Pattanayak and [2]P.L. Nayak

[1]Department of Chemistry, Faculty of Science,
Omar Al-mukhtar University, P.O. Box: 919, Al-bayda, Libya
[2]P.L. Nayak Research Foundation and Centre of Excellence in Nano Science and Technology, Synergy Institute of Technology, Phulnakhara, Bhubaneswar, Odisha, India

Abstract: The synthesis of eco-friendly nanoparticles is evergreen branch of nanoscience for biomedical application. Low cost of synthesis and non toxicity are main features make it more attractive potential option for biomedical field and elsewhere Gold nanoparticles are traditionally synthesized by reducing metallic agents. There are a number of reducing agents reported in the literature for the synthesis of AuNps. These methods are toxic methods. In the present investigation, green synthesis of gold nano particles has been carried out using eco-friendly method such as the plant extract of Dhania. The nano particles so synthesised were characterized by Uv-visible and TEM analysis. The Cellular Internalization studies of AuNps provide new opportunities for probing cellular processes via nanoparticulate-mediated imaging. The cytotoxicity studies clearly demonstrate that the phytochemicals within these herbs provide a nontoxic coating on AuNps.

Key words: Green synthesis · Gold · Dhania · Cytotoxicity studies

INTRODUCTION

Nanotechnology has dynamically developed as an important field of modern research with potential effects in electronic and medicine (Glomm 2005, Chan 2006, Boisselier and Astruc 2009). Nanotechnology can be defined as a research for the design, synthesis and manipulation of structure of particles with dimension smaller than 100nm. A new branch of nanotechnology is nanobiotechnology. Nanobiotechnology combines biological principles with physical and chemical procedures to generate nano-sized particles with specific functions. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanopaticles formation. These methods of synthesis can be divided on intra cellular and extracellular (Ahmad *et al*. 2005). This integration of nanoparticles with biological molecules has lead to the development of diagnostic devices, contrast agents and important tools in cancel therapy. Nanobiotechnology describes an application of biological systems for the production of new functional material such as nanoparticles. Biosynthetic methods can employed either microorganism cells or plant extract for nanoparticles production. Biosynthesis of nanoparticles is an exciting recent addition to the large repertoire of nanoparticles synthesis methods and now, nanoparticles have entered a commercial exploration period. Gold and silver nanoparticles are presently under intensive study for applications in optoelectronic devices, ultrasensitive chemical and biological sensors and as catalyst.

Recently Nayak and coworkers have extensively studied the use of plant extracts for the green synthesis of gold nano particles [1, 2, 3, 4]. The use of phytochemicals in the synthesis of nanoparticles is an important symbiosis between nanotechnology and green chemistry [5, 6, 7]. As the nanorevolution unfolds, it is imperative to develop 'nano-naturo' connections between nanotechnology and green domains of the nature. Production of nanoparticles under nontoxic green conditions is of vital importance to address growing concerns on the overall toxicity of nanoparticles for

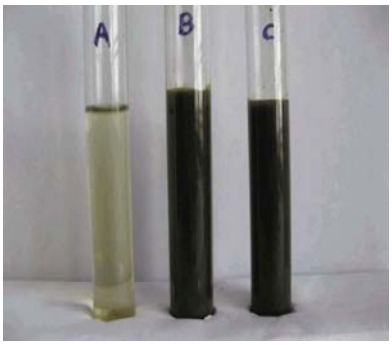
Corresponding Author: Dr. P.L. Nayak, P.L. Nayak Research Foundation and Centre of Excellence in Nano Science and Technology, Synergy Institute of Technology, Phulnakhara, Bhubaneswar, Odisha, India.
Tel: +91 9238859425.

medical and technological applications [8, 9, 10]. The power of phytochemicals, which initiate varieties of chemical transformations within biological systems, is well known [9, 11, 12, 13]. For example, a high level of genistein found in plant materials is both a phytoestrogen and antioxidant and has been extensively used to treat conditions affected by estrogen levels in the body [14, 15]. The tremendous health benefits of chemical cocktails present within Dhania is beyond doubt, the actual applications of the chemical reduction power of the myriad of chemicals present in herbs and spices is still in infancy. Therefore we investigated the synergistic potentials of polyphenols, flavonoids, catechins and various phytochemicals present in Dhania for the reduction reactions of gold salts to produce AuNps which have potential applications in the diagnosis and therapy of various deadly diseases including cancer.

