STUDIES OF DIFFERENT PLANTS IN HIMACHAL PRADESH FOR THEIR THERAPEUTIC USAGE (ANTIBACTERIAL ACTIVITY)

Dissertation submitted in partial fulfilment of the requirement for the degree of

Bachelor of technology

In

DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS

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STUDENT DECLARATION

I hereby declare that the work presented in this project titled **"Studies of different plants in Himachal Pradesh for their therapeutic usage (Antibacterial activity)"**, submitted in the partial fulfilment of the requirements for the degree of Bachelor of Technology in Biotechnology at Jaypee University of Information Technology; Waknaghat is an authentic record of my work carried out under the supervision of **Dr. Jitendraa Vashistt,** Associate Professor, Department of Biotechnology and Bioinformatics.

I also declare that no part of this thesis has previously been submitted to any University or any examining body for acquiring any degree. I am fully responsible for the contents of this project report.

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CERTIFICATE

This is to certify that the work reported in the B. Tech. thesis entitled **"Studies of different plants in Himachal Pradesh for their therapeutic usage (Antibacterial activity)"**, submitted by Yashkirti Garg in the partial fulfillment for the award of degree of **Bachelor of Technology in Biotechnology** at Jaypee University of Information Technology, Waknaghat, India, is an authentic record of work carried out under my supervision.

This report has not been submitted partially or fully to any other University or Institute for the award of this or any other degree or diploma.

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ACKNOWLEDGEMENT

First and foremost, I am very grateful to, **Prof. Sudhir Kumar, Head of Department of Biotechnology and Bioinformatics** at **JUIT Solan (H.P)** for allowing me to carry out this project, with his kind support and guidance.

I would like to extend my thanks and sincere gratitude to my mentor **Dr. Jitendraa Vashistt**, Associate Professor, Department of Biotechnology and Bioinformatics, Jaypee University of Information technology. His constant motivation, guidance and encouragement in the times of Covid-19 pandemic were essential for a positive mindset and the completion of this project. He steered me in the right direction whenever I came across any difficulty throughout the work.

I would also like to thanks my project partner Naveeta Karar and Ms. Monika Choudhary (PhD. Scholar) who has helped me through this project and it would not have been possible to finish it without their help.

I thank anyone who was involved directly and indirectly in the completion of my project.

Thank you

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

m	Meter
AST	Antimicrobial Susceptibility Test
MIC	Minimum Inhibitory Concentration
CLSI	The Clinical and Laboratory Standards Institute
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
ml	Millilitre
CFU	Colony Forming Unit
PMC	Pubmed Central

ABSTRACT

Medicinal herbs have been used for their therapeutic uses since time immemorial. It is amazing to know that weeds growing on the side of the road can have so many medicinal uses. This project reports digs deeper into the plants spotted in the state of Himachal Pradesh for their antibacterial and other therapeutic properties. Around 40 plants have been studied through text mining approach along with the altitude on which they are found. In the first part of the methodology, it summarises the phytochemical constituents of these plants that may be responsible for these properties. In the second part, it reports the use of the extracted components by different people in various fields. In the review of literature section, various bioassays that lead to the detection of the antibacterial and antifungal properties have been reviewed. The results have been displayed in a tabular form for easy follow though and future use.

CHAPTER 1

INTRODUCTION

Since ancient times, medicinal plants have been used in almost all cultures to cure a lot of health issues. The extensive use of plants as medicines can be seen described in the Vedas and Bible. These traditional medicines are still used in a lot of parts of the world and are still an essential part of Indian healthcare as a result of its wide availability and low cost. The medicinal herbs or traditional plants synthesise a lot of chemical compounds, also known as phyto-chemicals; these account for numerous functions like antagonism towards the bacteria, fungi and viruses, and even some insects. A common example of it being the intake of turmeric powder extracted from turmeric plant that consists of the compound Curcumin, acting as an antimicrobial and also an antihypertensive agent. The pharmacological activity has been reported from almost all the plant parts. [1]

A lot of these phytochemicals have now been used in the modern medicines because of their established pharmacological activities. Approximately a fourth of today's medicines are extracted from plants after testing. A reported drug called Arecoline is derived from the plant *Areca catechu* that is prescribed as an antihelmintic. Another drug called Rhomitoxin comes from the tree of Rhododendron, prescribed for its antihypertensive and tranquilizing effects.

