Miranda House

University of Delhi

Final Progress Report of DU Star innovation Project MH – 02

Therapeutic Potential of Medicinal Plants: Culture, Extraction, Physicochemical Characterization and Testing their Cytotoxic or Immunostimulatory Properties

Utilization Certificate
Star Innovation Project MH-02

Project Title: Therapeutic Potential of Medicinal Plants : Culture, Extraction, Physicochemical Characterization and Testing their Cytotoxic or Immunostimulatory Properties

College: Miranda House

Project Investigators: Dr. Saloni Bahri (Department of Batony) , Dr. Sadhna Sharma (Department of Zoology) & Dr. Smriti S. Bhatia (Department of Chemistry)

Grant sanctioned	13,85,000/-					
S. No.	Budget Head	Amount Received Rs	Amount Utilized Rs. 2016-17	Amount Utilized Rs. 2017-18	Total Amount Utilized Rs.	Balance Amount Rs.
1	Consumables	2,67,000	2,39,203	17,797	2,57,000	10,000
2	Equipment	7,44,000	5,97,575	1,46,250	7,43,825	175
3	Salary	1,44,000	52,835	40,204	93,039	50,961
4	Contingency	60,000	32,207	27,793	60,000	Nil
5	Stipend	1,20,000	94,000	-	94,000	26,000
6	Field Trip	50,000	5,000	45,000	50,000	Nil
Total Amount Utilized Rs.		**13,85,000**	**10,20,820**	**2,77,044**	**12,97,864**	**87,136**

Certified the out of Rs.13,85,000/- (Rs. Thirteen Lac Eighty Five Thousand Only) sanctioned under the Star Innovation Projects Scheme MH-02 Rs. 12,97,864/- (Rs. Twelve Lac Ninety Seven Thousand Eighty Hundred Sixty Four Only) has been utilized during the period of the project.

Dr. Saloni Bahri
Department of Batony

Dr. Sadhna Sharma
Department of Zoology

Dr. Smriti S. Bhatia
Department of Chemistry

For Gupta Nandan & Associates
Chartered Accountants

Gupta Nandan & Associates ★ Chartered Accountants ★ New Delhi M. No. 086537

Pawan Kumar Gupta
(Proprietor)
Membership No. 086537
UDIN: 22086537AOAPLG8342
Date : 28/07/2022
Place : New Delhi

Miranda House, Star Innovation Project, University of Delhi

Project MH-02

Therapeutic Potential of Medicinal Plants: Culture, Extraction, Physicochemical Characterization and Testing their Cytotoxic or Immunostimulatory Properties.

Principal Investigators:

Dr.Saloni Bahri, Department of Botany

Dr.Sadhna Sharma, Department of Zoology

Dr.Smriti Sharma Bhatia, Department of Chemistry

Summary of the project:

Several studies have elucidated the potential of a number of naturally derived phytochemicals as therapeutic agents. Plant secondary metabolites, their semi-synthetic and synthetic derivatives are vital sources of anticancer drugs. It is estimated that more than 50% of antitumor drugs which are under clinical trials, are from plants.

Alkaloids such as vinblastine and vincristine isolated from *Catharanthus roseus* are well known examples of plant derived anticancer agents. There are a number of cytotoxicity determining assay, MTT and TUNEL assay are two of them. These also describe the mechanism of killing. Moreover, immune system enhancement is required to fight any infection or cancer. Many plant derived compounds act as immunomodulators. Nitric oxide and Tumour Necrosis Factor-alpha (TNF-alpha) are two such immunomodulators.

We used human cell lines for our experimental set up as these cell lines have been shown to be a good model system for evaluating candidate anticancer agents/immunomodulators. A large number of these can be grown reproducibly with the advantage of no donor variability of cellular function. The other objectives achieved were screening of plants for their medicinal properties in order to select three plants for carrying out tissue culture, acquainted students with the basic principles, requirements, methodology and various general and industrial applications of plant tissue culture technology, students were trained for in-silico determination and characterization of active principle of plant extract and students were acquainted with the methodology of animal cell culture & MTT assay.

Detailed outcomes:

We have screened some plants for their medicinal potential. Depending upon the availability and their amenability to in vitro conditions, an economically important leguminous plant *Acacia senegal* (Gum Arabic) has been selected. The medicinal uses of the plant are:

- used in treatment of a number of disorders like leprosy and gonorrhea, minor gastrointestinal problems such as indigestion and diarrhea.
- a very good source of fiber and can help alleviate constipation and discomfort associated with digestive disorders.
- It is a demulcent, a substance that relieves irritation in the mucus membranes of mouth by creating a protective film.
- Reduces the blood sugar and cholesterol level as well.
- It cures sore throat, cold and coughs too. It's an ingredient in many cold medications such as throat lozenges and cough medicines.
- It has antibacterial properties that help control harmful bacteria in the mouth that cause gum diseases.