In the present research programme, gold nano particles have been synthesised by the plant extract of Dhania. The nano particles have been characterized by using Uv-Visible and TEM studies. The cytoxicity study of the nano particles have also been studied.

(a)

Fig. 1: a) DHANIA LEAVES



(b)

Fig. 1: b) Tube A- Auric acid,
Tube B- Dhania extract,
Tube C- Dhania gold
nanoparticle solution.

MATERIALS AND METHODS

Synthesis of Dhania Gold Nanoparticles (Dhania-AuNps):

Coriander or Dhania (Coriandrum Sativum): It is a small herb belongs to family *Apiaceae* (Fig. 1. e). Major active constituents of *coriandrum sativum* are essential oils and fatty oil. The essential oil content of the weight of ripe and a dried fruit of coriander varies between 0.03 and 2.6% and the content of fatty oil varies between 9.9 and 27.7%. The juice of coriander is use for treating nausea and morning sickness. It is also used in the treatment of colitis and some of the liver disorders. Coriander seeds also help to reduce acid peptic disease and it is also used as ayurvedic medicine in the treatment of Dysentery [25].

Step 1: Dhania Extract Preparation: Intact Dhania (8 g) were washed with distilled water to remove any traces of contaminants. Dhania leaves were then soaked in 50 ml of DI water at room temperature for 72 hrs. The supernatant was decanted and centrifuged at 8000 rpm for 10 min at room temperature and was stored at 40°C and for use within 3 days.

Step 2: Four ml of Dhania supernatant were diluted to 8 ml in DI water and was heat bed to simmer for 1 min.

Step 3: To this solution, 100 µl of NaAuCl4 (0.1 M) were added and further heated to simmering with constant stirring. Within 20 minutes, the color of the solution turned to ruby red indicating the formation of gold nanoparticles (Dhania-AuNps).

Cytotoxicity Studies (MTT Assay): Cytotoxicity evaluation of Dhania-AuNps was performed using MTT assay as described by Mosman [20]. Approximately 1 × 105 ml-1 cells (MCF-7 and PC-3) in their exponential growth phase were seeded in a flat-bottomed 96-well polystyrene coated plate and were incubated for 24 hrs at 37°C in a 5% CO2 incubator. Series of dilutions (10, 30, 50, 70, 90, 110 and 150 µM) of AuNps in the medium was added to the plate in hexaplates. After 24 hrs of incubation, 10 µl of MTT reagent was added to each well and was further incubated for 4 hrs. Formazan crystals formed after 4 hrs in each well were dissolved in 150 µl of detergent and the plates were read immediately in a microplate reader (Spectramex, 190 Molecular Devices Inc., USA) at 570 nm. Wells with complete medium,

nanoparticles and MTT reagent, without cells were used as blanks. A control experiment with series of dilutions of NaAuCl4 was performed using the same MTT kit to validate the assay.

RESULTS AND DISCUSSION

Synthesis of Green Gold Nanoparticles: Our new *green* process for the production of gold nanoparticles uses direct interaction of sodium tetrachlroaurate (NaAuCl4) with Dhania powder in the absence of man-made chemicals and thus, satisfies all the principles of a 100% green chemical process. Various phytochemicals present in Dhania is presumably responsible for making a robust coating on gold nanoparticles and thus, rendering stability against agglomerations. Absorption measurements indicated that the plasmon resonance wavelength, λmax of Dhania-AuNps, is 535 nm respectively. The size of Dhania-AuNps is in the range of 12±4 nm; respectively as measured from TEM techniques (Figures 1).

XRD of Gold nano Particles: Figure 2. Shows the XRD patterns obtained for gold nanoparticles synthesized in present research work. The crystalline nature of the gold nanoparticles is clearly shown in XRD pattern. Bragg reflections corresponding to lattice planes (111), (200), (220), (311), (222) are observed in XRD pattern.

