

STUDIES ON ANTIBACTERIAL ACTIVITY OF SOME SELECTED FLOWERS

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF SCIENCE
IN
APPLIED BIOLOGY

SUBMITTED BY
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UNDER THE GUIDANCE OF
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MUMBAI

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**“STUDIES ON ANTIBACTERIAL ACTIVITY OF
SOME SELECTED FLOWERS.”**

A

Thesis

**Submitted To The
University Of Mumbai
For The Degree Of
Master Of Science
(Research)**

In

Applied Biology

By

**Pallavi Telang,
(B.Sc., Hons)**



**Clinical Pathology Department
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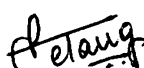
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*Dedicated to my
Loved Parents*

“Statement order the University Rule R.2190”

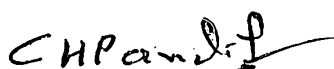
As required by the University regulation number **R.2190**, I wish to state that the work embodied in the thesis titled **“STUDIES ON ANTIBACTERIAL ACTIVITY OF SOME SELECTED FLOWERS.”** forms my own contribution to the research work carried out under in the guidance of **Dr. (Mrs.) C. H. Pandit M.D.** Head, Clinical Pathology Department, Haffkine Institute for Training, Research and Testing Parel, Mumbai. The work in the thesis has not been submitted for any other degree of this or any other university.

Wherever, the references have been made to previous work of others, it has been clearly indicated as such and included in the Bibliography.


Signature of the candidate.

(Ms. Pallavi Telang)

Certified that the above statement made by the student is correct.


Signature of the Guide.

(Dr.(Mrs.)C.H.Pandit)

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As I write these words, I realize myself on the threshold of ones greatest moment that every student has to go through. As I reach the twilight of my academic career and welcome the dawn of my professional career, I cant help but reflect back on this phase of my life.

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I sincerely apologize for any omission.

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(Research Candidate)

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Chapter 1

Introduction

CHAPTER ONE :- INTRODUCTION

Nature abounds in a rich and diverse plant life, having a tremendous medicinal potential worth to be tapped. In recent years there has been an upsurge in the clinical use of indigenous drugs. Polyherbal preparations, originally used in the traditional systems of medicine, are now being investigated and effectively tried in a variety of pathophysiological states. Side effects and expenses associated with allopathic drugs have provoked the need for research into drugs that are without the side effects, especially those belonging to the traditional systems of medicine like Ayurveda, Homeopathy, Siddha, Unani to name a few. An emphasis is also laid on the integration of indigenous health care systems with modern health facilities [Pandit H, 2003].

Since the ancient times, man has turned to the bonanza of nature viz., plants and plant products for promoting human health. Long before the discovery of microbes as etiological agents of infections, herbal medicines were used all over the world. For example plaster of figs for the treatment of abscesses and cedar wood oil for disinfecting the wounds of lepers were the remedial examples of Biblical times. Ancient developed the art of preventing decay of tissues by using gums, matting and vegetable oils. Plant drugs were described in "Pen Tsao" meaning 'The great herbal' written by the Emperor Shen nung about

3000 years B.C. He recommended the use of separate medicinal plant for each day of the year. [Staurt 1911]

Ayurveda, the Indian traditional system of medicine, lays an emphasis on health promotion-a concept of prevention of diseases and strengthening of both physical and mental health. The prophylactic approach necessitates the ways to strengthen tissues, so that they can withstand both endogenous and exogenous stressors. The Ayurvedic medicines are largely based upon herbal and herbo-mineral preparations, either single drug or in combination (Polyherbal) having specific diagnostic and therapeutic principles. The Tridosha theory represents three causative principles of the human body. The Vatadosha represents the functions of propulsive movements (breathing, heartbeats, and common actions like lifting, catching and throwing). The Pittadosha represents the functions of interchange and conversion and the perception of knowledge and memory. The Kaphadosha depicts the inter-linking functions and constituents of the body, involving the growth and the secretory processes. When the functions controlled by the doshas are in perfect harmony with each other, a person remains in a healthy state. One of the main approaches in Ayurvedic medicine is to increase the body's natural resistance to the disease/stress. "Rasayana" implies the circulation of 'Rasa' or the nutrients. The concept of Rasayana aims at optimizing the circulation of the essential nutrients and has been promulgated by all Ayurvedic treatises as being useful in disease prevention. Ayurvedic literature

recognizes a substantial number of plants to act as Rasayanas. So as to assess the potential of Rasayanas, various studies have been undertaken on a variety of plants. It was recognized in the Ayurveda that the immune system was involved in the etiology and pathophysiologic mechanisms of various inflammatory diseases of the skin, gut, respiratory tract, joints and central organs. It held the doctrine that modulation of the immune response would alleviate the diseases and the concept of Rasayana in Ayurveda was based on related principles. Ayurvedic medicine thus constitutes a rich source of active substances for immunotherapy based on herbal preparations [Nadkarni A. K., 1976].

Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago. From that point, the development of traditional medical systems incorporating plants as a means of therapy can be traced back only as far as recorded documents of their likeness. However, the value of these systems is much more than a significant anthropologic or archeologic fact. Their value is as a methodology of medicinal agents, which, according to the World Health Organization (WHO), almost 65% of the world's populations have incorporated into their primary modality of health care. The goals of using plants as sources of therapeutic agents have been a) to isolate bioactive compounds for direct use as drugs, e.g., digoxin, digitoxin, morphine, reserpine, taxol, vinblastine, vincristine; b) to produce bioactive compounds of novel or known structures as lead compounds for semi-synthesis to produce

patentable entities of higher activity and/or lower toxicity, e.g., metformin, nabilone, oxycodon (and other narcotic analgesics), taxotere, teniposide, verapamil, and amiodarone, which are based, respectively, on galegine, Δ^9 -tetrahydrocannabinol, morphine, taxol, podophyllotoxin, khellin, and khellin; c) to use agents as pharmacologic tools, e.g., lysergic acid diethylamide, mescaline, yohimbine; and d) to use the whole plant or part of it as a herbal remedy, e.g., cranberry, echinacea, feverfew, garlic, ginkgo biloba, St. John's wort, saw palmetto etc. The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated at 250,000, with a lower level at 215,000 and an upper level as high as 500,000. Of these, only about 6% have been screened for biologic activity, and a reported 15% have been evaluated phytochemically [Fabricant .D, 2001].

Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test and a substantial number of new antibiotics introduced in the market are obtained from natural or semi synthetic resources. It has been reported that between the years 1983 and 1994 of 93 antibacterial agents submitted to the analysis by FDA six were natural products 45 were semi synthetic products modeled on a natural product and 7 antivirals were synthetic compounds based on natural product models. The systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds

with the potential to act against multi resistant bacteria.

Plant extracts have been studied against bacteria for years, but in the more intensified way in the last three decades [Suffredini I.B, 2004]. Plants have been one of the important sources of medicine ever since the dawn of human civilization. In spite of tremendous developments in the field of allopathy during the 20th century, plants still remain one of the major source of drugs in modern as well as traditional system of medicine through out the world. Drugs obtained from the plants and animals are called as drugs of natural origin and are produced in the living cells of plants or animals [Khanum A,2005]. Historically plants have provided an important source of anti-infective therapeutic agents like quinine, berberine and emetine and have remained highly effective instruments in the fight against infection. Plants are known to synthesize a variety of secondary metabolites capable of providing protection against the infectious agents. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases cancer and opportunistic AIDS infection [Iwu M W,1999]. In the context of providing efficient and inexpensive medicine to the masses and built up public confidence in indigenous medicine it is necessary that the traditional medicine is evaluated in the light of current concepts and modern research in medicine and medical biochemistry. Such an effort will make the Indian traditional medicine acceptable countrywide at all

social and economic levels. It is also the first step in making Indian systems internationally recognized and accepted.

Abbe Kneipp in his book has said, "there is a plant for every illness". The Indian physicians of ancient eras had knowledge of the use of plants in the disease cure. Sushruta Samhita summarizes the objectives and the advantages of the Rasayanas in a more practical way. Accordingly, many herbs were found to be anti-aging, enhancing the life span, promoting the intellect, strength, immunity and thereby ensuring longevity. Many manuscripts of Ayurveda had a mention of a large number of plants used for the restoration of health and vigor. Historical records indicate that, as long as 2,600 years ago, the Chinese already developed drugs using a number of forms of phytotherapeutic agent elaboration.

In light of the recent emergence of bacteria, which are resistant to multiple antimicrobial drugs, posing a challenge for the treatment of infections, the need to discover new antimicrobial substances for use in combating such microorganisms becomes patent. On the other hand, due to the lack of knowledge concerning the potential toxic activity and adequate usage, medicinal herbs are often used incorrectly, thereby not yielding the desired effect.

Of the currently used allopathic drugs, many over a periodic use have shown carcinogenic properties. Also, most of them possess potentially harmful side effects. The need of the hour is, therefore, to screen such pharmacologically active substances that pose a minimal risk to the subject being treated. All this has brought about a worldwide awareness regarding the benefits of herbs, minerals and natural remedies based on them. A large section of the western world has turned to the use of the traditional systems of medicines in disease prevention and health promotion. These systems of medicine, lay a lot of importance on herbs for rejuvenating the body functions and stresses their use for maintaining a healthy life. Although a large number of plants with medicinal value exist, only a few have been exploited in modern medicinal therapies. The main reasons for the same are lack of standardization, isolation, characterization of active principles, difficulty in assessing the pharmacokinetics, and lastly the paucity of controlled clinical studies.

The medicinal plants continue to be of interest both in India and worldwide. In many of the developing countries, the use of plant drugs is increasing because modern life - saving drugs are beyond the reach of a majority of world population who cannot afford them. This is despite the fact that many such countries spend more than 50% of their total health budget on such drugs. (Handa S. S.,1993).

