



Biotic Response Modulating Plant Growth

Mehak Maheshwari¹ & Dr Gurjeet Kaur²

¹Scholar, Amity Business School, Amity University Uttar Pradesh, Lucknow Campus

²Professor, Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus

ABSTRACT

Lantana americana is invasive species and grows over large areas in land. In recent years there have been vast diversity in microbes and diseases caused by them. On generating natural sources of antimicrobial medicines. Lantana is plant I have been working with. Scientist are working hard to find new People are suffering to a much larger extent from diseases and there is no good treatment for them. Doctors are suggesting antibiotics but these are of less importance as microbes have developed antimicrobial resistance. This is a major concern in recent times. I with help of my guide is working for generating new sources of antibiotics to overpower bacterial diseases. My study is focused discovering natural medicines.

INTRODUCTION

Doctors often prescribe antibiotics to us on viral and bacterial diseases, but some of these are not much effective, have you thought of reason for same?

Probably because microbes are resistant to these medicines.

My guide has explored this area and given me guidance to work on plant Lantana americana and search its antimicrobial properties. Seen this as a field with immense researches-

In our surroundings we are surrounded by millions of microbes. some of these are pathogenic to us and may cause huge damage to our health.

Scientist and environmentalist are finding sources of pathogens in ecosystem and sources to cure diseases caused by theses.

My guide has seen this as an emerging field with immense potential, often we see doctors prescribing antibiotics to people for bacterial infections, but sometimes these don't work have you thought why?

Probably because these microbes are resistant to these medicines.

My guide has seen this as an emerging field for researches, she explained me to work on Lantana americana as a plant to search for antimicrobial properties.

OBJECTIVES

Primary objective of his is experiment is to find new sources of antimicrobial plants and microbes. This will help cure problem of AMR. It is very important to work on this area to help people get rid of deadly microbial disease.

Traditional Indian plants have huge potential to be used as antibiotics and as herbal drugs. Many good bacteria also help cure diseases and prevent colonization of harmful bacteria. Some commonly used are penicillium notatum, yeast strains, candida. Penicillin was first antibiotic to be used.

My primary goal is to contribute in this novel research for finding new sources of drugs, various un searched plant species have potential to act as herbal medicines. There are several new drugs on way of discovery but the point is they are mainly synthetic, and people are now interested in more herbal sources to lessen effect of side effects.

Several medicines give early cure but there side effects are devastating, ageing early, hair fall, allergy, and many more. This problem along with AMR has focused attention of researchers in this field of science. Our biotechnology has immense potential to develop potentially curable drugs connecting principles of biochemistry, pharmacology, and cell biology. With brilliant guidance of my guide and fruitful discussions I have worked on Lantana americana and find how it can help in this core of research.

LITERATURE REVIEW

This experiment is focused on antibiotics and pharmacological drugs. For this work I have gone through journals, including principles of pharmacology, prominent books by Guyton and Hall on human physiology, Wilson and Walker principles of biochemistry, and P.K. Gupta molecular genetics, not to miss textbook on microbiology. All these books and immense guidance by my guide has helped me go deep in this topic. Also through this work I learnt about biochemical techniques. Journals play a pivotal role in one getting knowledge of recent trends in research on drugs in this field. I learnt about many techniques including spectrophotometer. Literatures in newspaper has also given me knowledge about government support in this field and investments done by companies in educational institutes for researches. Many educational institutes have started project on how bacteria are evolving new means to grow in human bodies and hence overpowering traditional antibiotics. Genetics are searching how genes are responsible for adaptations by bacteria for this traditional drug. As a large people from all fields are working to find new novel ways for treating infections. We have always faced this problem of co evolution of bacteria and human. There is need to increase good microbiota and decrease colonization by pathogenic microflora. Marketing companies in fact have growth to immense heights using this health as base, they are producing PROBIOTICS to help people increase their levels of good bacteria. It is important to educate people on use of immunity boosting foods.

METHODS AND MATERIALS

My way of going through this experiment includes detailed in-depth discussions with guide, reading journals, learning experimental techniques and principles, and reading recent researches on same. It is very important to learn about previous researches before beginning to start new work. Earlier results help us to understand how trend is going for particular microbe. Research involves detailed study of topic and principle to work on important parameters. This experiment involves work on spectrophotometer, use of principle of sterilization, work in LAF, use of centrifuge and autoclave. All these are important biochemistry and microbiological instruments.

In this experiment I studied about growth curve of soil bacteria. It also involves principle of serial dilution. Soil contains thousands of pathogens. It serves as important reservoir of microbes.