Cellular Internalization Studies: Results of cellular internalization studies of AuNps solutions are key to providing insights into their use in biomedicine. Their selective cell and nuclear targeting will provide new pathways for their site-specific delivery as diagnostic/ therapeutic agents. A number of studies have demonstrated that phytochemicals present in Dhania have the ability to penetrate the cell membrane and internalize within the cellular matrix [21, 22]. Cancer cells are highly metabolic and porous in nature and are known to internalize solutes rapidly compared to normal cells [22]. Therefore, we hypothesized that Dhania derived phytochemicals, if coated on AuNps, will show internalization within cancer cells. TEM images of prostate (PC-3) and breast tumour (MCF-7) cells treated with AuNPs unequivocally validated our hypothesis. Significant internalization of nanoparticles via endocytosis within the MCF-7 and PC-3 cells was observed (Figures 2; 3). The internalization of nano particles within cells could occur via processes including phagocytosis, fluid-phase endocytosis and receptor mediated endocytosis. The viability of both PC-3 and MCF-7 cells post-internalization suggests that the phytochemical coating renders the nanoparticles non-toxic to cells. Such a harmless internalization of AuNps will provide new opportunities for probing cellular processes via nanoparticulate-mediated imaging.

Cytotoxicity Studies: Untreated PC-3 and MCF-7 cells as well as cells treated with 10, 30, 50, 70, 90,110 and 150 µM concentrations of various AuNps for 24 hrs were subjected to the MTT assay for cell-viability determination. In this assay, only cells that are viable after 24 hexposure to the sample are capable of metabolizing a dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) efficiently and produce a purple colored precipitate which is dissolved in a detergent and analyzed sphectrophotometrically. After 24 hrs post-treatment, PC-3, MCF-7 cells showed excellent viability even up to 150 µM concentrations of Dhania -AuNps (Figures 11 a, b;). These results clearly demonstrate that the phytochemicals within these herbs provide a nontoxic coating on AuNps and corroborate the results of the internalization studies discussed above. It is also important to recognize that a vast majority of Gold (I) and Gold (III) compounds exhibit varying degrees of cytotoxicity to a variety of cells (Figure 4). The lack of any noticeable toxicity of Dhania-AuNps provides new opportunities for the safe application in molecular imaging and therapy.

Cellular Internalization Studies: Results of cellular internalization studies of AuNps solutions are key to providing insights into their use in biomedicine. Their selective cell and nuclear targeting will provide new pathways for their site-specific delivery as diagnostic/ therapeutic agents. A number of studies have demonstrated that phytochemicals present in Dhania have the ability to penetrate the cell membrane and internalize within the cellular matrix [21, 22]. Cancer cells are highly metabolic and porous in nature and are known to internalize solutes rapidly compared to normal cells [22]. Therefore, we hypothesized that Dhania derived phytochemicals, if coated on AuNps, will show internalization within cancer cells. TEM images of prostate (PC-3) and breast tumour (MCF-7) cells treated with AuNPs unequivocally validated our hypothesis. Significant internalization of nanoparticles via endocytosis within the MCF-7 and PC-3 cells was observed (Figures 4; 5; 6). The internalization of nano