Extraction of the plant material: The plant material was dried, weighed and ground as fine as possible, then soaked overnight in 50/50. Then heated until boiling and simmered for an hour or so, then the liquid filtered off and kept. Then recover the material with water, or more of the above solution if readily available, and repeat this step, without soaking overnight. All the liquid was combined and reduced through simmering to a workable amount, about 500ml.

Students have been thoroughly trained in plant tissue culture techniques. They have learnt stock solution preparation, medium preparation, sterilization of medium, seeds, inoculation, maintenance of cultures, and their observations, recording of data and transfer of axenically raised plants to soil. In addition to that they have also learnt Microphotography. The students were acquainted with the methodology of animal cell culture both for primary cells and continuous cell lines. They were trained in flow cytometry technique to identify different cell markers; MTT assay for cytotoxicity and nitrite assay as one of the mechanisms of apoptosis. Students have been adequately trained in in-silico experiments. They have learnt basic computational techniques like building 3-D models of simple molecules, visualizing the 3-D

arrangement of the atoms in the molecule and optimizing the structures. Apart from this they are also being trained in various characterization techniques.

Methodology followed:

(i) Plant tissue culture

- Stock solution preparation:

- Major, Minor, Iron and Organic stock solutions were prepared for Murashige and Skoog's nutrient medium according to the concentrations of salts given in the following table and stored in the refrigerator. The salts were added sequentially and dissolved by magnetic stirring.

Sterilization of seeds:

The seeds were transferred into a beaker which was half-filled with water. To this, Teepol (detergent) was added and the resulting solution was stirred well. The seeds were then washed under running water till all the foam disappeared. The water was then removed from the beaker and the seeds were transferred to a small beaker. This beaker was kept in the laminar air flow and 0.5% Mercuric chloride (HgCl2) was added such that all the seeds were immersed in the solution. Mercuric chloride is used as to sterilize the surface of the seed. The seeds were kept in this solution for 10 minutes. The $HgCl_2$ solution was drained off and the seeds were washed 3 to 5 times with sterilized distilled water to remove any traces of mercuric chloride. This process was carried out in the laminar air flow too. Once the seeds were thoroughly washed, they were transferred into a Petri dish (which had Whatmann filter paper on it to soak the moisture on the seeds).

Inoculation of seeds:

- Two seeds of Acacia senegal were inoculated in each culture tube using forceps.
- The forceps were dipped in 90% ethanol and were heated using the spirit lamp before they were used to pick up a seed.
- It was ensured that each culture tube was sealed properly by placing the cotton plug on top of it.
- A total of 48 culture tubes (96 seeds) were inoculated.
- Maintenance of cultures:
- Cultures were kept in the culture room at a temperature of 26±20C and photoperiod of 8 hours light and 16 hours dark for further observations.

The parameters studied were length of root, number of root laterals, length of shoot, and overall growth of the seedlings.

(ii) Animal cell culture

- THP-1 Cell Line

Human promonocytic cell line THP-1 (National Centre for Cell Sciences, Pune) was grown in RPMI 1640 supplemented with10% fetal bovine serum, 1% HEPES, 1% L- glutamine, and 50 µg/ml of Gentamycin. The cells were treated with 20ng/ml phorbolmyristate acetate overnight and then were washed three times. Cells were be rested for a day.

- Primary Culture of PBMC

Peripheral blood was collected from a subject. Peripheral Blood Mononuclear Cells (PBMCs) were isolated by density gradient centrifugation on Ficoll-hypaque. Briefly, heparinized peripheral blood was layered over a histopaque cushion and centrifuged at 420xg for 30 min at 22°C. Interface cells (PBMCs) were recovered, washed and resuspended in RPMI-1640 with 10% Fetal Calf Serum (FCS).

Both types of cells were used for further experimentation.

- MTT Assay

For the study of cytotoxic effect of the plant extract tetrazolium-dye, MTT assay is to be performed, so students were trained in the assay. Briefly, the cells were seeded in 96-well plates at a density of 5×10^3cells/well in 200 µl culture medium. Following 24 h incubation and attachment, the cells were treated with a recombinant protein for different time points for training, for actual experiments extract will be used. After treatment, media was replaced with MTT solution (10 µl of 5mg/ml per well) prepared in PBS and will be incubated for 3 h at 37°C in a CO2 incubator. The yellow MTT dye was reduced by succinate dehydrogenase in the mitochondria of viable cells to purple formazan crystals. To solubilize the formazan, 50 µl of isopropanol was added to each well. The plates were be gently shaken for 1 min and absorbance was measured at 600 nm, with reference 490 nm, by Multimode microtiter plate reader (Molecular probe).

