University of Missouri - University of the Western Cape

The UM-UWC Linkage Program Report

Visit to Columbia: July 6-Aug 6, 2017

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Overview

I express absolute delight and deepest gratitude to the University of Missouri South African Education Linkage Program (UMSAEP) and the University of the Western Cape for supporting my research visit to University of Missouri, Columbia. I feel extremely honoured and appreciative to have been selected for this award. This award will be an important impetus for me to continue my research in Green Nanotechnology and Cancer treatment.

The primary purpose of this trip was to work with our current collaborator, Prof. Kattesh Katti (Director, Green Nanotechnology Centre), to maintain and strengthen a *long-term* collaborative research between DST/Mintek *Nanotechnology Innovation Centre (NIC)* (Department of Biotechnology, UWC) and Institute of Green Nanotechnology and Cancer Nanotechnology Platform (Department of Radiology, MU). Prof Katti and his team at university of Missouri have performed innovative and pioneering research on the green synthesis of gold nanoparticles. The main objective of this research visit was to get training in green synthesis of nanoparticles. A collaborative research grant proposal(UM-UWC) was submitted to Department of Science and Technology. During this research visit, experimental work was discussed with Prof Katti's research team, which will help us to refine the planning for executing the project. During my stay of four weeks in 2017, I visited Proteomics core, Cytology core labs, participated in a collaborative workshop and attended a scientific meeting. Additionally, my visit included presentations and discussions related to other scientific pursuits. UMSAEP award has provided me much needed motivation and career development through providing network opportunities with experts in the parallel research field. I was extremely delighted to showcase our research proposal to such an engaging group of people. I also connected with other researchers in the same field, which will open-up future collaboration opportunities. Being a part of MU community has been one of the best experiences of my life. I have gained a life enhancing experience that will forever enrich my personal and academic development.

Project Title: Evaluating the anticancer activity of Gold Nanoparticles synthesised from Honey Bush using green synthesis methods.

Aims of the Research:

The aims of the research, which is being carried out in collaboration with Prof Kattesh Katti at the University of Missouri were to synthesize, characterize and evaluate gold nanoparticles AuNPs from Honey Bush (HB-AuNPs).

The proposed work was divided into three segments.

Segment 1: The preparation of Honey bush extractions and assessment of Mangiferin content in the extracts.

Segment 2: The synthesis, characterization and evaluation of HB-AuNPs.

Segment 3: Testing of HB-AuNPs on various human cancer cell lines.

METHODOLGY and RESULTS:

Segment 1: The preparation of Honey bush Extracts:

Extracts of Honey bush plant materials were prepared in the laboratory of Prof Meyer (DST/MINTEK Nanotechnology Innovation Centre (NIC), Department of Biotechnology).

Methodology:

The green/unfermented Honeybush (*Cyclopia intermedia*) (Family: Fabaceae; Tribe: Podalyrieae), plant material was purchased by Rooibos Limited, Clanwilliam. Honey bush extracts were made using boiled distilled water (50mL of distilled water added to 5g of each plant powder). The extract was centrifuged at 3750 rpm for 2 hours. A stock solution of 64mg/ml was freshly prepared for each extract before the screening step.

Segment 2: The synthesis, characterization and evaluation of HB-AuNPs.

This part of work was performed in in the laboratories of Prof Meyer and Prof Katti (Green Nanotechnology Center, MU).

Methodology

HB-AuNPs were synthesized from Honey Bush extract as well as dried plant material. Various concentrations of extracts (64mg/ml to 0.005mg/ml) were screened for the synthesis of gold nanoparticles. A different method described by Prof Katti and his co-workers was also used to synthesize HB-AuNPs using the dried plant material instead of the extract for the synthesis. Once HB-AuNPs were synthesized they were further characterized with UV-vis spectra and the The hydrodynamic size of the nanoparticles were measured using the Zetasizer.

Results

After successful screening of various concentrations of Honeybush plant material, 12mg/ml plant concentration was selected for synthesis of nanoparticles and subsequent work. The synthesized nanoparticles were characterized using UV-vis spectroscopy. The surface plasmon resonance peak for the Hb-AuNPs was at 536 nm (Figure i), indicating the reduction of Au^{3+} to Au. The hydrodynamic size of HB-AuNPs was 146.3dnm.

