Green synthesis of plant mediated metal nanoparticles and their anti-angiogenic activity

Project Reference No.: YSS/2014/000888

Final Report under scheme for young scientists

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Final Report under scheme for young scientists
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1. Project Title Green synthesis of plant mediated metal nanoparticles and their anti-angiogenic activity	**2. File No.:** YSS/2014/000888
3. PI(Name &Address): Dr. L. MALLESHA Asst. Professor & HOD PG Department of Chemistry JSS College of Arts, Commerce and Science B.N. Road, Mysuru-570025 Karnataka, India	
4. Broad Area of Research: Life Sciences **4.1. Sub Area:** Nanotechnology **5. Approved Objectives of the Proposal:** ➢ Screening of medicinal plant for synthesis of nanoparticles. ➢ Extraction of plant source will be carried using the protocol. ➢ Synthesis of metal nanoparticles from extracted plant source. ➢ Characterization of synthesized nanoparticles will be carried out using spectral studies such as UV-Visible Spectroscopy, FT-IR and X-ray Diffraction. ➢ Evaluation of anti-angiogenic activity from synthesized nanoparticles.	
Date of Start: 16-11-2015	**Total Cost: Rs. 19,85,000.00** **Released amount: Rs. 18,00,000=00** **(Capital: Rs. 6,00,000=00,** **General: Rs. 12,00,000=00)**
Date of Completion:15-11-2018	**Expenditure as on 15-11-18** **Capital: Rs. 5,61,627=00** **General: Rs. 12,28,665=00**

6. Methodology

Medicinally important plants such as Memecylon malabaricum, Heliconia rostrata and *Euphorbia cotinifolia* were selected. Fresh leaves of above plants were collected and washed with double distilled water. About 10 gram of leaves were weighed and boiled with 100 ml of double distilled water for about 10 min. The extract was filtered using muslin cloth and Whattman no.1 filter paper. Then the leaf extract was collected in separate conical flask and the extract was stored at 4°C for further experimental analysis.

100 mL of 1 mM solution of silver nitrate and gold chloride were prepared in an Erlenmeyer flask, respectively. Then 2, 5, 7 & 10 ml of plant extract was added separately to 10 mL of silver nitrate solution and gold chloride solution, keeping its concentration at 1 mM. Silver and gold nanoparticles

were also synthesized by varying concentration of $AgNO_3$ and $HAuCl_4$ solution (1 mM, 2 mM and 3 mM) keeping extract concentration constant (1 mL). To optimize the yield of nanoparticles, the experiment was carried out using different parameters like pH (pH 3, pH 9 and pH 7) and temperature (8°C, 37 °C & 40 °C) .This setup was incubated in a dark chamber to minimize photo-activation of silver nitrate at room temperature. Reduction of Ag^+ to Ag^0 was confirmed by the colour change of solution from colourless to brown and reduction of Au^{3+} to Au^0 was confirmed by change in colour further formation was also confirmed by using UV–Visible spectroscopy.

After complete reduction the synthesize medium was centrifuged at 4000 rpm for 10 min. Methanol and petroleum ether solvent wash were given about 5-6 times. The pellet were collected and dried in oven at 45 °C. The obtained nanoparticles were characterized by various spectroscopic methods and the same were used to study *in vivo* anti-angiogenic activity by

a) Shell less chorioallontoic membrane assay
b) *In vivo* peritoneal angiogenesis assay using EAT cells in Mice model

7. Salient Research Achievements

7.1 Summary of Progress

The proposed work involves the synthesis of metal nanoparticles using medicinally important plant sources. *Memecylon malabaricum, Heliconia rostrata* and *Euphorbia cotinifolia* were found to have ability to form metal nanoparticles as observed in change in colour of the reaction. The positive results as observed by the formation of nanoparticles were maintained throughout the 72hr period of observation. The colour change observed for the extracellular samples were further confirmed by UV-vis spectral analysis as part of primary confirmation. Silver nanoparticle known to have an intense absorption peak in UV absorption spectra due to its surface plasma resonance excitation. Absorption peak was observed at 450 and 460 nm and indicated formation of silver nanoparticle. Gold nanoparticles formation was confirmed by UV-Vis spectral analysis, absorption was found at 560 nm.