About fifteen years ago, very few of the world's top pharmaceutical companies had a research program involving plants. However, currently more than halves of these undertake such investigations. The recent increased commitment towards plant derived products have resulted in the discovery of vincristine, etoposide, artemisinin, ginkgolides, and their successful therapeutic application.

In UK and USA, almost 25% of the active pharmacological components of the currently prescribed medicines were first identified in higher plants. The pharmacological properties of these agents have been elucidated by the use of modern synthetic methodology culminating in randomized clinical trials. So, recently there has been a change in the attitude of both the medical and the scientific fraternity that fosters a spurge towards research in medicinal plants and herbals. (Sci. Comm., 1995). This was of course based on the old practice of using plants and their extracts in the treatment of various microbial infections but to a greater extent, the credit goes to the advances made in modern chemistry and to the research workers who developed the techniques to demonstrate the antimicrobial activity both invitro and in vivo.

Hence, there is an urgent need that the researchers from different disciplines in India come together to share their valuable experience in an open environment and work in association for the betterment of our traditional herbal]

medicine in the interest of mankind. If we fail to do so, we should not be surprised if the traditional medicines in the nearest future are manufactured and packed in attractive and colorful packing and sold to us by the foreign multinational companies at cost we will not be able to afford.

In the wake of dramatic discovery of Penicillin from fungus Pencillium notatum by [Fleming (1929)] and the realization of the importance of this antibiotic in the therapeutic medicine [Chain and his associates 1940 and Abrahm et al 1941] plants were also extensively investigated for the presence of antimicrobial principles.

The beginning of this new era was marked by the work of [Osaborne ,1944] who examined the juices of 2300 plants species belonging to 266 families and found that the plants from 68 genera from 28 families exhibited activity against S.aureus and E.coli. [Rao et. Al (1946)] observed that the alcoholic extract of the plant *Moringa pterigosperma* exhibited marked activity against various Gram - positive and Gram - negative pathogens as well as M. tuberculosis.

[Nai Chu Chang et al (1950)] observed that crude extracts of *Coptis chinensis* in combination with berberine bisulphate inhibited growth of wide spectrum of bacteria including S.aureus, S.haemoliticus, C.diphtheria,

M.tuberculosis, Vibrio cholerae, Brucellus melitensis and other bacilli. Further [Bishop and McDonald (1951)] tested extracts of 940 species of *Nova scotran* plants for antibacterial activity and observed that 146 extracts were active against S.aureus and E.coli. In the last 3 decades crude extracts as well as purified forms (fractions) of large number of medicinal plants have been prepared and tested both in vitro and in vivo and many of these have attained their use in therapy.

Many drugs used to treat ailments and diseases have severe side effects especially if used in long term treatment. Pathogens also develop resistance to the drugs. It is thus necessary to constantly improve and develop new drugs. There is a trend in developed countries towards using natural drugs rather than synthetic drugs. This is not a new concept with many cultures having a history of traditional medicine [Stirk W A, 2003]. Ever since antibiotics became widely available about 50 years ago, these have been hailed as miracle drugs – magic bullets, able to destroy disease-causing bacteria. But with each passing decade, antibiotic - resistant infectious diseases have created a public health crisis worldwide. In industrialized nations, despite the progress made in understanding of microbiology and their control, incidences of epidemics caused by drug - resistant microorganisms are on the increase and the emergence of diseases caused by drug - resistant microbes is of public health concern [Linda B, 2002]. It is one of the major problems faced by the world in public health sector, and this

has been the subject of a series of high-level international conference 'The Microbial Threat' held in 1998.

Infectious diseases are responsible for one quarter of all deaths in the world, second only to cardiovascular diseases within the past twenty- five years, these have been associated with the new strains of bacteria and viruses which are highly resistant to available drugs and antibiotics. Infections become more difficult to treat, the severity of illness increases as does the duration of infectiousness, adverse reactions, the length of convalescence and costs. Antibiotics have been reported to inhibit the Gram - positive and Gram-negative organisms through varied mechanisms of actions.

However, the development of bacterial resistance to presently available antibiotics has necessitated search for newer antibacterial drugs. Though antibiotic resistance is not a new phenomenon, resistance to new drugs is appearing with increasing rapidity, often within a year of its introduction.

Few years after the introduction of penicillin, penicillin - resistant infection caused by S. aureus emerged. At present S.aureus has also developed resistance even to methicillin (strongest arsenal of drugs) and vancomycin (a potent drug against resistant pathogens). E.coli presents with resistance against sulfonamides, ampicillin, chloramphenicol and cephalothin. S.pyogenes has been

reported to be resistant to clindamycin and erythromycin. It means little or no returns to drug companies that manufacture these novel antibiotics [Report, 2001]. One way to identify finding new agents is by screening natural products, which have historically been used in various cultures for medicinal purposes.