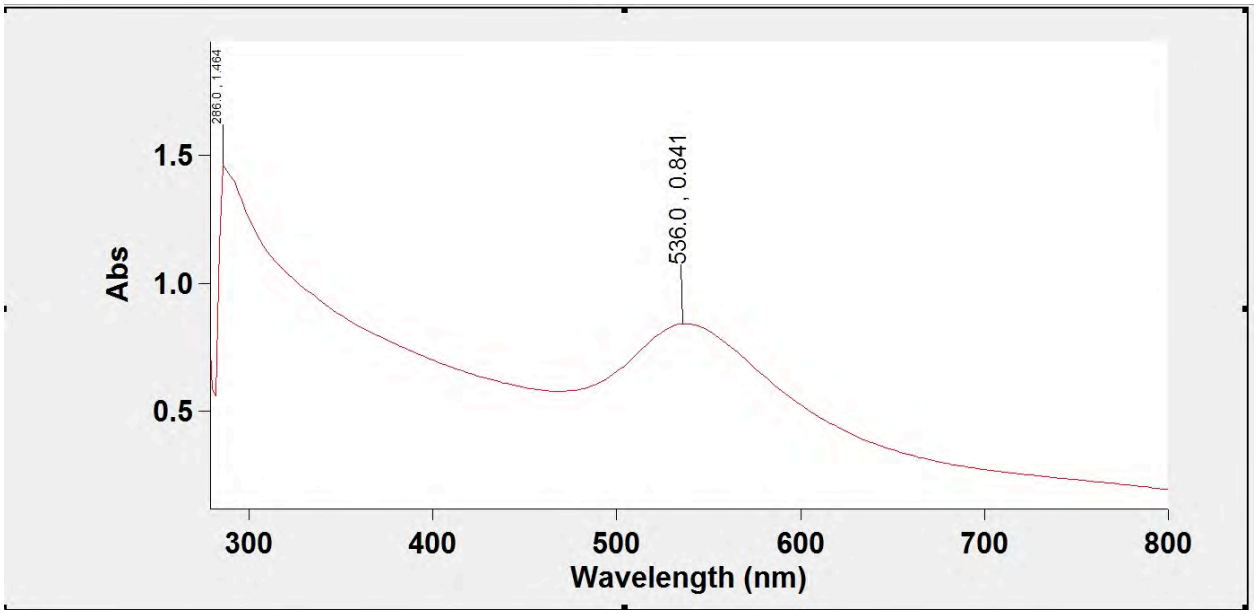


Figure (i) : UV-Vis absorption spectrum of synthesized HB-AuNPs

Segment 3: Testing of HB-AuNPs on various human cancer cell lines.

Our main aim in this segment was to evaluate in vitro cytotoxicity of HB-AuNPs on various cancer cell lines.

Methodology

Cell lines and culture conditions

The cells (PC3, PANC3, MIA PACA-1) were cultured and maintained in their respective media, supplemented with 10% fetal bovine serum and antibiotics (2% penicillin–streptomycin 100 IU ml−1). The cells were maintained in a monolayer culture at 37°C under a humidified atmosphere of 5% CO_2. The cells were sub-cultured by trypsinization (0.025% trypsin and 0.0025% EDTA) and maintained in a cell culture laboratory at the Green Nanotechnology Center, MU. Cell number and viability were monitored by standard trypan blue dye exclusion procedures.

Cell treatment and cytotoxicity assay (MTT assay):

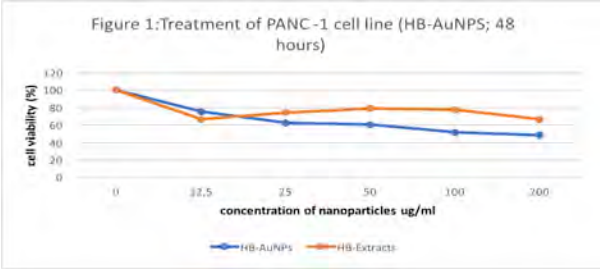
HB-AuNPs were evaluated for cytotoxicity against PC3, PANC3 and MIA PACA-1. Cells were seeded in 96 well plates ($1*10^4$ cells/ml). After 24 hours, the medium was aspirated and cells were treated with HB-AuNPs for 24, 48 and 72 hrs. Various concentrations (200, 100, 50, 25, 12,5 and 6µg/ ml) of HB-AuNPs were used. At the end of the treatment period the medium was aspirated and medium containing MTT3-(4,5-dimethly thiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) salt (0.5 mg/ml) was added and cells were incubated for 4 hours at 37°C in a CO_2 incubator. The medium with MTT was then discarded and 100 µL of DMSO was added to dissolve the crystals. The absorbance readings of the purple blue formazan dye were measured at 570 nm. The experiment was carried out in triplicate and the average viability of the viable cells was calculated. A graph was plotted between the percentage cell viability and various concentrations. Positive and negative cytotoxicity controls were present in each plate. Negative controls (untreated cells) were set as 100% viability. To determine the cytotoxicity, % cell viability was calculated as per the following formula:

