

Green Synthesis of Gold Nano Particles VIII: Green Synthesis and Characterization of Gold Nano Particles Using the Extract of Podina (*Mentha piperita*) and Study of its Cytotoxicity Properties

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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 Podina. 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 • Podina • Cytotoxicity studies

INTRODUCTION

The term “nano” is derived from the Greek word “nanos” which means small and it is used as the prefix for one billionth part (10⁻⁹). According to American Society for Testing and Materials (ASTM international 2006), nanoparticles are those particles which have two or more than two dimensions and are in the size range of 1 - 100 nm. These particles have special and enhanced physical and chemical properties as compared to their bulk materials due to their large reactive and exposed surface area and quantum size effect as a result of specific electronic structures. These particles have been widely used in many fields such as electronics, Inorganic nanoparticles and combination of inorganic nanoparticles with organic materials to form hybrids possess unique physical, chemical, photochemical, biomedicine and chemistry optical and electrical properties which make them different and more applicable than large size materials. Nanoparticles are proved to be promising multi-functional platform because they can be used for many imaging and

therapeutic functions. These types of platforms can be synthesized by different organic, inorganic or hybrid of organic and inorganic materials but among all these inorganic platforms are of most important for diagnosis and simultaneous therapy due to their easy modification, stability and high drug loading capacity

Applications of nanoparticles in diagnosis, treatment and monitoring of biological systems are slowly coalescing into a new field, often referred to as ‘nanomedicine’. Materials with nanoscale dimensions are of great interest in biomedical applications because their size is comparable to, or smaller than, that of many important biological entities such as genes (2 nm wide and 10–100 nm long), proteins (5–50 nm), viruses (20–450 nm), or cells (10–100 μm). These tiny particles can access otherwise unreachable regions of the organism and engage in interactions at molecular level or deliver a therapeutic load. For these reasons, it is widely accepted that systems incorporating either inorganic or organic nanoparticles have the potential to change dramatically the landscape of the biomedical field. A

confirmation of this prediction is the large body of research focused on the development of nanoparticles and nano-structures for bio-sensing, diagnostics, drug delivery and therapeutic purposes. Indeed, hundreds of nanomaterials-based imaging and diagnostic devices and a similar number of drug delivery systems are already in preclinical, clinical, or commercial development stage [3] and their number increases rapidly each year.

Recently Nayak and coworkers have extensively studied the use of plant extracts for the green synthesis of gold nano particles [1-4]. The use of phytochemicals in the synthesis of nanoparticles is an important symbiosis between nanotechnology and green chemistry [5-7]. 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 medical and technological applications [8-10]. The power of phytochemicals, which initiate varieties of chemical transformations within biological systems, is well known [9, 11-13]. 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 Podina 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 Podina 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 Podina. The nano particles have been characterized by using Uv-Visible and TEM studies. The cytotoxicity study of the nano particles have also been studied.

MATERIAL AND METHODS

Synthesis of Podina Gold Nanoparticles (Podina-AuNps):

Podina mint or Podina (*Mentha arvensis*): Belongs to *Lamiaceae* family (Fig. 1. a), it yields an essential oil and mentho., Mint is rich in many chemicals, vitamins and minerals such as Niacin, Carotene, Folic Acid, Thiamine, Riboflavin, Magnesium, Protein, Fat, Minerals, Carbohydrates, Calcium, Phosphorus, Iron, Magnesium, Copper, Manganese, Zinc, Chromium, Oxalic Acid, Menthol and Phytin Phosphorus. The plant possesses carminative, antibacterial, antifibrile, stimulative, stomachic, diaphoretic and antispasmodic properties that enhance the medicinal value of podina to a large extent [39].

Step 1: 100mg of Podina leaves were added to 6 ml of distilled water and the reaction mixture was stirred continuously at 25 °C for 15 min.

Step 2: To the stirring mixture, 100 µl of 0.1 M NaAuCl₄ solution (in DI water) were added. The color of the mixture slowly turned Dark brown from Dark green within 10 minutes, which indicates the formation of gold nanoparticles (Podina-AuNps).

Step 3: The reaction mixture was stirred for an additional 15 minutes and the gold nanoparticles thus formed were separated from residual Green tea leaves immediately using a 5µ filter and analyzed using UV-Visible spectroscopy and TEM.