WHO officials said poorly - planned or haphazard use of medicines has caused drug to lose effectiveness almost as quickly as scientist have been able to discover them. Antimicrobial resistance is naturally occurring biological phenomenon-bugs develop resistance to drugs that don't kill them but misuse and neglect of antimicrobial drugs amplify the process. Antimicrobial resistance can reduce the power of once life saving medicines to that of sugar pill. " We currently have effective medicines to cure almost every major infectious disease," said Dr. Gro Harlem Brundtland, WHO's director –general." But we risk losing these valuable drugs and our opportunity to eventually control many infectious diseases because of increasing antimicrobial resistance." In the developing countries, resistance commonly stems from under use of drugs. For example, some patients may begin an antibiotic regimen, begin to feel better before finishing all of the medicine, then save the remaining tablets for another time because the body is then able to build immunity to these.

In the United States, the situation stems from Disease Control and Prevention which have million prescriptions written for antibiotics each in

bacterial strains that become tougher than them. WHO drug-resistant bacteria account for up to 60 percent of hospital acquired infections. Illness caused by herbs is exceedingly rare. Government sources report zero deaths annually from commercial herbs despite their use by millions of people for decades. Unlike “approved” prescription drugs that only weaken the immune system and cause other diseases herbs are safely used for most health needs by a majority of world’s population. By administering vital phytonutrients (vitamins, minerals, phytochemicals, enzymes, antioxidants, etc...) we can strengthen the immune system so that the body is free to heal itself [CNN Report, 2000].

Over the centuries, plants and various preparations made from them have been used to treat human infections all over the world. In fact number of drugs which find their place in modern day therapeutics own their position to the knowledge derived from the primitive rights and common folk lore (passed down from generation to generation among the common folks). Chewing the bark of Cinchona tree by South American natives was found to cure malarial fever and it was in 1820, Quinine the antimalarial agent was isolated from it [Thattai U, 2002]. The Brazilian natives found ipecacuanha plant to be useful in the treatment of dysentery. ‘Emetine’ the alkaloid later isolated from this plant when tested proved to be antiamebic. The root of *Rauwolfia serpentina* used in native Indian medicine led to introduction of ‘Reserpine’ as tranquillising and antihypertensive

drug [Joshi,1985). The roots of *Arbenia euchroma* have for centuries been used as dyes for wound healing in Chinese and other Asian cultures [Harding A, 2002]. Another such example is the study of aeropoision employed by certain South American native tribes, which resulted in the isolation of 'Tubocurarine' [Snow G A, 1998].

During the middle age, there were series of publications named 'Herbals'. According to [Arber (1938)] systematic botany for the most part originated from the study of plants as healing agents. It was only by the early 19th century, it was realized that the medicinal properties of plant were due to active ingredients present usually in minute quantity in them. The systematic exploration of plants for detecting antimicrobial activity dates back as early as 1934 when an account of the first survey of plant material s exhibiting antibacterial activity was published. Since then A number of reports have appeared wherein juices, essential oils and extracts of plants as well as chemicals isolated from different parts of plants as well as chemicals isolated from different part such as flower, fruit, leaf, stem, bulb, callus etc. have been shown to exhibit antimicrobial activity.

Flowers of plant species may possess antibacterial activity as a natural protection mechanism for the reproductive organs of the plants to help perpetuation of the species through seed formation [Sereitii MRAI, 1999]. The present study has therefore been carried out to determine the antibacterial

activity of flowers of five selected indigenous plants against both the Gram-positive and Gram-negative organisms under standard laboratory conditions.

Chapter 2

Literature Review

CHAPTER TWO:- LITERATURE REVIEW

As reported by Horborne, 1984 the precise mode of extraction of the active principle from plant depends on three factors viz., the texture of the plant material, its water content and type of substance being isolated. The classical procedures for obtaining organic constituents from dried plant tissues like leaves, seeds and roots are by preparing infusion, decoctions, or maceration wherein water or suitable solvents like petroleum ether, ethyl acetate, alcohol benzene, chloroform etc. have been used. In such instances the extraction is carried out with or without applying heat. Extracted material is then strained and dried. In these procedures the residue of the plant material is not recycled for extraction with other solvent. However, the usual method of preparing the extracts is by continuous extraction of powdered plant material in Soxhlet apparatus with a range of solvents as described by Davis et. al.,(1950).The extraction is usually started with petroleum ether and followed by chloroform alcohol or ethyl acetate in succession. Finally, the residual plant material is subjected to hot extraction. Extraction with petroleum ether and then with chloroform brings about separation of lipids and terpenoids initially whereas extraction with alcohol or ethyl acetate is done for polar compounds. The extract obtained using different

solvent systems can then be further clarified by the use of filtration followed by concentration under vacuum using flash or spin evaporator. Crude concentrated extracts prepared from plants contain mixture of chemical substance and it becomes necessary to separate the constituents and purify them. Moreover, solvents used for extraction can be recovered and recycled. It has been observed that only one fifth of the solvent used for extraction is lost during the concentration of the extract. For phytochemical studies the separation and purification is usually carried out by column chromatography.