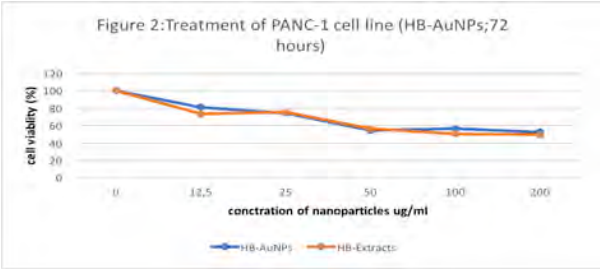
Viability % = mean OD of test sample mean / OD of negative control * 100.

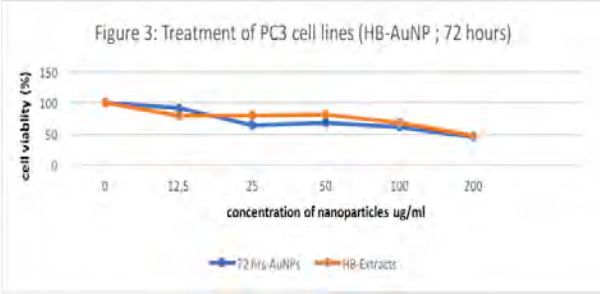
Results

Different cancer cell lines PC3 (Prostate cancer), PANC3 and MIA PACA-1 (Pancreatic) cancer cell lines were used to evaluate the anticancer activity of HB-AuNPs for 24, 48 and 72 hours. MTT cell proliferation essay has been used for the evaluation of cytotoxicity.

From the data, it is revealed that HB-AuNPs did not show any profound morphological changes in MIA-PACA-1 cell lines, even after 72 hours' treatment. No cytotoxicity was observed in MIA-PACA-1 cell lines as 80% of MIA-PACA-1 cells were still viable at highest concentration. Whereas, about 50% of PANC3 cells were viable after 48 hrs. This viability remained constant even after an extended period (72 hours) of treatment of HB-AuNPs (Fig 1 and 2). While in PC3 cells, growth inhibition was in 45% cells after 72 hours of treatment (Fig 3). A significant decrease in cell viability of PANC3 and PC3 cells as compared to control cells was observed.







Investigation of Gene regulation:

Once the cytotoxicity of HB-AuNPs was established, the next step was to explore the mechanism behind HB-AuNPs induced cytotoxicity using gene expression analysis. Genes previously shown to be involved in mangiferin cytotoxicity was selected for this study.

As a first step of gene expression analysis, primers have been designed to investigate the expression levels of nine genes in response to treatment of various cancer cell lines with HB-AuNPs. This part of work is carried out in University of Western Cape and is still under way.

Determination of total phenol content in plant extracts:

Training to perform the determination of total phenols in the plant extracts using the Folin-Ciocalteu method was undertaken at UM.

Training

The training undertaken are listed below:

Online training:

- Chemical management online training 13/07/2017
- Introduction to laboratory safety
- Basic Training for Animal Care and Use at MU (20/07/2017)
- DOT training test (21/07/2017)

Hands-on training:

- Introduction to laboratory safety (19/07/2017)
- Introduction to biosafety – (20/07/2017)
- ACQA/OAR Rodent Handling Workshops -03/08/ 2017

Additional noteworthy activities

In addition to the key objectives outlined above, a formal presentation about proposed work in UM-UWC joint grant proposal was given to Prof Katti's research team. Many experimental issues with respect to nanoparticle synthesis, characterization and their cytotoxicity were discussed in post-presentation discussion. These discussions will be very valuable for trouble shooting the experiments in lab.

I attended the **undergraduate research summer forum on 27th July 2017.** This event is a showcase of scholarly research activities conducted by undergraduate MU students. It was a deeply satisfying experience to communicate with young researchers as they showcased their

research work.

MU Veterinary Health Center, provides state-of-the-art facilities for conducting research relevant to animal and human health concerns. Prof Katti gave me an opportunity to visit Veterinary health center and discuss DST/Mintek NIC research projects with researchers in Veterinary Health Center, which would be quite helpful for future research collaborations.