- Nitrite assay

Concentration of NO_2 produced by cultured cells as a measure of the production of nitric oxide (NO) was determined at 540 nm using Multimode microtiter plate reader (Molecular Probe). 100 µl supernatant was removed from the culture wells, centrifuged at 400 x g for 10 min. to make it

cell free, and incubated with an equal volume (100 µl) of Griess reagent (1% sulfanilamide, 0.1 napthylethylene diamine dihydrochloride, 2.5% $H3PO_4$) at room temperature for 10 min. and absorbance was read. Concentration of NO2 was determined by using sodium nitrite as standard.

- Flow cytometry for cell markers

1 x 10^6 cells/ml were stimulated with 5 µg of Recombinant protein as well as unstimulated PBMCs were incubated for 48hrs at 37°C in 12 well culture plates supplemented with 10% FCS and antibiotics. Cells were harvested, washed in PBS supplemented with 0.5% BSA, and stained with fluorescently labeled antibodies. Antibodies used for phenotypic analysis were anti-CD3-FITC and anti-CD14-PE (Biolegend, USA). Cells were acquired using flow cytometer (BD Accuri C6, USA) and analyzed using software (De novo FCS express 5.0, USA).

(iii) Computational modeling

- Computational modeling of biostructures using open source softwares like ArgusLab by conducting regular training sessions.
- Introduction to various characterization techniques.

Future directions:

- *In vitro* studies on one of the selected medicinal plant.
- Selection of other medicinal plants and their in vitro studies.
- Extract preparation and characterization by TLC, FTIR and *in-silico* analysis.
- Exposure will be given to many cell lines to be used for the study.

Details of Student Researchers

Number of students involved:

Ten Students were involved in the project.

The courses/programme they are pursuing:

S. No.	Name of student	Course*
1	Divya	B.Sc. (H) Chemistry II year
2	Megha Pant	B.Sc. (H) Chemistry II year
3	M.Tomuilim Tontang	B.Sc. (H) Botany II year
4	Akanksha	B.Sc. (H) Botany II year
5	Pallavi Jha	B.Sc. (H) Botany II year
6	Shruti Acharya	B.Sc. (H) Zoology II year
7	Kanika Anabh	B.Sc. (H) Zoology II year
8	Anamika Naorem	B.Sc. (H) Zoology II year
9	Ariba Aziz	B.Sc. (H) Life Sciences II
10	Riya	B.Sc. (H) Life Sciences II

Responsibilities undertaken:

- Various techniques were used for testing cytotoxic and immunomodulatory role of selected medicinal plant.
- Adequate training in all the techniques pertaining to Plant tissue culture, Animal tissue culture and in silico analysis was done.
- Literature Survey

Results achieved:

- *Acacia senegal* (Gum Arabic) was selected after extensive literature survey.
- 10-day-old seedlings of *Acacia senegal* would be selected for extraction of active principle.
- A protocol was formalized for the in detail in silico analysis.

- Computational modeling of biostructures using open source softwares.
- *In vitro* studies on one of the selected medicinal plant.
- Selection of other medicinal plants and their *in vitro* studies.
- Extract preparation and characterization by TLC, FTIR and in-silico analysis.
- Exposure will be given to many cell lines to be used for the study.

Any other relevant information:

- The students of our project visited the Plant Tissue Culture Research Laboratory of the Department of Botany, University of Delhi on 17 October 2016. The students learnt about the tissue culture of various orchids and also about the extraction of chemical compounds through HPLC.
- Bahri, S., Bhatia, S.S., Moitra, S., Sharma, N., Bhatt, R., Borthakur, N.S., Agarwal, R. and Jain, D. 2016. Influence of silver nanoparticles on seedlings of Vigna radiata (L.) Wilczek. DU Journal of Undergraduate Research and Innovation, 2(1):142-148. 2395-2334.
- Dr. Smriti Sharma Bhatia presented a paper entitled "The economics of nanotechnology" at the National Seminar on Emerging Economics And Challenges to Sustainability towards developing Nations at Sri Aurobindo College, University of Delhi on 29-30 Mar, 2016.

Poster presentation on National Science Day, 28 February 2018

Certificate of Originality

This is to certify that the Project Investigators and the students of Innovation Project having Project code MH-02 and title 'Therapeutic Potential of Medicinal Plants: Culture, Extraction, Physicochemical Characterization and Testing their Cytotoxic or Immunostimulatory Properties' have carried out original research work submitted as Final Report to the University of Delhi. The work and the report are original. Any plagiarism dispute arising out of the project will be our responsibility.

Project Investigators