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Green Synthesis of Gold Nano Particles XIV: Green Synthesis and Characterization of Gold Nano Particles Using The Extract of Night-Flowering Jasmineor Gangasiuli (*Nyctanthes arbor-tristis*) and Study of its Cytotoxic 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 the main features make it more attractive potential option for biomedical field and elsewhere. Gold nanoparticles(AuNps) 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 nanoparticles has been carried out using eco-friendly method such as the plant extract of Gangasiuli.The nanoparticles 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 · Gangasiuli · Cytotoxicity studies

INTRODUCTION

Nanotechnology, shortened to "nanotech", is the study of the controlling of matter on an atomic and molecular scale. Nanotechnology, which has received considerable awareness in advanced biomedical science over the past decade, with dimensions similar to biomacromolecules, nanoparticles can be engineered to have specific or multiple functions and can be used for investigating and pursuing an in-depth understanding of the mechanisms involved in biochemical processes. In clinical research the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as polyethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy and their ability to deliver proteins, peptides and genes. The unique characteristics of particles in the nanometre range, such as high surface-to-volume ratio or size-dependent optical and magnetic properties, are drastically different from those of their bulk materials and hold pledge in the clinical field for disease diagnosis and therapeutics. More over other characterstics like controlled release and particle degradation are significantly important is drug delivery system. In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particle particleaggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.

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The amalgamation of nanotechnology with biological systems has been made feasible by combining the intrinsic properties of nanoparticles with immobilization of specific ligands, such as oligonucleotides and proteins, on the numerous recognition surfaces. Thus, the development of multifunctional nanoparticles, which integrate diagnostic (quantum dots, magnetic, metallic, polymeric and gold nanoparticles) and/or therapeutic (magnetic and metallic nanoparticles) properties, as well as specific targeting capability by surface modification with biomolecules, is a continuous topic of research. Thus this article will focus on gold nanoparticles (AuNPs) used as basis for the development of methodologies suitable for application in clinical diagnosis.

Recently Nayak and coworkers have extensively studied the use of plant extracts for the green synthesis of gold nano particles [1-13]. The use of phytochemicals in the synthesis of nanoparticles is an important symbiosis between nanotechnology and green chemistry [14, 15, 16]. 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 [17, 18, 19]. The power of phytochemicals, which initiate varieties of chemical transformations within biological systems, is well known [20, 21, 22, 23]. 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 [24, 25]. The tremendous health benefits of chemical cocktails present within Gangasiuli 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 Gangasiuli 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 Gangasiuli. The nano particles have been characterized by using Uv-Visible and TEM studies. The cytoxicity study of the nano particles have also been studied.

MATERIALS AND METHODS

Synthesis of Gangaseuli Gold Nanoparticles (Gangaseuli-AuNps):

Night-Flowering Jasmine or Gangasiuli (Nyctanthes Arbor-Tristis): Belongs to family Oleaceae (Fig. 1a), commonly known as Night Jasmine. Active components present in leaves are D-mannitol, â-sitosterole, Flavanol glycosides- Astragaline, Nicotiflorin, Oleanolic acid, Nyctanthic acid, Tannic acid, Ascorbic acid, Methyl salicylate, trace of volatile Oil, Carotene, Friedeline, Lupeol,mannitol, Glucose and Fructose, Iridoid glycosides, Benzoic acid. Extensively used by Ayurvedicphysicians for analgesics, antipyretic along with ulcerogenic potency have also been observed. This plant has also been found to possess anti-allergic, antimalarial, leishmanicidal, amoebicidal and anthelminthic activities.

Step 1: Gangasiuliextract preparation: Intact Gangasiuli (8 g) were washed with distilled water to remove any traces of contaminants. Gangasiuli leaves (*Nyctanthes arbor-tristis*) 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: AboutFour4 ml of Gangasiuli 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 $NaAuCl_4$ (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 (Gangasiuli-AuNps).

Cytotoxicity Studies (MTT Assay): Cytotoxicity evaluation of Gangasiuli-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% CO_2 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

Fig. 1a: Gangasiulileaves

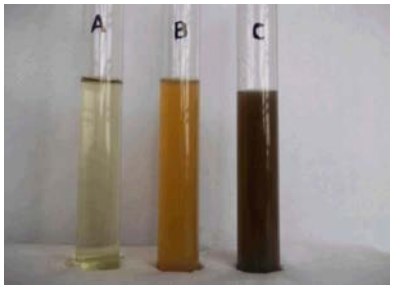


Fig. 1b: Tube A- Auric acid,
Tube B- Gangasiuli extract and
Tube C- Gangasiuli gold nanoparticle solution.