I attended a workshop animal handling and had my first ever experience with animal handling in an experimental set-up.

Through Prof Katti's efforts, I got a chance to visit various other labs (Proteomics core facility; Molecular Cytology Core Facility) at the UM campus. I was fortunate to discuss my research projects with Prof Brain P. Mooney (Associate Director, UM Proteomics Center) and got to learn about their interesting current research work. I could discuss proposed objectives in my research lab and joint grant proposal. I could also get information about treatment, storage and shipping procedures of samples (if required) for proteomic analysis. I also could visit their lab and got information about the various instruments, laboratory methodology etc.

I believe that this meeting will be quite instrumental and crucial for future collaborative work.

A meeting with Prof Alexander Jurkevich (Associate Director, Molecular cytology Core Facility), provided me with invaluable information about his fascinating research.

I could also join UM recreational activity centre, which was an exciting experience. My UM visit was really an enlightenment for both body and mind.

Summary

My first experience with MU-UWC linkage program will have great impact on my career development. At UM campus, my experience as a researcher extends far beyond the Department of Radiology because of the strong collaborations that exist among the colleges across campus. It was quite interesting to do various lab trainings. There are many aspects that can be incorporated in our research labs as well. Our group and Prof Katti's group are working in parallel fields so this visit has been a source of useful feedback, valuable research ideas and insights into different methodological approaches. We aim to publish one research paper and a conference presentation from this collaborative research work generated from MU-UWC partnership. UMSAEP will surely contribute in capacity building of future young researchers in both universities. The partnerships like UMSAEP will help both institutions to achieve the goal of preparing their students to be real "global citizens".

Apart from research work, this unique visit also gave me an opportunity to explore a new country and to be dazzled by new culture and customs. I wish UMSAEP program to be continued forever!

Acknowledgements

I would like to extend my sincere gratitude and appreciation to the Prof Rodney Uphoff (Professor Emeritus of Law and Director, UM) for organizing this visit, which was a platform for networking, exchange of valuable ideas lessons learning and to make a strategic research plan in field of Green Nanotechnology under supervision of Prof Kattesh Katti.

I am eternally grateful to Ashley Rhode (Project Specialist, Office of the President, MU) for all the planning and arrangements for the trip.

I would also like to extend this opportunity to acknowledge Prof Ramashwar Bharuthram (Executive Director: Special Projects in the Office of the Vice Chancellor, UWC) for his continued support for postgraduate research. His constant efforts are not only helping promising young researchers to achieve their dreams, but are also likely to cultivate the new generation of scientists.

Words can neither qualify nor quantify Prof Kattesh Katti's encouragement, support and guidance. Thanks for opening my eyes to new stages of opportunity and strength. I will remain forever grateful for the knowledge imparted to me. It would be a great asset throughout my career. I am proud to be able to learn from you. I don't have adequate words to express my feelings for Mrs Kavita Katti for her kind hospitality. I admire her for kindness and support.

I would like to thank Dr Menka Khoobchandan, and Mr Velaphi Thipe. They are amongst the most thoughtful people I know. I will always remember the friendship they extended to me. Thank you for your kindness and taking care of me in every aspect to the best of your ability. Thank you for sharing my shopping adventure. Words are not enough to express you how grateful I am for the training sessions and practical tips in the lab. I hope I will be able to return the favor to you in future.

I would like to say thanks to Ms Liz Abbott (Coordinator, Animal Care), Ms Anna Hamilton (Coordinator Occupational Health and Safety Program) and Ms Sherrie E Neff , for organizing animal training for me.

I would like to express my sincere appreciation to the work done behind the scenes by the

International Relations Office at UWC (Ms Debra Lamson, Mr Leolyn Jackson, Ms Hilda Wilson and Ms Rushni Salie).

I would also take this opportunity to thank Prof Martin Onani (Chemistry Department, UWC), who also visited MU while I was there. His presence in MU was quite helpful for me getting acquainted with the new place.

I want to express my deepest gratitude to Prof Mervin Meyer to give me this wonderful opportunity. I am incredibly fortunate to have him as my Mentor. I truly appreciate and value everything I have learnt from him, which will forever be a major contribution behind my success and achievements. I believe that I will be able to lead towards a successful career with his sincere support and mentorship. I have the utmost respect for him. He has been, and continued to be an excellent role model to his students.