The following report consists of a collection of plants found in varying altitudes of Himachal Pradesh. The Himalayan region is a floral hotspot. The state of Himachal Pradesh in India has a wide attitudinal range of 200m – 7109m representing a very diverse ecosystem. [2] This means that there are a large number of plants in the mountains that are used for a lot of their medicinal properties Plants have been used by local communities for a variety of purposes since ancient times, including food, medicine, fodder, wood, agricultural tools, and religious and other purposes. They make money by trading some of the high-value medicinal plants.

Figure 1: the state of Himachal Pradesh

These plants contain a wide array of bioactive compounds that result in the antibacterial, antifungal, antiviral and hepatoprotective activities. These multiple biological activities are a result of the bioactive compounds present in the plants. The compounds are the secondary metabolites like the polyphenols, flavonoids, alkaloids, lignins, carotenoids, terpenoids whereas some of them are vitamins and amino acids.

The compounds are extracted from the plant source and tested on bacteria, fungi, and viruses as a crude extract. A biologically active compound can be isolated and identified by this extract. The presence of antibacterial properties in raw extracts can be detected using a variety of antimicrobial tests. For this particular project, bioassay that can be used in a laboratory setup like the antibacterial susceptibility test has been reviewed.

CHAPTER 2

REVIEW OF LITRATURE

There is a huge inclination towards plant based medicines in health care and Himachal has been a medicinal hotspot for a long time now. These traditional medicines were used by our ancestors to maintain their health. According to reports, a majority of the Indian population particularly rural areas relies on traditional medical methods. As the technology advances, these herbs have made a swift entrance into the modern medicine. After a lot of laboratory testing, the phytochemicals extracted from these plants are used in the medicines that are quality assured. These biological compounds are tested for several biological properties before they serve our purpose. A few papers testing for antibacterial activity of the herbal extracts were reviewed for this project. These papers focus on the use of antimicrobial testing methods as potential antimicrobial agents for in vitro evaluation of extract and refined drug drugs. [3]

Antibacterial agents: The compounds that acts against the bacteria by either inhibiting their growth (bacteriostatic) or by killing them (bactericidal). Examples: Tetracycline, Cephalosporins etc.

6.1 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Also abbreviated as AST, it is a test used to determine the specificity of antibiotic towards a group of organism. It is a procedure in the clinical microbiology laboratory. It determines at what concentration does the antimicrobial inhibits the microbial growth in vitro.

Minimum inhibitory concentration: Abbreviated as MIC, determines the threshold for the inhibition of growth of the micro-organisms. It is the lowest concentration of antimicrobials

that after an overnight incubation will inhibit the visible growth of microorganisms. **Minimum bactericidal concentration:** It is the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic free media. [4]

Following are a few tests that are used as standard tests for the antibacterial and antifungal assay.

6.1.1 Diffusion methods

Agar disk- diffusion method

This method is used in clinical microbiology laboratories for AST. It tests the antibacterial action of the compounds on various bacterial pathogens like *streptococci, haemophililus influenza* etc, and against filamentous fungi like *Aspergillus*, *Fusarium* etc. The procedure uses specific culture media and different incubation conditions for the bacteria to grow. [6]

The test involves the incubation of the agar plates with the inoculums containing the test organism. The test compound is added to the filter paper at the required concentrations and placed on the surface of the agar plate. As the Petri dish is incubated at the desired conditions, the test compound diffuses into the agar medium and if it contains any antibacterial agent it inhibits the growth of the test microorganism. The disadvantage of this method is that as the zone of inhibition cannot tell if the organism has died or simply prevented from growing. It is also difficult to measure the MIC as it if difficult to determine the amount of test compound that diffused into the agar medium.