However for such procedure a suitable solvent system has to be evolved (in order to achieve maximum separation) by chromatographic techniques, which include paper chromatography, thin layer chromatography, Gas liquid chromatography (GLC) and High Performance Liquid Chromatography (HPLC). Paper chromatography is applicable to water - soluble plant constituents and Gas liquid Chromatography finds its main application with volatile components. GLC and HPLC technique may not be available with all laboratories. Besides these technique require special training. For laboratory investigations, TLC is simpler and is the method of choice for separating all lipid components that are lipids, steroids,

carotenoids, simple quinones, chlorophynes etc. This technique is also useful for evolving a suitable solvent system for a further purification of separated constituent of crude extract. Several in vitro techniques are in practice for evaluation of antimicrobial activity. Each method has its advantages and disadvantages. In the review on "The screening methods for natural products with antimicrobial activity." Rios et al. (1988) have given good account of different techniques employed in the evaluation. All the various techniques are reviewed here and in order to unify the different criteria and parameters, standard methods to study the antimicrobial activity of medicinal plants are proposed.

These are classified into two groups namely diffusion and dilution methods. The diffusion methods are well suited for preliminary evaluation of pure substances, which form solutions of homogenous nature and diffuse easily in the test medium. The disadvantages of these methods are these methods are of low creditability for the samples that are difficult to diffuse in the medium. Further these methods are not useful for determining the minimum inhibitory concentration (MIC). The dilution methods include dilution of the substance (active principle) in the liquid medium or in the solid medium and are employed to determine MIC of the antimicrobial

compound. Of the various test procedures, broth dilution technique is the most acceptable test for the compound. By this method MIC of any product against several test organisms can be tested at any time (Cruck Shank).

Several plant extracts possess antimicrobial activity against variety of organisms and different screening methods have been employed to determine this activity (Mitscher et. al.,1972 ;Taninguchi and Kubo,1993; Grien and Brantner,1994). Lately,the disc diffusion method has been used to determine activity in the methanolic extract of *Acacia xanthophyloea Benth. (Mimosaceae)* (Chhabra and Uiso, 1991), while the broth dilution method for *Erythina abyssinica DC* (Papilionaceae) (Taninguchi and Kubo, 1993) and the agar dilution method for *Pimpinella major (L.) Huds. (Apiaceae)* (Grien and Brantner, 1994). It has been observed that the results particularly the MICs obtained from the plant extracts vary and are dependent on the method used. No comparative study has so far reported to evaluate the suitability of these methods in quantifying antibacterial activity of plant extracts as has been the case of antibiotics (Ampel etal.,1984 ; James,1990; Okemo,1997).

Many medicinal plants have been investigated for their chemical components and some of the isolated compounds have

been shown to possess interesting biological activity. Some of these plants are discussed below.

Chakraborty et al (1998) studied different crude extracts of *Plumbago zeylanica* Linn against selected number of microorganisms in which the chloroform extract of the plant showed antibacterial activity.

Gehlot (1998) observed that aqueous leaf extracts of *Aerva persica* showed antibacterial activity against S.typhi. Aqueous and alcoholic flower extracts and aqueous extracts of leaf and stem of this plant exhibited antibacterial activity against *S.aureus*.

In Africa Eloff (2001) found bark and leaves of Marula plant (*Sclerocarya birrea*) were extracted with acetone and MIC values were determined using microplate serial dilution technique with S. aureus, P. aerogenosa, E. coli, Enterococcus faecalis as test organism. All organisms were active with MIC values from 0.15 to 3 mg/ml. Marula bark from previous times is widely used for bacteria related diseases by indigenous cultures in Africa.

Perez G.S.(2001) tested antimicrobial activities of chloroform, methanol and aqueous extracts of bark of *Gymnanthes lucida*, Gliri

cidia sepium, *Lesilomadivariata*, *Lysiloma tergemina* and *Coccoloba cozumelensis*. Methanol extracts of two *Lesiloma* species and *G.sepium* exhibited antimicrobial effects against S.epidermidis, S.aureus, P.aeruginosa, B.pumillus and V.cholareae at doses of 200 microgram/ml. The major inhibitory effect was observed with *L.tergemina*, which showed bacteriostatic effect on S.epidermidis at doses of 400 microgram/ml.

Aly elaagbe (2001) found that hexane, ethyl acetate, chloroform and methanol successive extracts of yellow root bark, red root bark, and root wood effectively inhibited growth of B.subtilis and S.aureus at concentration of 200 microgram/disk.

K. C. Ofokansi (2003) revealed that the leaf extracts of *Ocium gratissimum* (family *Labiatae*) exhibited some degree of antibacterial activity against isolates of E.coli and B.subtilis but showed no activity against isolates of S.aureus and P.aeruginosa. The column fractions however showed no antibacterial activity against test organisms. The probable antimicrobial activity of the plant is likely to be associated with its volatile oil contents.

Vahanwala S.J.(2000) screened aerial parts that is flowers, leaves, bark, fruits of *Couropita guanensis aubl* for their antimicrobial

activity. Petroleum ether extracts of *C.guainensis* have exhibited promising antibacterial activity suggesting useful leads for further phytochemical study on *C.guainensis*.

