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Biogenic gold nanoparticles synthesis mediated by *Mangifera indica* seed aqueous extracts exhibits antibacterial, anticancer and anti-angiogenic properties

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ABSTRACT

During the last few decades, gold nanoparticles (AuNP's) have gained considerable attention in nanomedicine and expanded its application in clinical diagnosis and as therapeutics. Employing plant extract for synthesising gold nanoparticles proves to be an eco-friendly technology for large scale production. It is highly economical and suitable for biological applications by negating the use of chemicals involved in conventional route. In this study, AuNP's was prepared by a simple one step method of employing aqueous Mangifera indica seed extract as a reducing agent. Scanning electron microscopy and transmission electron microscopy revealed spherical shaped nanoparticles and dynamic light scattering analysis indicated the AuNP's to be approximately 46.8 nm in size. AuNP's efficiently inhibited the growth of *E. coli* and *S. aureus* by its inherent ability to generate reactive oxygen species (ROS) and exhibited detrimental effects towards the tested bacterial species. Biocompatibility assessment indicated the non-toxic nature of AuNP's towards mesenchymal stem cells at 25 µg/ml and interestingly, suppressed the growth of human gastric cancer cells under in vitro culture conditions. AuNP's significantly exhibited anti-angiogenic property in chick chorioallantoic membrane model (CAM) by downregulating Ang-1/Tie2 pathway. Overall, the synthesized AuNP's exhibited antibacterial and anti-angiogenic properties with high biocompatibility thereby supporting its candidature for various biomedical applications. It can be employed in suppressing tumor growth, combat inflammatory diseases that necessitate the involvement of angiogenesis suppression, and antibacterial activity is suitable for its clinical translation to negate surgery associated infections.

1. Introduction

During the last few decades, gold nanoparticles are widely investigated for its efficient role in the field of biomedicine owing to its distinct physico-chemical and biological properties. It has been widely investigated for its broad spectrum properties which include antibacterial activity, drug delivery, gene transfer, detection of human pathogens, nucleic acid labelling, cosmetics, and as molecular theranostics [1–3]. The conventional methods for synthesising AuNP's often involve the use of toxic chemicals and expensive technologies that possess increased environmental risks and render its clinical translation despite its fascinating properties [4]. The methods for synthesizing gold nanoparticles include chemical reduction, hydrothermal, sol-gel, reverse micelle, ion sputtering, etc. Synthesis methods involving the use of fungi, bacteria and plants as green factories have gained considerable interests and motivated researchers to allow better control on size and shape of gold nanoparticles. Amongst these alternative routes for synthesis, in the view of simplicity, plant extracts are easier to scale up the synthesis, proves to be economical and safe for its use in humans [4–7]. Presence of terpenes, citric acid, flavonoids, phenols, ascorbic acid, alkaloids and reductase acts as potential reducing agents in nanoparticles synthesis [8]. Plant extracts may act as stabilizers and reducing agents in the synthesis of metallic nanoparticles [8,9]. Apart from this, employing plant agents is found to be faster than conventional techniques for gold nanoparticles synthesis. For instance, reduction of auric chloride took place within 10 min at room temperature by using *Cassia aruiculata* aqueous extract [10]. Biogenic gold nanoparticles synthesis is approaching popularity due to its antibacterial

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activity and easy reduction from the precursor salt solutions. Gold nanoparticles has been synthesised from extracts of various portions of the plants (stem, leaves, flower, seed, fruit, pectin etc) and various plants have been explored which includes *Mangifera indica*, *Gymnocladus assamicus, Cacumen platycladi, coriander, Nerium oleander, Pea nut, Solanum nigrum, Hibiscus cannabinus, Eucommia ulmoides, Salix alba, Sesbania grandiflora, Pogestemon benghalensis, Morinda citrifolia*, etc [11]. Considering the fact there are no reports on utilizing aqueous extracts of *Mangifera indica* seeds, we propose a single pot synthesis of AuNP's using the seed extract as a plant source.

Mangifera indica is an indigenous herb in medical and ayurvedic formulations for over 4000 years with various pharmaceutical properties. It has been used to treat various ailments due to its antioxidant, antibacterial, antiviral, antidiabetic, antiparasitic, antiallergic, cardiotonic, hypotensive and anti-inflammatory properties [12]. For instance, the mango fruit is rich in polyphenols, mangiferin, catechins, rhamnetin, gallic and epigallic acids, quercitin, kaempferol, alkylresorcinols etc which is responsible for the reduction of gold cations for AuNP's synthesis [13]. Fresh/dry leaf extract of M. Indica was utilized for gold nanoparticles synthesis with sizes of 20 nm and 17 nm [14]. In another study, gold nanoparticles of size around 432.30 nm were effectively synthesized using leaf extract [15]. Mangifera indica flower extract was used for biogenic green gold nanoparticles formation with sizes ranging between 10–60 nm [13]. However, the seeds of Mangifera indica is unexplored for its reducing properties in metal nanoparticles synthesis and they are rich source of flavanoids, tannins and polyphenols derivatives. Henceforth, we chose the aqueous seed extracts for biogenic reduction of gold nanoparticles in the current study. The synthesized gold nanoparticles were aimed for reducing microbial infections, inhibiting angiogenesis in malignancy and potentially safe for mammalian applications.