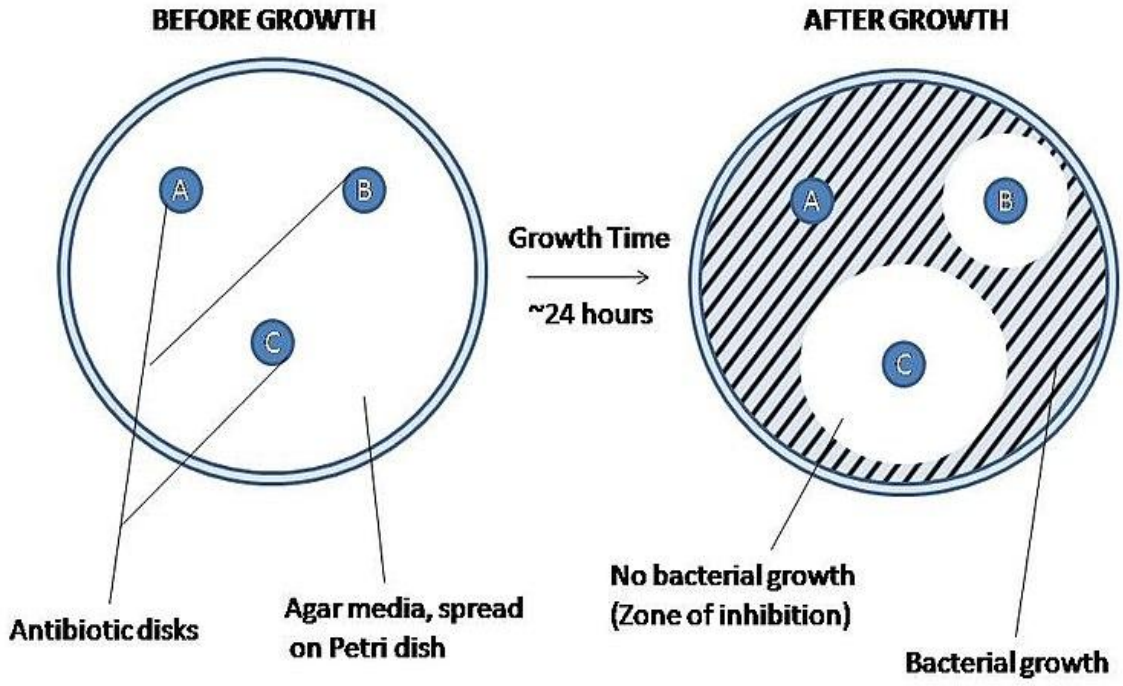


Figure 2: A diagram showing the process of agar disk diffusion method [5].
[https://en.wikipedia.org/wiki/Disk_diffusion_test#/media/File:Agar_Diffusion_Method_1.jpg; Author: Sommer36, CC BY-SA 4.0]

Agar well diffusion method

It is a similar method like the disk diffusion that evaluates the plant extract for antimicrobial properties. After the agar plat is inoculated with the test organism, holes are punched into the agar surface with a cork borer, and the test compound or extract is filled into the well. After required incubation, the zone of inhibition is checked. Similar to the disk diffusion, the plant extract diffuses from the well into the agar and shows either bacteriostatic or bactericidal effect.

Antimicrobial gradient method:

The principle of this test is a combined form of diffusion and dilution methods carried out to determine the MIC value. The principle is based on the ability to create a concentration gradient in the agar medium of the antimicrobial agent being studied. A strip is deposited on

the agar surface impregnated by increasing concentration gradient of the antimicrobial agent from one end to the other. The active compound diffuses into the medium and inhibits the growth of the bacteria at certain zones (zones of inhibition). At the intersection of the MIC strip and at the end of the zone of inhibition of antifungal compounds and antibiotics is determined.

6.1.2 Dilution methods

These are mostly used for the determination of MIC values in the laboratories and clinics. Not only these assays tell us whether the plant extract has any antimicrobial effect, it also helps in the estimation of its concentration. These help in the quantitative measurement of in vitro antimicrobial activity on fungi and bacteria. CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have established certain criteria for BIOS for a similar experiment in laboratories.

Broth dilution method:

There are two types of broth dilution assay namely Micro and Macro dilution assay. These assays require the preparation of two fold dilutions of the antimicrobial agent or extract taken from the plants, in a liquid growth medium. The whole preparation can be tested in either test tubes containing 2ml of volume (macro-dilution) or in 96 well plates (micro-dilution). Certain dyes like alamar blue dye is used as a growth indicator.

Agar dilution method

In this method, the molten agar medium is performed by twofold serially diluted concentrations of the antimicrobial agent to be tested. To this concentration gradient, the inoculum is inoculated on the agar surface and the MIC is recorded.

Both fungi (eg: *Candida sp., fusarium, aspergillus*) and bacteria (eg: *Helicobacter sp*.) can be tested for susceptibility using this method.

Time kill test:

Also known as time kill curve is used for the determination of bactericidal and fungicidal effect of the test compound. The test can be either time-dependant or concentration-dependent. The percentage of dead cells is done at different concentrations and time intervals, as CFU/ml stands for colony forming unit per ml of the medium. The bactericidal effect is achieved with a lethality percentage of 90% for 6 hours, which is equal to 99.9% of lethality for 24 hours. [3]

The plant kingdom promises us with a large diversity of secondary metabolites as well. The above experiments are done on plant extracts to check their antibacterial activity. If we dig deeper, we find that there are certain secondary metabolites present in those extracts that are actually responsible for all the reported activity. The number of secondary metabolites that have been extracted from the plants is huge. There are more than 12000 known alkaloids, more than 25000 terpenoids and more than 8000 phenolic compounds reported till now. [7]