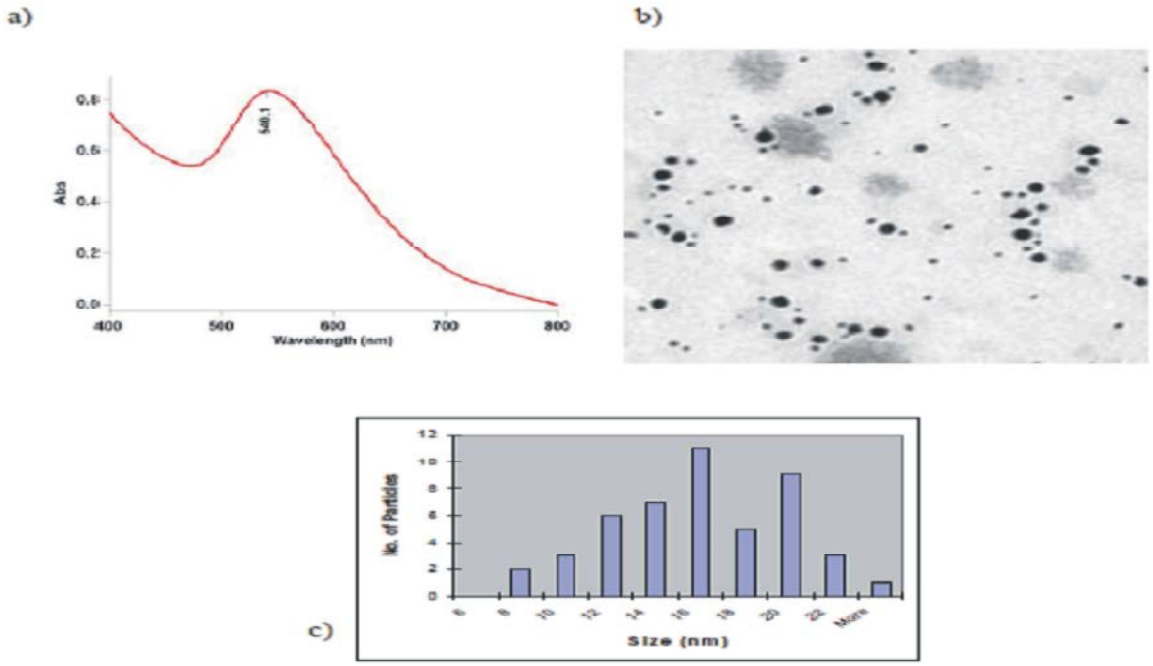


Fig. 1: a) UV-Visible absorption spectrum, b) TEM Image, c) size distribution of Dhania Gold Nanoparticles

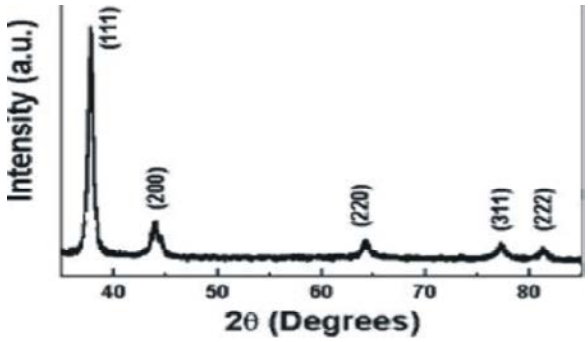


Fig. 2: XRD of Gold particle

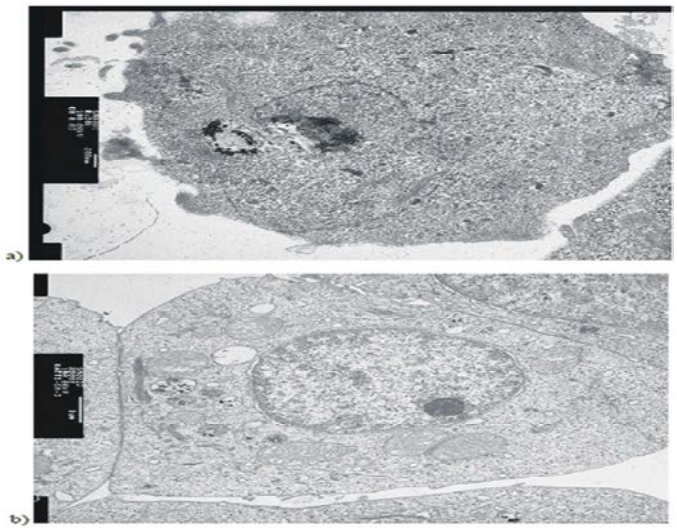


Fig. 3. a, b: TEM Images of different MCF-7 cells showing uptake of Dhania-AuNps in to the lysosomes