Microbial infections pose serious risks to outcome of complex surgeries despite aseptic surgical procedures. Surgery associated infections are often difficult to be eradicated owing to its resistance for antibiotic therapy and biofilm formation. Inability to minimize the systemic spread of infection and delayed infections are the major draw backs associated with repeated administration of broad spectrum antibiotics following major surgeries involved in tissue correction, for instance, especially bone tissue regeneration in patients with cancer metastasized to bone. Hence, a multifunctional system for bone cancer treatment often necessitates urgency in developing materials to combat microbial growth and limit cancer progression to promote long term bone regeneration [16].

Angiogenesis is the formation of new blood vessels from a pre-existing one and it is a necessary step for tumor growth and progression. Treatment for cancer by blocking angiogenesis was proposed by Judah Folkman [17] and now it has become as a widely accepted approach for effectively inhibiting tumor growth. Therefore, blocking angiogenesis in tumors could be a therapeutic option to treat and halt cancer progression. A preclinical study indicated that the inhibition of angiogenesis alone by drugs that block angiogenic factors and by generic inhibitors show robust anti cancer activities [18]. Angiostatin and endostatin are some of the generic inhibitors available in the human system. The endogenous inhibitors avert the mature vasculature for further development [19]. Now there is an urge to develop a solid angiogenic inhibitor that globally targets angiogenic pathway.

AuNP's are widely preferred in nanotechnology based medicine because of their low cyto-toxicity, conjugation ability with several biomolecules, such as proteins, enzymes, amino acids and DNA, and holding of high optical extinction coefficients [20–23]. AuNP's also exert antiangiogenic potential by interacting with the heparin-binding domain of VEGF [24]. AuNP's have been investigated with respect to their usefulness in the treatment of the various angiogenesis related pathological conditions to suppress cancer progression by inhibiting neovascularisation [25,26]. These reports confirm that the anti-angiogenic properties of AuNP's, and demonstrate that these might be an alternative and cost-effective approach for the cancer treatment.

Since there are no reports on the use of *Mangifera indica* seed extract to synthesize AuNP's, therefore, in the current study, the antibacterial properties and antiangiogenic effects of AuNP's were investigated using a chick chorioallantoic membrane model. Hence, we speculate that the synthesised nanoparticles has the ability to minimize bacterial infections, inhibit tumor growth and can be used along with biomaterial based constructs for tissue engineering applications in organ specific metastatic cancer patients.

2. Materials and methods

2.1. Preparation of Mangifera indica seed extract

The seeds of *M. indica* were chosen to synthesize gold nanoparticles owing to its cost effectiveness and vast availability. The seeds were collected from Chennai, India and shade dried. The dried seeds were chopped into small pieces and powdered using a mechanical blender. 10 g of mango seed powder were soaked in 100 ml of distilled water for 5 h under constant stirring at room temperature. The solution was then filtered with Whatman filter paper no.1 and filtrate of the aqueous solution was stored at −20 °C until use. Aliquots of extract were filtered using 0.45 μm filter prior to AuNP's synthesis.

2.2. Green synthesis of gold nanoparticles

For the biosynthesis of AuNP's, 1 mM $HAuCl_4$ x $4H_2O$ (precursor for gold ions) solution was combined with aqueous *M. indica* seed extracts at different ratios (v/v in ml) 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 (*M. indica* seed extract: Auric chloride solution). Among the ratios, formation of AuNP's was highly seen at a ratio of 6:4. 60 ml of aqueous extract was mixed with 40 ml of 1 mM $HAuCl_4$ and incubated at room temperature. The formation of AuNP's was indicated by the appearance of a deep purple red/ruby red colour.

2.3. Characterization of nanoparticles

UV–visible spectra of the synthesized nanoparticles were recorded using Shimadzu UV-1800 spectrometer scanned between 300–800 nm. The as synthesized AuNP's were centrifuged at 15,000 rpm for 30 min and the pellet was washed thrice with double distilled water, and dried at room temperature for further characterization. Fourier Transform Infrared (FTIR) spectra were recorded for AuNP's and *M. indica* seed extracts using IR Perkin Elmer Spectrum1 spectrometer. X-ray diffraction (XRD) patterns for the AuNP's were obtained at room temperature and spectrum was recorded in the range of 2Θ –20° to 80° with CuKa radiation (λ = 0.15 nm) at 2° min^{-1} (BRUKER AXS, D8 Discover). High Resolution Scanning Electron Microscopy (SEM) images and Energy dispersive X-ray (EDAX) analysis were taken using Hitachi-S4800. High Resolution Transmission Electron Microscopy (TEM) image Analysis was carried out using Technai 20 G^2 HRTEM. Dynamic light scattering (DLS) measurements were recorded using Zetasizer Nano ZS90 for predicting the size distribution of gold nanoparticles.

2.4. Anti-bacterial activity by minimum inhibition concentration (MIC) method and ROS assay

The anti-bacterial activity (bacterial cell growth inhibition) of synthesised nanoparticles was analysed by MIC method using ATCC strains of *Staphylococcus aureus* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria) [27]. A loop of a fresh colony was obtained from the agar plate and inoculated into 100 ml of LB broth, and incubated overnight. 2.5 μl of overnight culture (concentration of 10^8 colony-forming units (CFU)/ml of medium) was incubated in 5 ml of LB broth with different concentrations of nanoparticles, and cultured overnight at 37 °C in an incubator at 120 rpm, and OD was taken at