Secondary plant metabolites: Specialised chemical compounds that are produced by the plants for its survival in the environment. These compounds do not aid the plant in its growth and development. These are produced in the plants subsequent to the growth phase through specialised metabolic pathways. The main functions of these metabolites have been observed to be:

1. Protection of the plant from pathogens as they have antibiotic, antiviral and antifungal properties.
2. Prevent leaf damage from the sun as they absorb UV light. [8]

The main classes of secondary plant metabolites are as follows:

1. Alkaloids
2. Phenolics (Tannins, flavonoids, Coumarins, Lignans, Stilbenes, Simple phenolics)
3. Saponins
4. Terpenes
5. Lipids
6. Carbohydrates

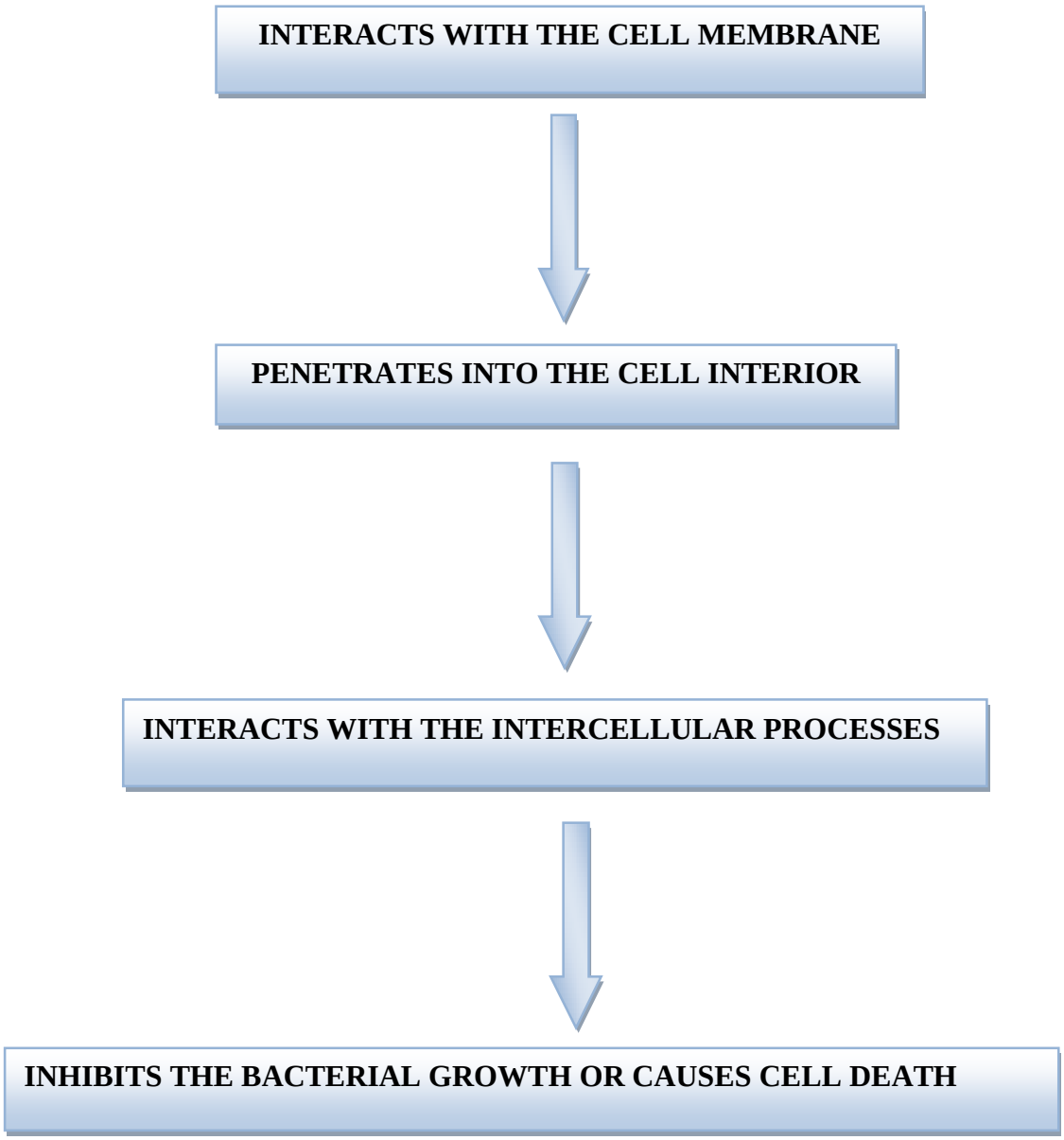
2.2: MECHANISM OF ACTION

The following part of this review focuses on the mechanism of action of these secondary metabolites. A better understanding of these mechanisms of action of different metabolites gives us a deeper insight of how these actually affect the microorganism and how can these compounds be incorporated into our health care system to benefit the people suffering from different bacterial infections. [7]