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 $NaAuCl_4$ 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 ($NaAuCl_4$) with Gangasiuli leaves extract in the absence of man-made chemicals and thus, satisfies all the principles of a 100% green chemical process. Various phytochemicals present in Gangasiuliis 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 Gangasiuli-AuNps is 535 nm. The sizes of Gangasiuli-AuNpsis in the range of 12±4 nm; as measured from TEM techniques (Fig. 1).

XRD of Gold nano Particles: Fig. 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 Gangasiuli have the ability to penetrate the cell membrane and internalize within the cellular matrix. Cancer cells are highly metabolic and porous in nature and are known to internalize solutes rapidly compared to normal cells. Therefore, we hypothesized that Gangasiuli 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 (Figs. 4, 5, 6). 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 Gangasiuli-AuNps (Figs. 1 a, b; 2 a, b; 3 a, b). These results clearly demonstrate that the

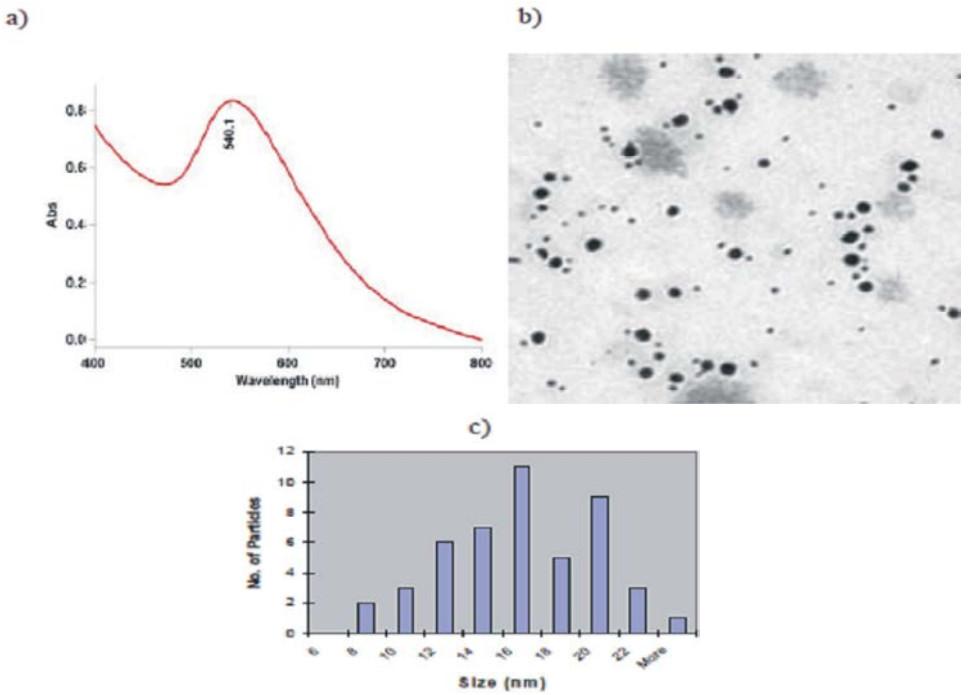


Fig. 1: a) UV-Visible absorption spectrum, b) TEM Image, c) size distribution of Gangasiuli Gold Nanoparticles