The angioinhibitory activity of the compounds exhibit significant positive results in the shell less CAM assay model of developing embryos. The data shown represents the results using a minimum of six eggs in each group. The investigation of anti angiogenic activity of gold and silver nanoparticles from plant sources showed a significant reduction of proliferation of capillaries around the zone of application of the discs loaded with the metal NPs as compared to the site where only the saline was applied. These results indicate that the two metal NPs (Ag and Au), from each plant leaves are potent antiangiogenic molecules *in vivo*.

The peritoneal linings of tumor-bearing mice treated with metal NPs and the control group on inspection for anti angiogenic effect showed a significant difference in vascularisation of the peritoneum. The decrease in the number of capillaries evident on the peritoneal surface as compared to

the control groups, which indicates the prominent in vivo antiangiogenic activity of metal NPs on the peritoneum of tumor bearing mice.

7.2 New Observations:

The synthesised metal nanoparticles are (Ag & Au NPs) are eco-friendly, simple, cost effective, easy to scale up, thus the green production of nanoparticle using biological resource has great potential. *Memecylon malabaricum, Heliconia rostrata* and *Euphorbia cotinifolia* leaf extract was found to be more potential in producing stable gold and silver nanoparticles. The gold nanopartcles forms cube in shape, which is confirmed by SEM and XRD.

The EAT tumor bearing mice when treated with gold and silver NPs synthesised from leaves of plant sources showed a significant reduction in the body weight, ascites volume and tumor cell burden demonstrating the antiproliferative activity of gold and silver NPs. Micro vessel density (MVD) counts have become the morphological gold standard to assess the neovasculature in tumors. MVD counts are reflective of the angioarchitectural properties of the tumor, in that they represent the average intercapillary distance. This is in fact an important parameter as it is the goal of an anti-angiogenic tumor therapy to increase the intercapillary distance to a degree that it becomes rate-limiting for the growth of the tumor. The gold and silver NPs treated animals showed a drastically decrease in the MVD counts, proving evidence for the anti angiogenic activity of the compound.

7.3 Innovations:

So for nobody has tried the synthesis of metal nanoparticles from *Memecylon malabaricum, Heliconia rostrata* and *Euphorbia cotinifolia* in a particular region. We standardized the maximum yield of NPs by using various parameters, like temperature, pH and concentration of the reactants. Capping of the metal NPs by various secondary metabolites would synergize the activity of the respective metal NPs. Gold forms cube shape nanoparticles.

7.4 Application Potential:

7.4.1 Long Term

Silver and gold are safe nontoxic inorganic materials used as antimicrobial agent. Ag and Au NPs can be used in the preparations of ointments, bone cement, biosensors, cell imaging, anticancer and antiviral agents. Metal nanoparticles can be used as catalytic agents as well as in designing the diagnostic kits.

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1. INTRODUCTION

Nanotechnology is enabling technology and it will be developed at several levels such as materials, devices and systems.[1] Nanoparticles were synthesized using plant extracts because of its cost effectiveness, eco-friendly, easily scaled up for large scale synthesis, non-toxic and there is no need to use high pressure, energy, temperature and toxic chemicals.[2–4] Green synthesis consists of a myriad of promising approaches for the production of metal nanoparticles (MNPs) with desired properties. Plants signify the most explored group of living organisms for the green synthesis of MNPs. Several species of the *Memecylon genera* have already been used to synthesize metal nanoparticles. *Memecylon edule* leaf extract mediated green synthesis of silver and gold nanoparticles has been reported.[5] Green-synthesized silver and gold nanoparticles were produced rapidly by treating silver and gold ions with an extract of *Memecylon umbellatum* leaf has been reported.[6] *Memecylon malabaricum*, endemic to Western Ghats, has many medicinal properties, methanol extract of the plant has antimicrobial property.[7] The plant extract selectively kills colon cancer cells in the *in vitro* experiments. *Memecylon umbellatum* can act as a lead for the development of potential anticancer agents with selectivity towards cancer cells.[8] The leaves of *Memecylon malabaricum* have good antipsoriatic potential, which provides scope for further detailed research in this plant for psoriasis.[9] The leaves of *Memecylon malabaricum* which are primarily rich in a wide group of natural multipotent molecules like tannins with manifold chemopreventive activity represent a promising class as anticancer drugs anticancer drugs memecylaene molecule from the same plant has anti-inflammatory property too.[10, 11]