Ahmad I (2003) studied the flower of gulmohar (*Delonix regia*) extracted in 70 % ethanol and further fractionated against 9 bacteria both Gram positive and Gram negative and all test fungi except Aspergillus niger. Antimicrobial potency of active extracts was determined in terms of MIC against S.aureus, S.typhimurium and C.albicans by macrotube broth dilution assay. MIC being in the range of 4.5-12.5 mg/ml against S.aureus and S.typhimurium and 1.25 – 12.5 mg/ml for C.albicans.

Saied Kianbakht of Iran (2003) studied *Tribulus terrestris* used as urinary anti-infective in folk medicine. The invitro antibacterial activity of methanolic extracts of different parts (fruits, stems plus leaves and roots) was evaluated against four reference bacteria by broth dilution assay and agar diffusion assay. MIC value of methanolic extracts of fruits and stem plus leaves against all bacteria was 2 mg/ml and MIC value of roots against S.aureus, E.faecalis and E.coli was 4 mg/ml and the MIC value of the roots against P. aeruginosa was 2 mg/ml. In agar diffusion assay, the

methanolic extracts of all parts of the plant showed considerable activity against all bacteria.

Naqvi B S (1994) studied on crude 50 % ethanolic extracts of leaves, stem and flowers of *Nerium indicum* Linn and *Hibiscus Rosasinensis* Linn which showed antibacterial activity against 10 Gram positive and Gram negative both pathogenic and non pathogenic strains.

Vapours of the colourless oil named Allicin obtained from *Allium sativum* when tested for antibacterial activity but also proved toxic to wide variety of bacteria (Cavellito et al 1945).

Studies on natural products and their use as herbal drugs during the last decades have indicated them to be possible alternative to synthetic drugs / antibiotics. However more concreted efforts has been made into these studies to discover new molecular structures as lead compounds from the plant kingdom.

S.emarginatus showed strong activity against the tested bacterial strains that is Pseudomonas testosteroni, Staphylococcus epidermidis, Klebsiella pneumoniae, B subtilis, Proteus morganii and

Micrococcus flavus. Its leaf extract can also be used as lead molecule in combating the diseases caused by these bacterial strains.

Crescentia cujete juice and fruit of the plant shows antibacterial activity against B subtilis, S aureus, S typhi and S pneumoniae.

Merih Kivanc (1997) studied fresh juices of 17 plants and tested for inhibition of bacteria and yeast in culture media. Garlic was the most inhibitory plant on the bacteria. Onion, red pepper, garden radish and horseradish inhibited the growth of 1 to 3 bacteria. Garlic, onion, dill and cabbage juice inhibited growth of all 10 yeasts.

Lee (2003) evaluated the antibacterial activities of various fruit and vegetable extracts on common potential pathogens including antibiotic resistant strains. All purple and red vegetable and fruit juices had antibacterial activities in dilution from 1:2 to 1:16. Tea also had significant activity with bactericidal action in concentration ranging up to 1.6 mg/ml against a spectrum of pathogens including resistant strains such as methicillin and ciprofloxacin resistant S aureus, vancomycin resistant enterococci and ciprofloxacin resistant P aeruginosa. Melo Cawalcante et al (2003) found out that fresh and

processed cashew (*Anacardium occidentale*), apple juice (CAJ) are among the most popular drinks in Brazil. Besides then nutritional benefits these juices have antibacterial and antitumor potential.

Sow A I (1995) said that 3 species of *Mentha* gender under name Nana essential oil were used to study the antibacterial potency (MIC) by agar dilution method. The survey has been carried out on 10 bacterial strains among which 3 ATCC reference stubs and one fungus (*Candida albicans*). The MIC found was less than 25 microgram/ml. The results show an antibacterial potency according to NCCLS norms (MIC < 256 microgram/ml). Tested strains were more sensitive to essential oil of common mint than fass mint. About 1200 flowering plants mostly native to Australia were screened for antibacterial activity by Atkinson (1946). Of these 50 were found active against *S aureus* and 5 against *S typhi*. In many cases the activity was due to presence of essential oils.

Chapter 3

Aims and Objectives

CHAPTER THREE: - AIMS AND OBJECTIVES

RATIONALE:-

India has one of the oldest, richest and the most diverse cultural traditions associated with the use of medicinal plants. After the extensive use of totally or partially synthesized molecules isolated from nature there is a gradual global shift from synthetic medicines to natural products. India has necessary infrastructure, expertise trained manpower heritage and natural resources to become a major player in the global market for the natural products for commercial and therapeutic utilities. It is believed that India has a share of Rs. 600 crores in global herbal market and has potential to increase it to Rs.3000 to 4000 crores during the next decades. Keeping in mind all these forthcoming possibilities things have been rapidly moving not in India but all over the world [Desai S,2002]. Hence, an attempt has been made here to develop a new safer drug of plant origin in a country like India.