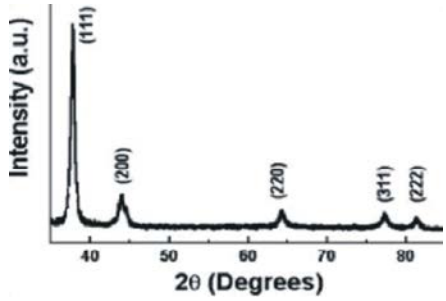
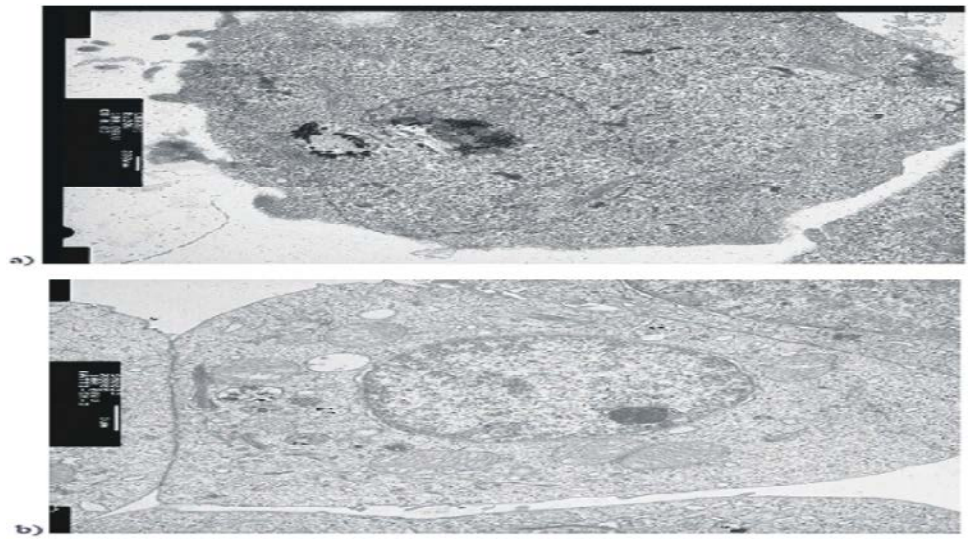


Fig. 2: XRD of Gold particle



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

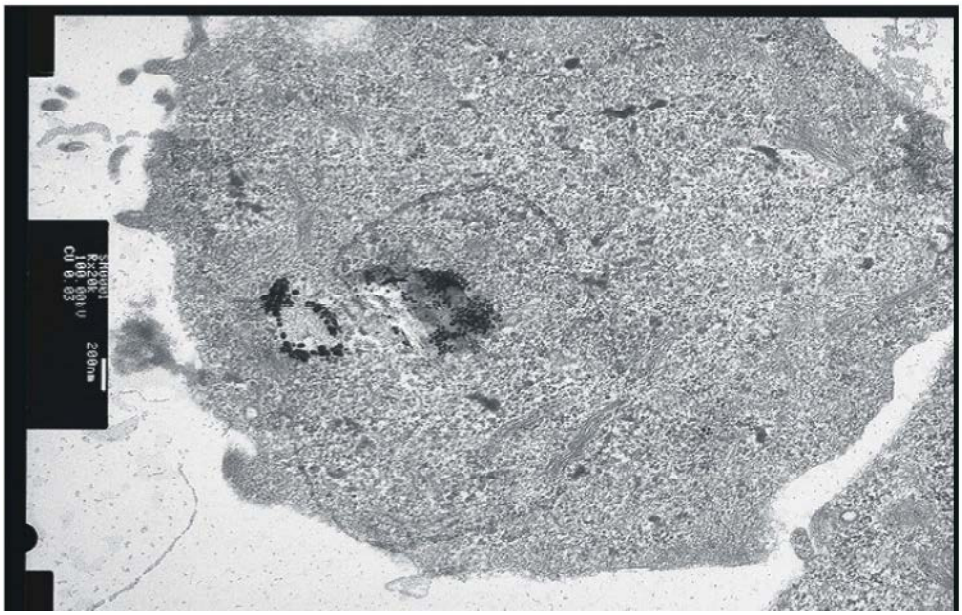
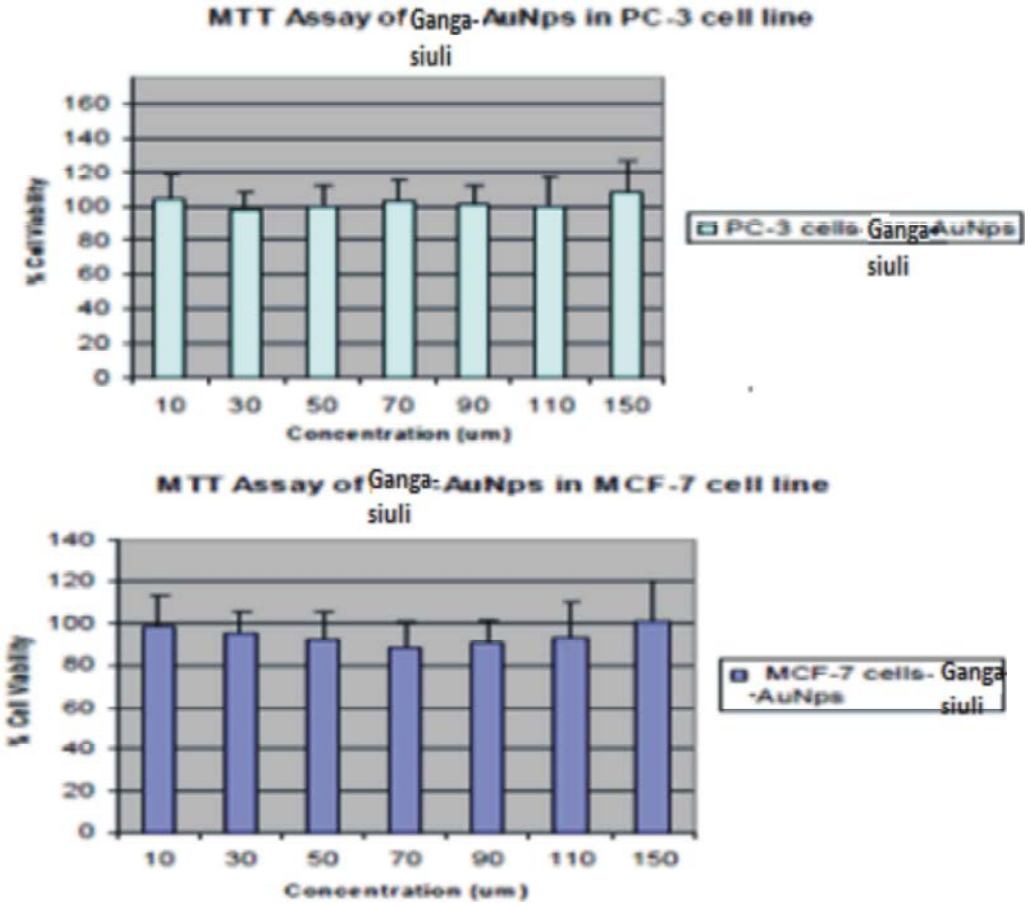