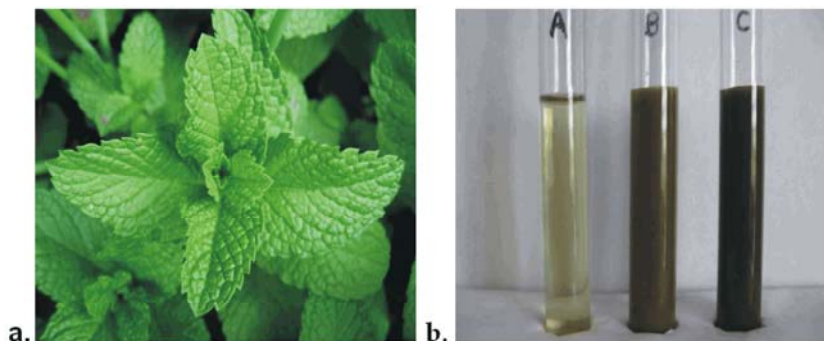


Fig. 1 a): PODINA LEAVES

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



Fig. 2: Synthesis of gold nanoparticles

Cytotoxicity Studies (MTT Assay): Cytotoxicity evaluation of Dhania-AuNPs was performed using MTT assay as described by Mosman [20]. Approximately 1×10^5 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 tetrachloroaurate (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 Podina 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, λ_{max} of Podina-AuNps, is 535 nm respectively. The size of Podina-AuNps is in the range of 15 ± 5 nm; respectively as measured from TEM techniques (Figures 1).

XRD of Gold nano Particles: Figure 4. 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/

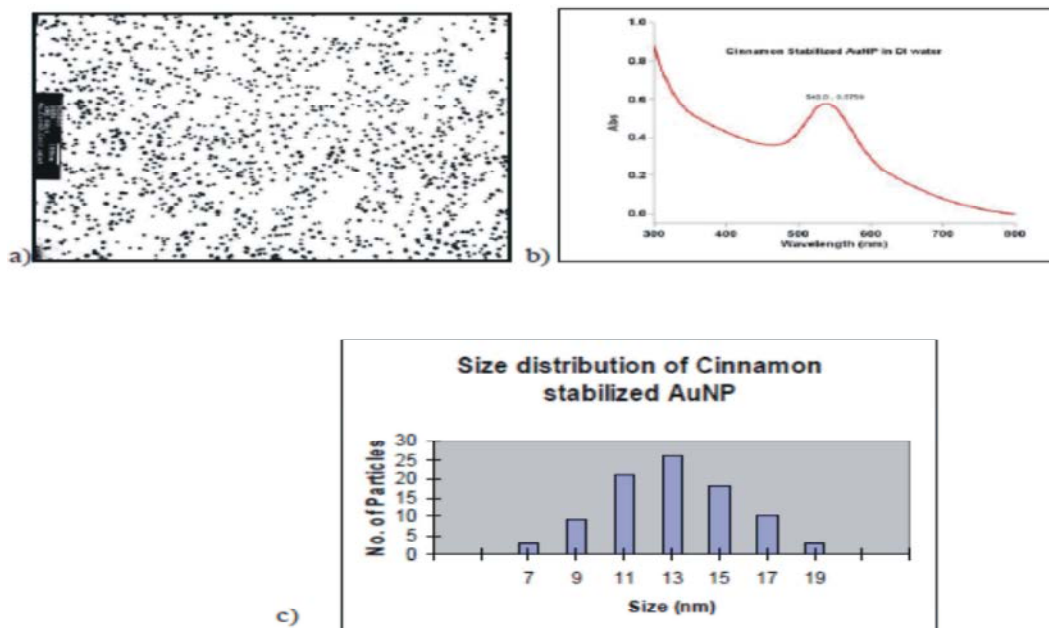


Fig. 3: a) UV-Visible absorption spectrum, b) TEM Image, c) size distribution of Podina Gold Nanoparticles.

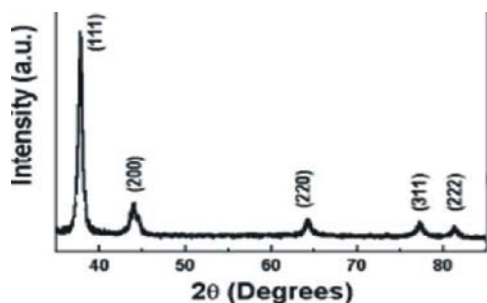


Fig. 4: XRD of Gold particle

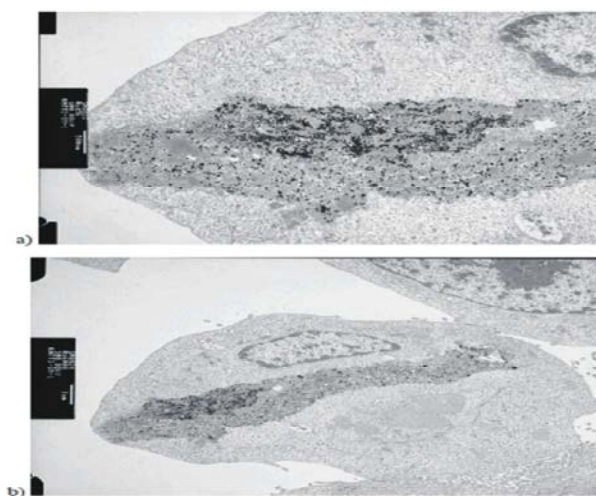


Fig. 5: a, b: TEM Images of different MCF-7 cells showing uptake of Podina-AuNPs in to the lysosomes.