Plant products are invariably cheaper and are claimed to be fairly safe. These, as compared to modern synthetic drugs, may also provide promising leads for developing new synthetic therapeutic agents. Historically, plants have provided an important

source of anti-infective therapeutic agents like quinine, emetine, and berberine and have remained highly effective instruments in the fight against microbial infection. The use of plant preparations as foodstuffs, flavoring agents, dyes, insecticides, cardioactive, hypolipidaemic, antimicrobial and antitumour agents are some of the examples of immense chemical diversity. A variety of drugs exhibiting moderate to powerful antibacterial action have been grouped together and are now designated as non-antibiotics [Seretii MRAI,1999] . During the last decade, infectious diseases have been treated with plant extracts / isolates. Today these are still used extensively for primary health care in developing countries where traditional medicine plays an important role.

Plants are still the almost an exclusive source of drugs for a majority of the world's population. Major pharmaceutical companies have shown renewed interest in higher plants like *Azadirachta indica*, *Nycanthes arbor tritis*, *Hibiscus rosasinensis* etc. as a source of possible novel active substances [Magama S, 2003].

India has the necessary infrastructure, expertise, trained manpower, heritage and natural resources to become a major

player in global market for natural products for commercial and therapeutic utilities. However, in India very few investigations have been reported to achieve a suitable alternative antibacterial drug of plant origin. Hence an attempt was made here to screen some of the plants for possible antibacterial activity.

India possesses a distinct identity due to its varied geography resulting in a great diversity of its natural ecosystem [Biodiversity profile]. The Himalayas have given India a wealth of plant life, in the form of herbs, fruits & flowers with incredible properties, yielding to us some of the most precious active principles, with powerful healing & rejuvenating qualities. Their virtues have been extolled in our ancient Vedic texts and these have become a part of the Indian herbal tradition of Unani and Ayurveda [No1 Global herbal Exporters].

AIMS AND OBJECTIVES: -

- To collect, botanically identify, dry and powder the flowers of the plants under study.
- To extract the powdered plant material using polar and non-polar organic solvents.
- To prepare aqueous extracts of plant material.
- To evaluate these extracts for their antibacterial activity.
- To fractionate further isolate and purify the active extracts.
- To carry out physico – chemical characterizations of active constituents.
- To study the synergism of active plant extract and synthetic drugs.

Chapter 4

Materials

and

Methods

CHAPTER FOUR: - MATERIALS AND METHODS

Collection of plants

Authentic flowers from various plants under study were obtained from the local market and used for experimental purpose.

Bacterial cultures employed in the study

The following bacterial cultures were employed to study antibacterial activity of crude extracts and their fractions. All the cultures were available in lyophilized form in Department Of Bacteriology, H.I.T.R.T., Mumbai. These cultures whenever required were lyophilized in peptone water and maintained on nutrient agar slants with periodic sub culturing or storing in the refrigerator.

- 1 Staphylococcus aureus standard [NCTC 3750]
- 2 Staphylococcus aureus clinical isolate [pus sample]
- 3 Streptococcus pyogenes clinical isolate [pus sample]
- 4 Escherichia coli standard [NCTC10148]
- 5 Escherichia coli clinical isolate [water isolate]
- 6 Salmonella typhi standard [NCTC T786]

Media used

1 Peptone water

The medium was used to revive lyophilized cultures of different bacteria to be employed in screening of various extracts and purified fractions for their antibacterial activity.

2 Nutrient Agar

Slants of Nutrient agar were used to maintain (sub culturing) bacterial cultures.

3 Muller Hinton broth

The medium was employed to test the antibacterial activity of extracts and their fractions.

Extraction of plant material using non- polar and polar organic solvents

The flowers were sun dried and powdered in a grinder mixer (80 – 100 mesh). Extraction of powdered material of the flowers was carried out using non-polar and polar solvents. The dried plant material was first extracted in Soxhlet with pet ether (60-80 °C) and then with methanol in succession. The extraction was carried out till it was complete as indicated by no coloration in the side arm of the Soxhlet apparatus. The petroleum ether extract obtained was filtered through the Watman filter paper no 1 and the suspended matter obtained on the paper was mixed with the residue, which was left in Soxhlet apparatus after petroleum ether extraction. The solvent from the filtered extract was removed under reduced pressure. The extract was further dried on boiling water bath. Before allowing the second extraction with methanol the left over residue and suspended matter from the previous extract were first dried by spreading on the filter paper (to avoid traces of solvent). This was then extracted with methanol following the procedure described above for the petroleum ether.

% Yield of the individual extractives was calculated [Partridge D (1950), Vahanwala S (2000)]. All these extracts were made free from solvents and then subjected to antibacterial activity by standard procedures. The crude extraction using acetone as a solvent was also done in the same manner.

Aqueous Extraction

The flower powder was subjected to aqueous extraction in distilled water using hot decoction method described by [Indian herbal pharmacopoeia, 1998]. To 5 g powder 50 ml of boiled water was added in 80 °C in a stoppered flask. It was shaken well and allowed to stand for 10 minutes. After cooling 2 g of kieselgurh was added and filtered. The filtrate was transferred to trade evaporating dish (7.5 cm in diameter) and was evaporated on a water bath, for 30 minutes. Finally it was dried in a steam oven for 2 hrs and the residue was weighed. The percentage of water-soluble extractives with respect to air-dried drug was calculated.