Fig. 4: TEM Images of different MCF-7 cells showing uptake of Gangasiuli-AuNps in to the lysosomes



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

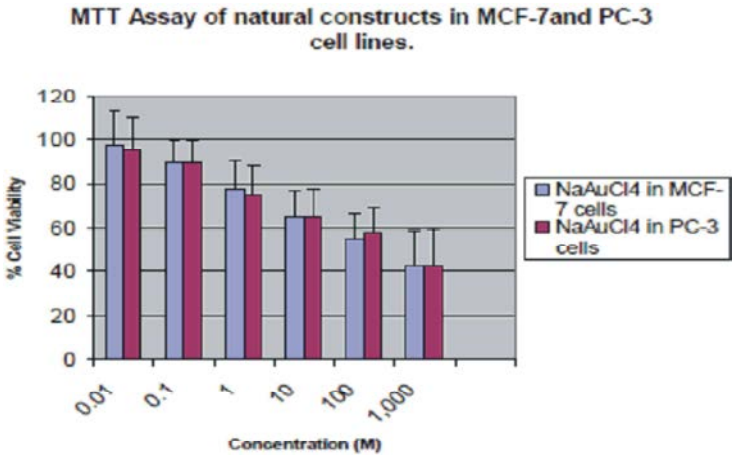


Fig. 6: Dose dependent cytotoxicity of NaAuCl4 in cultured PC-3 and MCF-cells after 24 hrs of exposure using MTT assay

phytochemicals within these herbs provide a nontoxic coating on AuNps and corroborate the results of the internalization studies discussed above[write here the referencenumbers?]. 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 (Fig. 4). The lack of any noticeable toxicity of Gangasiuli-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 Gangasiuli have the ability to penetrate the cell membrane and internalize within the cellular matrix. Cancer cells are highly metabolic and porous in nature and are known to internalize solutes rapidly compared to normal cells. Therefore, we hypothesized that Gangasiuli 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 (Figs. 4, 5, 6). 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.

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 Gangasiuli.

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