Secondary plant metabolites affect the bacterial cell in the following number of ways:

1. They disrupt the structure, function and the efflux system of the bacterial membranes.
2. Interrupts the synthesis of RNA and DNA.
3. They interfere with their intermediately metabolism.
4. They coagulate the constituents of the cytoplasm and this result in the disruption of usual cell-to-cell communication.

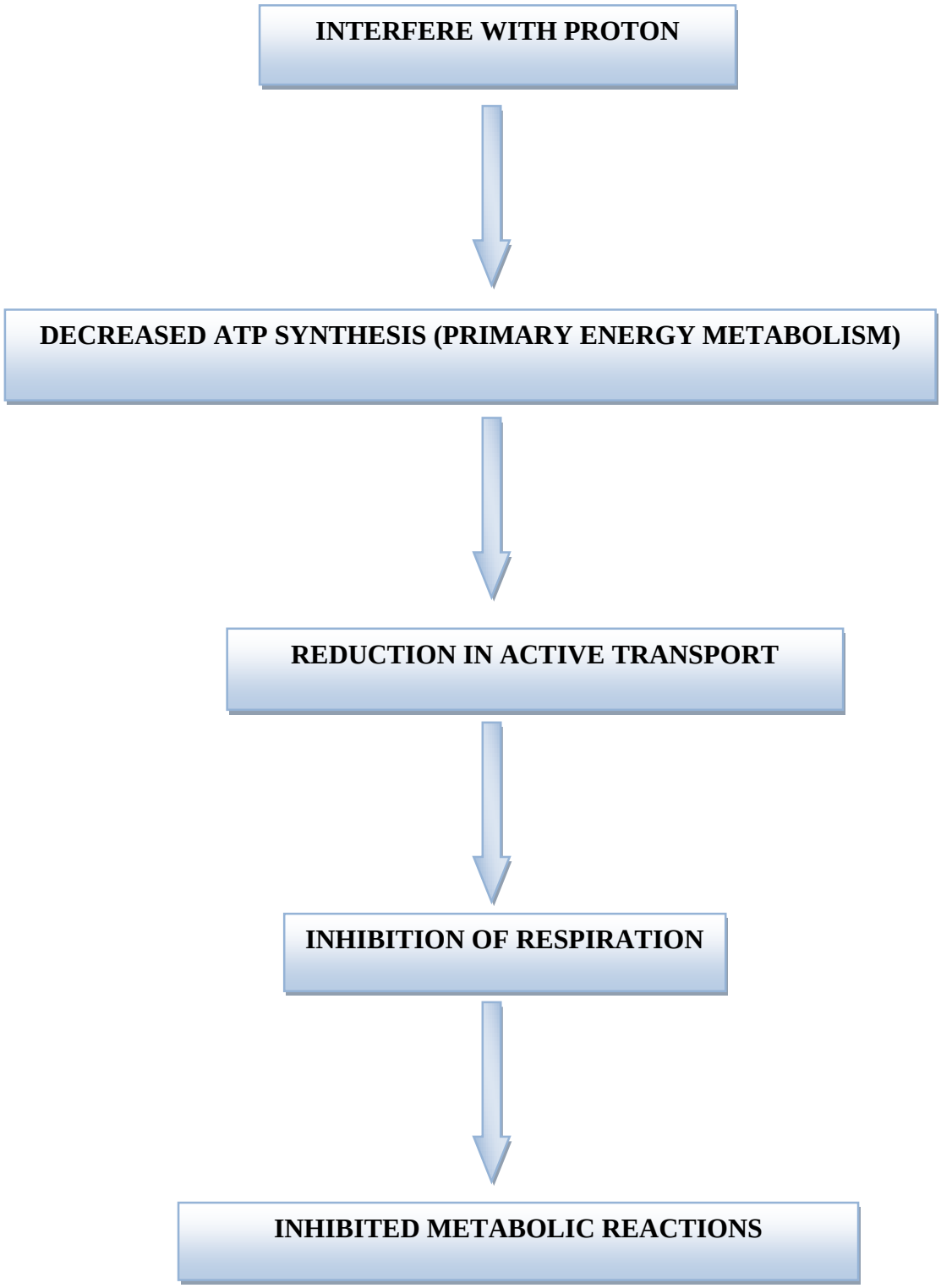
The following flow chart shows the mechanism of action of Thymol that is a monoterpene phenol. It is known to be one of the most active metabolites in the plant.