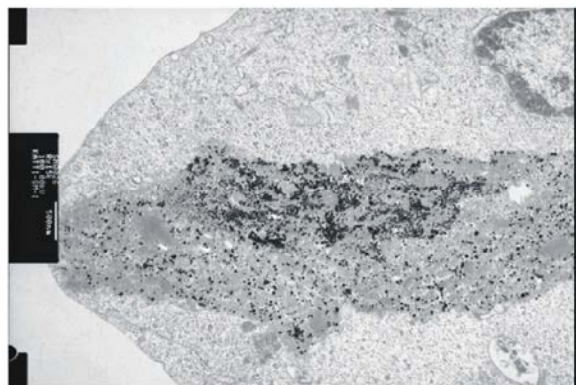


Fig. 6: TEM Images of different MCF-7 cells showing uptake of Podina-AuNPs in to the lysosomes.

therapeutic agents. A number of studies have demonstrated that phytochemicals present in Podina 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 Podina 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 nanoparticles 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 μ M 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 hrs exposure 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 spectrophotometrically. After 24 hrs post-treatment, PC-3, MCF-7 cells showed excellent viability even up to 150 μ M concentrations of Podina -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 Podina-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 Podina 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].

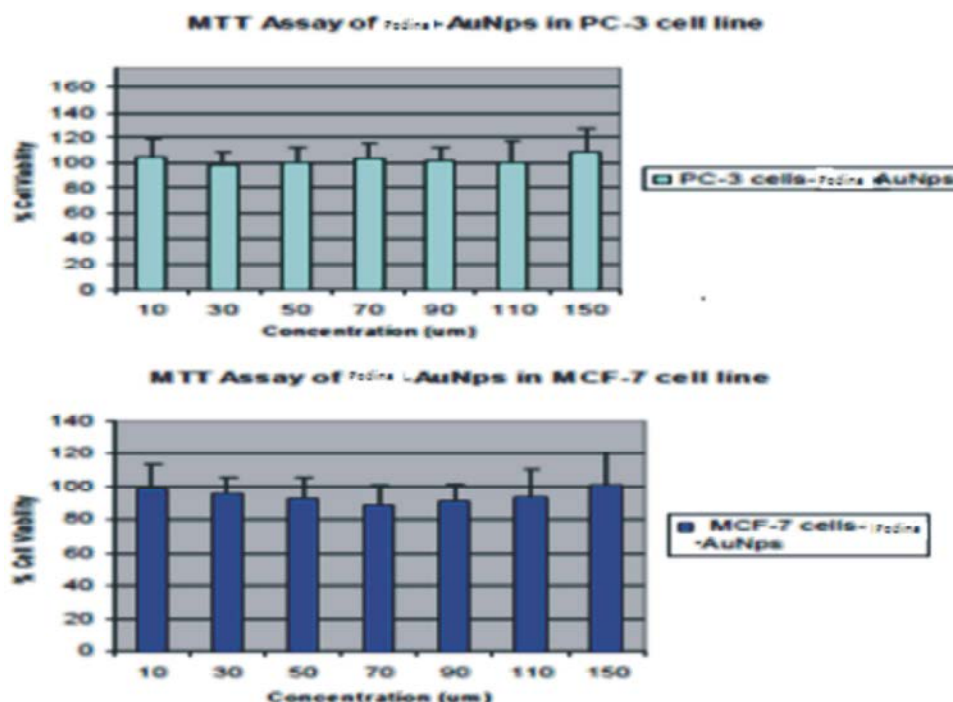


Fig. 7: a, b: Dose dependent cytotoxicity of Podina-AuNPs in cultured PC-3 and MCF-cells after 24 hrs of exposure using MTT assay.

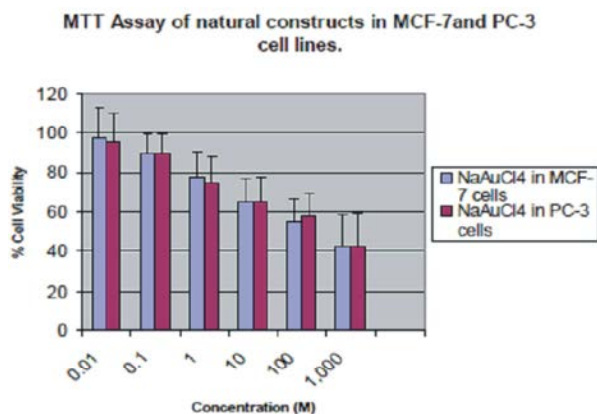


Fig. 8: Dose dependent cytotoxicity of NaAuCl₄ in cultured PC-3 and MCF-cells after 24 hrs of exposure using MTT assay.

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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 Podina.

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. Paridal, 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, J.R. Peralta-Videa, J.G. Parsons, H. Troiani and M.J. 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, 2007 and 2008. Cytotoxicity of nanoparticles. Small., 4: 26-49.
12. Espín, J.C., M.T. García-Conesa and F.A. Tomás-Barberán, 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 *Msn* 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. Suppakitporn, 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.