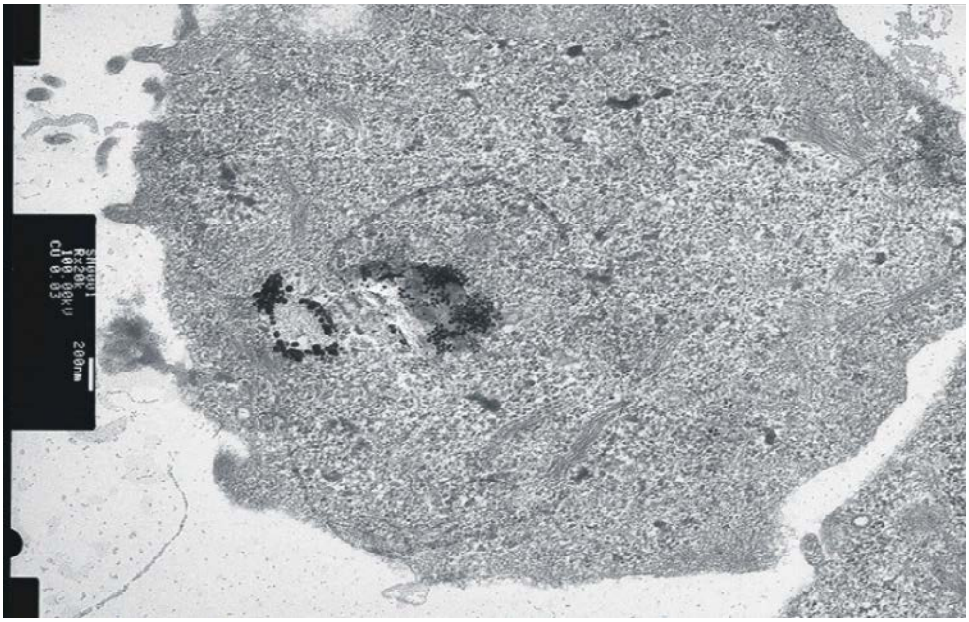


Fig. 4: TEM Images of different MCF-7 cells showing uptake of Dhania-AuNps in to the lysosomes