Thymol can also up- or down regulate the genes involved in outer membrane protein synthesis, inhibit the enzymes responsible for protection against thermal stress, synthesis of ATP, citric metabolic pathways, etc.

The plant secondary metabolite increases their antibacterial activity as their lipophilicity increases. It happens because they can then better interact with the cell membrane of the bacterial cell and disrupt their stability and structure. This disturbs the membrane integrity

and results in an increased permeability of ions inside the cell. Eventually, the electrochemical potential of the membrane changes along with the membrane enzyme activity.



The antimicrobial potential and mechanism of action of different secondary metabolites depend on various factors like the target cells features i.e. whether the microorganism is a bacteria or a fungi or whether the bacteria is gram positive or negative. Additional to this the environment of the microorganism also plays a great role i.e. the temperature, concentration, pH, hydrophilicity of the bacterial membrane etc. Following is a collection of different secondary metabolites and their mechanism of action on various microorganisms as has been reported.

Table1: Bioactive compounds and their antibacterial mechanism of action.

Compound	Mechanism of action
FLAVONOID	Inhibits DNA gyrase. Inhibits beta- hydroxyacyl - acyl carrier protein activities. Reduction of membrane fluidity. Stops O_2 consumption, as a result of bonding to the inhibition site on the respiratory electron transport chain.
SAPONINS	Formation of a molecular complex with sterols in fungal membranes, resulting in pore formation therefore causing loss of membrane integrity and function.
ALKALOID	Intercalates with the bacterial DNA. Inhibits the activity of the histidine decarboxylase enzyme.
TANNINS	Act on the bacterial cell membrane, and bind to enzymes and polysaccharides causing inactivation.
TERPENES	Interacts with the membrane by inserting between acyl chains of phospholipids. This Increases the membrane permeability and results in an efflux of ions and ATP, disturbs membrane potential and pH gradient of the cell.

CHAPTER 3

OBJECTIVES

OBJECTIVE 1: To screen and categorize different medicinal plants based on the altitude they are found in and their bioactive compounds.

OBJECTIVE 2: To investigate the plant part from where the active compounds resulting in antibacterial activity have been extracted.

OBJECTIVE 3: To investigate the presence of these plants in other parts of the Himalayan region/India

OBJECTIVE 4: To investigate and note whether these plants have other therapeutic properties.

CHAPTER 4

METHODOLOGY

1. Through the text mining approach, various medicinal plants that grow at different altitudes in the state of Himachal Pradesh, India; were screened for different medicinal properties and therapeutic uses.
2. From more than 1800 plants found in the region, 40 were shortlisted.
3. The major criteria of screening were based on the antibacterial properties of the plant.
4. The bioactive compound responsible for the same was noted.
5. The plant part used for the antimicrobial assays was also notes.
6. Different activities of these plants were checked to see if they were already reported in any significant scientific literature at other places along with additional therapeutic activities.

CHAPTER 5

RESULTS

Following are the results of the data collected during the project.

Table 2: List of plants found Himachal Pradesh with their bioactive compounds and therapeutic properties.

ALTITUDE	BOTANICAL NAME OF THE PLANT	ALKALOID	PHENOLICS	SAPONINS	TERPENES	OTHER COMPOUND	PLANT EXTRACT FROM
300-1800m	**Euphorbia helioscopia**	–	–	–	+	Euphoscopoids Helioscopianoids	Leaves
300-1800m	**Commiphora wightii**	+	–	–	+		Gum resin
500-800m	**Centella asiatica**	–	–	+	+	Asiatic acid Madecassic acid	Leaves
800-1000m	**Tribulus terrestris**	+	+	+	–	Steroidal neotigogenin	Whole plant body
800-1100m	**Terminalia chebula**	–	+	–	–	Chebulic acid Ellagic acid	Fruit, bark
800-1500m	**Tinospora cordifolia**	–	–	–	+	Isoniazid, Tinosporide tinosporaside cordifolide cordifol Heptacosanol	Stem
~1200m	**Derris indica**	+	+	+	–	Steroids	Roots, leaves
~1300m	**Acacia catechu**	–	–	+	+	Catechin, Taxifolin	Leaves
1300-1800m	**Bauhinia variegata**	–	+	–	+	steroids glycosides	Stem bark