- Initially I started by weigh 1g soil and add 9ml of water.
- Take 6 test tubes and add 1ml to each containing 9ml of water and we add 1ml of soil solution from 1st test tube to 2nd one.
- Repeat this steps and make dilutions with soil sample from banana plant.
- Preparation of agar plates-
- Using prepared sterilized agar media, bring sterile petri plates in LAF, pour melted agar media over plates, and leave for solidification.
- Now as we have already prepared serial dilutions we can streak over plates to isolate bacteria.
- Streaking, spreading and pour plating agar techniques to isolate bacteria from soil sample.
- Now seal plates using paraffin.
- Keep in incubator at 31 degree Celsius, for 1- 2 days.
- Preparation of plant extract-
- With leaves from Lantana americana we crush in methanol solution using mortar and pestle.
- Centrifuge it to obtain supernatant free from debris.
- Now we prepare methanol solution 8%.
- Keep in refrigerator.

At last we need to check antimicrobial activity of plant taken. Firstly we check type of microbe, gram positive and gram negative to find which antibiotic is needed. This is done using gram staining procedure.

- Bacteria under study was found to be gram negative.
- We can test antimicrobial activity using disc diffusion method.
- Take plant extract in LAF, and suitable antibiotic for gram negative bacteria. Using forceps put antibiotic on agar plates streaked agar plates. Add a drop over agar plates using dropper taking plant extract.
- Seal plates using paraffin.
- Keep in incubator. If our plant contains antimicrobial activity, then a zone of inhibition will be seen.

We also performed detailed procedure for testing pattern of growth microbe using spectrophotometer.

Procedure-

Make 7 flasks as follows-

- Flask1- master culture (media Luria broth and inoculated with pure colony from agar plate).
- Flask2- 100microlitre inoculum and 1ml plant extract and media 25ml.
- Flask3- 100microlitre inoculum and 2ml plant extract and media 25ml.
- (1 and 2 flask have aqueous plant extract and media 25ml.
- Flask3- 100microlitre inoculum and 1ml methanolic plant extract and media 25ml.
- Flask4- 100microlitre inoculum and 2ml methanolic plant extract and media25ml.
- Flask5- 100microlitre inoculum and 1mlmethanolic solution and media25ml.
- Flask6-100microlitre inoculum and 2ml methanolic solution and media25ml.
- Now we need to take absorbance at 600nm for each flask, at timings- 0hrs, 30min,, 1hrs , 2hrs.
- Flask7-blank it does not contain inoculum but only media.

First I inoculated flask for 4hrs reading, then after 2hrs I inoculated flask followed by 1hr,30min .

(0 hrs reading was taken just after inoculation).

DATA AND ANALYSIS

With completion of time frame I measured absorbance at regular intervals for each flask.

(each flask absorbance need to be taken at 4 time slot.)

Then I had discussions with my guide regarding absorbance obtained, we drew growth curve of bacteria using readings and found that it was explaining following pattern-

There were 4 growth phase as observed in microbe generally-

- Lag phase
- Log phase
- Stationary phase
- Decline phase

Shape of graph was sigmoid.

Hence we could easily see bacteria population was growing as usual and there was no effect of plant extract on its growth.

CONCLUSION

Through this experiment I found that plant species taken *Lantana americana*, lacks antimicrobial activity. It cannot be used to develop drugs for treating disease. I also got to learn about various principles of microbiology, biochemistry and molecular genetics and how these are related. With immense guidance from my guide and lab teachers I learned important laboratory techniques, importance of sterilization and application spectrophotometer. Although we did not get desired activity from this plant but my research under guidance of Dr. Gurjeet Kaur will continue to contribute for this novel cause. I wish to touch unturned stones for development of herbal medicines in cure of people. We all need to work together to develop more innovations in field of immense potential Biotechnology.

REFERENCES

<https://www.scopus.com/scopus/inward/record.url?partnerID=10&rel=3.0.0&view=basic&eid=2-s2.0-85163758383&md5=36d2d3297830645279424121c7ec46ad>.

Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health*. 2015;109(7):309-18. doi: 10.1179/2047773215Y.0000000030. Epub 2015 Sep 7. PMID: 26343252; PMCID: PMC4768623.

1. Atkins, Peter and Julio de Paula. *Physical Chemistry for the Life Sciences*. New York: Oxford University Press, 2006.
2. Chang, Raymond. *Physical Chemistry for the Biosciences*. USA: University Science Books, 2005.

-
3. Gore, Michael. Spectrophotometry & Spectrofluorimetric. New York: Oxford University Press, 2000.
 4. Price, Nicholas and Dwek, Raymond and Wormald, Mark. Principles and Problems in Physical Chemistry for Biochemists. R. G. Ratcliffe. New York: Oxford University Press, 1997.
 5. Irwin H. Segel, Biochemical Calculations (How to Solve Mathematical Problems in General Biochemistry), 2nd edition, John Wiley & Sons, 1975
 6. <http://www.nist.gov/pml/div685/grp03/spectrophotometry.cfm>