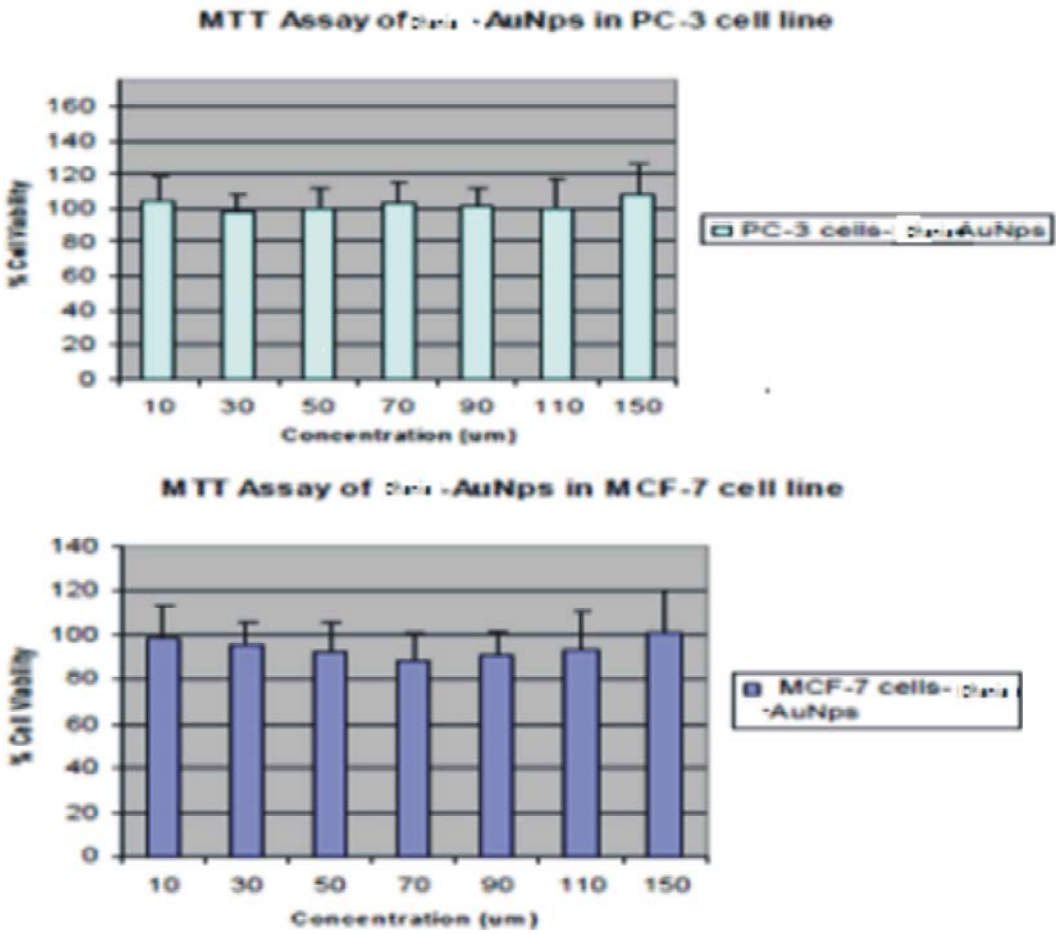


Fig. 5a,b: Dose dependent cytotoxicity of Dhania-AuNPs in cultured PC-3 and MCF-cells after 24 hrs of exposure using MTT assay

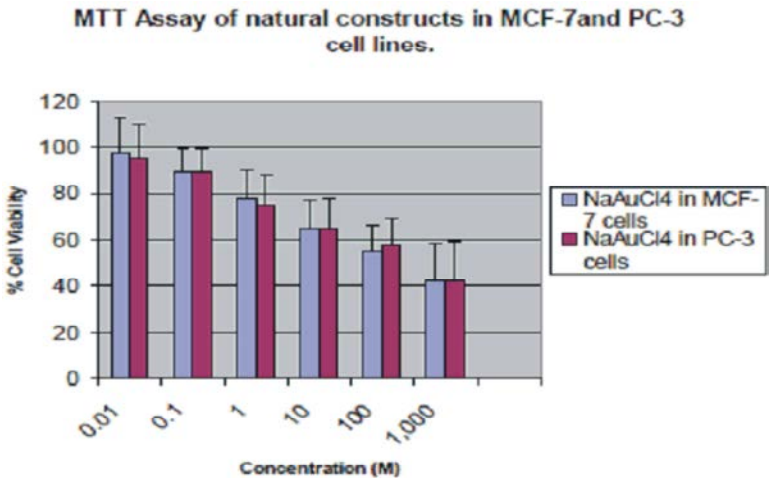


Fig. 6: Dose dependent cytotoxicity of NaAuCl4 in cultured PC-3 and MCF-cells after 24 hrs of exposure using MTT assay

particles within cells could occur via processes including phagocytosis, fluid-phase endocytosis and receptor mediated endocytosis. The viability of both PC-3 and MCF-7 cells post-internalization suggests that the phytochemical coating renders the nanoparticles non-toxic to cells. Such a harmless internalization of AuNps will provide new opportunities for probing cellular processes via nanoparticulate-mediated imaging.

SUMMARY AND CONCLUSION

Green synthesis of metallic nanoparticles is a successive alternative to chemical synthesis protocols for synthesizing gold nano particles. Gold nanoparticles are defined as stable colloid solutions of clusters of gold atoms with sizes ranging from 1-100 nm. At this nanoscale, AuNps possess different physicochemical characteristics when compared to the bulk gold, most obvious example being the colour change from yellow to ruby red when bulk gold is converted into nanoparticulate gold. This ruby red colour of AuNps is explained by a theory called “surface plasmonics”. Gold nano particles have been synthesised successfully by using green chemistry with the help of the plant extract like Dhania.

REFERENCES

1. Lal, S.S. and P.L. Nayak, 2012. Green synthesis of Gold nano particles using various extracts of Plants and Spices, International Journal of Science Innovations and Discoveries (*IJSID)*, 2(2): 325-350, ISSN: 2249-5347.
2. Pattanayak, M. and P.L. Nayak, 2013. Green Synthesis of Gold Nanoparticles using *Elettaria cardamomum* (ELAICHI) Aqueous Extract, World Journal of Nano Science and Technology (WJNST), IDOSI Publications, 2(1): 01-05.
3. Pattanayak, M. and P.L. Nayak, 2013. Green Synthesis and Characterization of Zero Valent Iron Nanoparticles from the Leaf Extract of *Azadiracchta indica* (NEEM). World Journal of Nano Science and Technology (WJNST), IDOSI Publications, 2(1): 06-09.
4. Parida1, U.K., B.K. Bindhani and P.L. Nayak, 2011. Green Synthesis and Characterization of Gold Nanoparticles Using Onion (Allium cepa) Extract, World Journal of Nano Science and Engineering (WJNSE), 1: 93-98.
5. Schellenberger, E.A., D. Sosnovik, R. Weissleder and L. Josephson, 2004. Magneto/Optical Annexin V, a Multimodal Protein. Bioconjugate Chem., 15(5): 1062 -1067.
6. Huang, J., Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Y. Wang, W. Shao, N.J. Hong and C. Chen, 2007. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. Nanotechnol., 18: 105104-105115.
7. Jorge, L., G. Torresdey, E. Gomez, JR. Peralta-Videa, JG. Parsons, H. Troiani and MJ. Yacaman, 2003. Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Langmuir., 19: 1357.