						anthraquinones.	
~1500m	**Asparagus racemosus**	+	+	+	–	glycoside/Sugar phenol steroid	Roots and tubers
1500-3000m	**Berberis lycium**	+	–	–	–	berberine, berbericine berbericinine	Root extract
~1600m	**Andrographis paniculata**	+	+	–	–	Andrographolides tannins Neophytadiene Phytol	Leaves, stem
1600-2500m	**Dioscorea deltoidea**	–	–	+	–	asperoside diosin	Tuber
<1800m	**Aegle marmelos**		+	+	+	carotenoids	Leaves, Seeds
<1800m	**Berberis aristata**	+	+	–	–	berberine oxyberberine aromoline protoberberine karachine palmatine	Roots
<1800m	**Cassia fistula**	–	+	–	–	anthraquinones Eugenol	Leaves
<1800m	**Glycyrrhiza glabra**	+	+	+	–	Glycosides carbohydrates starches	Leaves
<1800m	**Justicia adhatoda**	+	+	+	–	Vasicinone Vasicinol Adhatodine ,Anisotine Hydroxypeganine	Leaves
<1800m	**Mentha piperata**	–	+	+	+	Menthol limonene cadinene	Leaves
<1800m	**Rauvolfia serpentina**	+	+	+	+	ajmaline deserpidine indobine	Roots , rhizome

						indobinine reserpine serpentine yohimbine	
<1800m	**Terminalia bellirica**	–	+	–	–	Glucoside tannins gallic acid ellagic acid chebulanic acid	Bark, fruits
1800-2800m	**Valeriana jatamansi**	–	+	–	+	Iridoids steroids	Whole plant body
<1800m	**Withania somnifera**	–	+	–	–	WithanolideA Withanolide D withaferin A withaniamide	Roots
1801-2800m	**Heracleum candicans**	–	+	–	–	iridoid lignin	Roots, shoot.
1801-2800m	**Swertia chirayita**	+	+	–	+	Amarogentin mangiferin Swertiamarin	Whole plant
~1900m	**Boerhaavia diffusa**	+	+	–	+	Steroids ursolic Punarnavine punarnavoside caffeoyltartaric acid lignins	Leaves
~2000m	**Viola sepens**	+	+	+	–	Tannins Flavonoids	Leaves, roots
~2100m	**Achyranthes aspera**	–	–	+	–	Saponins A and B	Leaves, stem
~2100m	**Ajuga parviflora**	+	+	+	+	fatty acids withanolodes	Whole plant
~2200m	**Acorus calamu**	–	–	+	+	acorine lucenin calamusenone asaronaldehyde shyobunone Tannine	Rhizome, leaf

~2500m	**Zanthoxylum armatus**	–	–	–	–	Tambulin Prudomestin Ombuin	Bark
>2801m	**Aconitum heterophyllum**	–	+	+	+	glycosides quinones	Roots
>2801m	**Carum carvi**	+	–	–	–	Limonene carvacrol carvone carvenoe linalool p-cymene	Fruits, seeds
>2801m	**Dactylorhiza hatagirea**	–	+	–	–		Roots
>2801m	**Nardostachys grandiflora**	+	+	–	+	nardo-stachysin lignans neolignans	Roots, leaves
>2801m	**Picrorhiza kurrooa**	+	–	–	–	picroside I picroside II	Leaves, roots
>2801m	**Rheum australe**	–	–	–	–	chrysophanol emodin	Roots
3700m	**Cannabis sativa**	+	–	–	–	Cannabidiol	Leaves
-	**Burchellia bubalina**	+	+	–	–	α- and β-gardiols (iridoids)	Stem and leaves

- **+ Compounds present.**
- **- Compounds absent.**

Table 3: List of plants reported in various other places multiple times with different therapeutic activities.

Name of the plant	Other places where the plant was reported	Other therapeutic activities	References
Acacia catechu	Eastern Himalayan region in India	Antipyretic Hepatoprotective Antidiarroeal	[9,10]
Achyranthes aspera	North India	Antimalerial Diuretic Antileprotic Antispasmodic	[11,12]
Ajuga parviflora	North India	Antibacterial Insecticides (Red floor beetle).	[13,14,15]
Asparagus racemosus	North India	Nervous disorders Liver diseases Immunomodulatory activities	[16,17]
Aegle marmelos	North India	Antioxidant properties Antihypertensive properties Antidiabetic properties.	[18, 19]
Acorus calamus	North and Western India	Anticellular immunosuppressive Hypolipidemic activity	[20, 21]
Aconitum heterophyllum	North India	Anti-inflammatory	[22,23]
Andrographis paniculata	North and Southern India	Hepatoprotective	[24,25]
Bauhinia variegata	North and Southern India	Antioxident	[26,27]