In the present study, MNPs has been studied for the synthesis of metal nanoparticles. The optical properties of nanoparticles were determined by ultraviolet-visible spectra. The morphology of nanoparticles was characterized by transmission electron microscopy. Furthermore, X-ray diffraction and Fourier transform infrared spectroscopy were used to confirm the formation of nanoparticles. This simple but efficient approach has open up a new angle of view for the biosynthesis nanoparticles through plant media. *In vivo* angiogenesis by gold and silver nanoparticles from *Memecylon malabaricum* extract were studied by their anti-angiogenic activity. Histopathology and MVD count of mice peritoneum were also reported.

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Heliconia rostrata and *Euphorbia cotinifolia* plant, the plant which are endemic to western ghats and known to treat many skin diseases. So for nobody tried out the production of metal NPs from the same. We have optimised the different parameters to maximise the yield, further produced NPs and tested for their *in vivo* anti-angiogenic activity by shell less CAM assay and peritoneal angiogenesis. We have published 02 papers in international journals and presented the project work in 04 different events.

PhDs Produced no:	Technical Personnel trained:		
Under Ph.D. Thesis submission Stage	ONE (Project Assistant)		

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Synthesis of metal nanoparticles using *Heliconia rostrata* leaf extract and their antiproliferative and apoptotic property

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A B S T R A C T

The silver and gold nanoparticles were biosynthesized using leaves of a pharmacologically important plant *Heliconia rostrata*. A rapid, eco-friendly, cost-effective and one-step process of synthesis has been achieved, thus produced nanoparticles were characterized by UV–visible, FT-IR, XRD and TEM spectral studies. Further, newly synthesized nanoparticles were used to study the induction of apoptotic activity on EAT cells. These two type of nanoparticles showed antiproliferative and apoptotic property in mice model. The outcome of this study could be useful for the development of value added products from indigenous medicinal plants, which has biomedical applications.

1. Introduction

The growth of green biosynthesis for the production of nanoparticles is evolving into an important branch of nanotechnology.[1] Numerous methodologies are developed to synthesize noble metal nanoparticles of particular shape and size depending on specific requirements. Biosynthesis of nanoparticles has an emerging highlight of the intersection of nanotechnology and biotechnology which has received increased attention to a growing need to develop environmentally benign technologies in material syntheses. Biomolecules as reductants are found to have significant advantage over chemical reductants due to their biocompatible nature.[2] The scientific and technological significance of metal nanoparticles has made them the subject of intensive research, given their special chemical and physical properties. Presently, biological nanoscience has increasing attention due to its advanced nature and the efficacy of produced nanoparticles, which are used as catalyst in industry.[3] Silver nanoparticles have drawn the attention of scientists because of their extensive application in the development of new technologies in the areas of material sciences, electronics and medicine at the nanoscale.[4] In particular, gold nanoparticles are employed in many fields, biosensing,[5] electronics,[6] enzyme electrodes,[7] super conductors [8] and cancer therapy.[9]

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CERTIFICATE

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Department of Chemistry

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This is to certify that Mr/Mrs/Dr L. MALLESHA, JSS College, B.N. Road

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He/She presented paper in the ORAL/POSTER session.

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This is to certify that Dr. Lingappa Mallesha has Presented a Poster Presentation on "GREEN SYNTHESIS OF SILVER NANOPARTICLES USING HELECONIA ROSTRATA AND THEIR CHARACTERIZATION..." in Two Days International Conference entitled "Environment, Health & Policy Nexus" held on 27th & 28th July-2017 at Jagadguru Sri Shivarathreeshwara University, Mysuru, India.

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This is to certify that ~~Sri/Smt/Dr~~ **Dr. L Mallesha**, Assistant Professor, Pg Department Of Chemistry, Jss College Of Arts, Commerce And Science, Ooty Road, Mysuru-570025, Karnataka has presented a paper/~~participated~~ in Prof.K.V.Thomas Endowment **International Symposium** on **NEW TRENDS IN APPLIED CHEMISTRY (NTAC -2017)** organised by the Post Graduate and Research Department of Chemistry, Sacred Heart College, Thevara, Kochi, Kerala on 09-11 February, 2017

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