8. Gardea-Torresdey, J.L., K.J. Tiemann, J.G. Parsons, G. Gamez, I. Herrera and M. Jose Yacaman, 2002. Investigation into the Mechanism(s) of Au (III) Binding and Reduction by Alfalfa Biomass. Microchemical Journal, 71: 193-204.
9. Hardman, R., 2006. Toxicologic Review of Quantum Dots: Toxicity Depends on Physicochemical and Environmental Factors, Environ. Health. Perspect., 114: 165.
10. Curtis, J., M. Greenberg, J. Kester, S. Phillips and G. Krieger, 2006. Nanotechnology and Nanotoxicology: A Primer for Clinicians, Toxicol. Rev., 25: 245.
11. Lewinski, N., V. Colvin and R. Drezek, 2008. Cytotoxicity of nanoparticles. Small., 4: 26-49.
12. Espín, J.C., M.T. García-Conesa and F.A. Tomás-Barberán, 2007. Nutraceuticals: Facts and fiction, Phytochemistry, 68: 2986.
13. Rochfort, S. and J. Panozzo, 2007. Class targeted metabolomics: ESI ion trap screening methods for glucosinolates based on MS*n* fragmentation, J. Agric. Food. Chem., 55: 7981.
14. Setchell, K.D., N.M. Brown, P. Desai, L. Zimmer-Nechemias, B.E. Wolfe, W.T. Brashear, A.S. Kirschner, A. Cassidy and J.E. Heubi, 2001. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements, J. Nutr., 131: 1362S-75S.
15. Magee, P.J. and I.R. Rowland, 2004. Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer, Br. J. Nutr., 91: 513-520.
16. Limer, J.L. and V. Speirs, 2004. Phyto-oestrogens and breast cancer chemoprevention. Breast Cancer Res., 6: 119-127.
17. Bandele, O.J. and N. Osheroff, 2008. Epigallocatechin Gallate, A Major Constituent of Green Tea, Poisons Human Type II Topoisomerases, Chem. Res. Toxicol., 21: 936-43.
18. Shankar, S., S. Ganapathy and R.K. Srivastava, 2007. Green tea polyphenols: biology and therapeutic implications in cancer. Front Biosci., 12: 4881-99.
19. Dannemann, K., W. Hecker, H. Haberland, A. Herbst, A. Galler, T. Schäfer, E. Brähler, W. Kiess and T.M. Kapellen, 2008. Use of complementary and alternative medicine in children with type 1 diabetes mellitus - prevalence, patterns of use and costs. Pediatr Diabetes.
20. Suppapitiporn, S. and N. Kanpaksi, 2006. The effect of cinnamon cassia powder in type 2 diabetes mellitus, J. Med. Assoc. Thai., 89: Suppl 3: S200-5.
21. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol Methods., 65: 55-63.
22. Mizuno, H., Y.Y. Cho, F. Zhu, W.Y. Ma, A.M. Bode, C.S. Yang, C.T. Ho and Z.G. Dong, 2007. Theaflavin-3, 3'-Digallate Induces Epidermal Growth Factor Receptor Down-Regulation, Mol. Carcinog., 45: 204-212.
23. Sun, D.J., Y. Liu, D.C. Lu, W. Kim, J.H. Lee, J. Maynard and A. Deisseroth, Endothelin-3 growth factor levels decreased in cervical cancer compared with normal cervical epithelial cells, Human Pathology, 38: 1047-1056.