		Anticancer activities Antihyperlipidemic	
Berberis aristata	North India	Antiamoebic Antihelminthic Leishmanicidal Tuberculostatic properties. Antihyperglycemic	[28,29]
Berberis lyceum	Northern Himalayan region	Cholesterol reducing Wound healing activities	[30]
Burchellia bubalina	Region not reported	Antifungal Anti-inflammatory properties	[31]
Boerhaavia diffusa	Northern Himalayan region and South India	Antidiabetic Hepatoprotective Immunomodulatory activities	[32,33,34]
Cassia fistula	North, West and Southern India	Antioxidant potential Hepatoprotective activity.	[35,36]
Centella asiatica	South India	Wound healing Increased collagen production	[37,38]
Commiphora wightii	Central India	Nervous diseases Cardiac and circulatory Bronchial congestion Weak digestion Wound healing	[39,40]

Dioscorea deltoidea	Region not reported	Endothelial dysfunction in rats Contraceptive activity	[41]
Dactylorhiza hatagirea	North Himalayan region and Central India	Aphrodisiac activity Wounds and fractures Dysentery and diarrhoea	[42,43]
Derris indica	South India	Inhibitory affects in bronchitis Rheumatic joints Whooping cough	[44,45]
Euphorbia helioscopia	Region not reported	Vasodepressor activity Anticancer potential against human cancer cells	[46,47]
Glycyrrhiza glabra	North India	Antiviral Anticancer Anti-ulcer Anti-diabetic Anti-thrombic Antimalarial	[48,49]
Justicia adhatoda	East India	Ethno botanical use	[50,51].
Mentha piperata	Region not reported	Antioxidant activity Nausea and vomiting	[52,53]
Nardostachys grandiflora	North Himalayan region		[54]
Picrorhiza kurrooa		Antiulcerogenic Antichorestatic	[55,56]

	North India	Hepatoprotective Antidiabetic	
Rauvolfia serpentina	North India	Anti-inflammatory Antidiuretic Antiproliferative Anticholinergic activities	[57,58]
Rheum australe	Northern Himalayan region	Cytotoxicity Antioxidant capacity	[59]
Swertia chirayita	North India	Liver disorder Treatment of diabetes (hypoglycaemic effect)	[60,61]
Terminalia bellirica	North India	Wound healing properties Antioxidant Immunomodulatory Hepatoprotective Renoprotective Anti-hyperlipidemic Antidiabetic Anticancer activities.	[62, 63]
Terminalia chebula	Region not reported	Inhibition of cancer cell growth antioxidant	[64, 65]
Tinospora cordifolia	Eastern and south India	Anti- diabetic Anti- spasmodic Anti- arthritic Anti- leprotic Anti- malarial Hepatoprotective Anti- neoplastic activities	[66, 67]

Tribulus terrestris	Region not reported	Aphrodisiac properties Antidiabetic affects	[68, 69]
Valeriana jatamansi	North Himalayan region and South India	Insecticidal Antioxidant properties	[69,70]
Viola serpens	North Himalayan region	Antioxidant activity	[70,71]
Withania somnifera	South India	Antidepressor activity Immunomodulatory activity	[72, 73]
Zanthoxylum armatum	North and western India	Hepatoprotective activity	[74, 75]

CHAPTER 6

DISCUSSION

The Himalayan region is full with flora that can be used for their medicinal properties in a number of remedies. People have been benefitting from these plants for a long time. Table 1 and Table 2 reports the constituents and their therapeutic properties and it can be clearly seen that there is wide array of diseases that can be cured with these plants. For this project only 40 plants had been selected from a large collection of plants. Majority of phyto-constituents in these plants showed both antibacterial and antifungal properties as has been tested by a lot of people. Other additional therapeutic properties have also been suggested by people after carrying out the required bioassays.

The above information was extracted from the sources provided by PubMed, PMC and other sources in Google scholar.

CONCLUSION

The given projects lead to the understanding that almost all plants consists of some or the other therapeutic properties if used correctly. The Himalayan region is home to a large array of such plants and people have been living in harmony with the environment to protect these plants for a long time. These plants can be used in the traditional way and also be incorporated in the modern medicine to benefit a large section of the society.

During the research of all afore mentioned plants, it was observed that some of these plants were also on the brink of extinction, therefore a balance in the environment can be of a lot of benefit to the healthcare system.

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