Screening of Extracts for their antibacterial activity

The antibacterial in vitro activity of the extracts was studied by tube dilution method described by [Cruickshank R, 1973 ; Deshmukh S, 1993] the details of which are given below.

The antibacterial activity has been described in terms of 'Minimum Inhibitory Concentration' [M.I.C.] in $\mu\text{g/ml}$ required to inhibit the growth of bacteria. All the new compounds have been screened in vitro against Gram positive and Gram negative groups of bacteria respectively by tube dilution technique or broth dilution technique.

The standard drugs employed in the antibacterial testing of compounds were ampicillin and trimethoprim.

Method

10 mg of the test substance was dissolved in 0.1 ml Tween 80 and then diluted to 10 ml with DMSO [Nostro A, 2000]. The solution was sterilized by autoclaving and was added to the medium in such a amount to give a final concentrations of 500,200,100,40,20,10 $\mu\text{g/ml}$ of the medium.

The test solution was added in graded concentrations aseptically to the tubes containing sterile test medium (Muller- Hinton broth). To the each test tube was then added 0.1 ml of standardized inoculum (24 hr old cultures of the test organism diluted to 10^{-2} . After mixing the contents, the tubes were incubated at 37 oC and examined after 48 hrs for the presence or absence of the growth of the test organism. The lowest concentration of the test compound, which showed no visible growth, was taken as the minimum inhibitory concentration (MIC). The compounds showing inhibition up to concentration of 500 µg/ml and below were considered as active.

Fractionation of crude extracts

To ascertain the best solvent system to be employed for the fractionation of crude extracts, TLC was run using different permutations and combinations of solvents. This was essential to achieve best results of the extracts on the chromatogram. TLC was run by the method of [Bobbit (1963)] described below. Only those extracts i.e. showing antibacterial activity were subjected to TLC. Alumina sheets coated with silica gel were cut into pieces (plates) of size 4.0 cm x 1.2 cm and used for spotting the extracts. A small chamber i.e. coplin jar with a lid was first lined with filter paper and

then the solvent was introduced in the jar. The lid was closed in order to ensure complete saturation of the atmosphere in the jar with solvent vapors. This helped in faster separation, formation of straight solvent front and achieving round spots. The spotted plates before placing in the chamber were dried at room temperature in order to evaporate the solvent. Separation was carried out by ascending TLC in which sample spot was above the solvent level. Once the solvent reached to the required level the plates were removed from the jar and dried at room temperature in order to remove the solvent of the chromatogram. The dried chromatogram was then visualized after exposing it to iodine vapours. The TLC pattern obtained for 2 active extracts is shown diagrammatically.

Fractionation of active crude extracts

The fractionation of each active crude extract was carried out in a large column packed with 120 g of Silica gel (60-120 mesh) (E. Merk) and 2 g of crude extract was loaded on it. Fractions obtained using different solvent system for elution was checked for their antibacterial activity by tube dilution method as described earlier. The entire procedure adopted for fractionation is described in detail as follows- [Lattu R, 1994 ; Lederer E, 1957; Rane J, 2001]

1Preparation of column

Silica gel columns were filled with the organic solvents that gave the best resolution. The solvent was allowed to trickle down slowly through cotton by opening the tap of the column, allowing complete soaking of the cotton with the solvent and removal of air trapped in between.

Slurry of silica gel (60-120 mesh) was prepared in the same solvent system used in the beginning of column chromatography and was poured slowly in the column. At the same time excess solvent was removed from the tap. The silica gel in the column was then continuously washed with the same solvent system with gentle tapping in order to ensure tight packing. Both the packed columns of silica gel (37 g) gave the length of cm.

Once the columns were set, filter paper discs were put at the top of the column in order to prevent the disturbance of adsorbent while adding the solvent. The solvent was always kept above the adsorbent [Welcher F, 1963].

2 Preparation of the sample

The sample to be loaded was first dissolved in the least volume of the solvent used at the beginning of preparing the column. The dissolved extract was placed on the surface of the adsorbent using long Pasteur pipette and then allowed to trickle down into the column.

3 Elution

For eluting different fractions from petroleum ether extract in column three solvent systems were employed. These were Petroleum ether, chloroform and ethanol. The same was used for acetone extract. These solvents being polar to non polar in origin were used for fractionation of active crude extracts.

The yield of each fraction obtained on column chromatography is shown in table.

Using different solvent systems three fractions were obtained from petroleum ether (60-80 °C) extract and three from acetone extract. All these six extracts were tested for their antibacterial activity in the

manner described for the crude extracts and the results were presented in table.

Attempts to isolate, purify and characterize the active constituents from the fractions

Antibacterial activity of active crude extracts and their fractions is often attributed to the presence of certain chemical groups such as phenolic compounds, terpenoids, organic acids and alkaloids. Thus, phytochemical screening of the extracts and their fractions for detecting the presence of these chemical compounds correlates the antibacterial activity. Presence of these compounds in active crude extracts and their fractions was detected by phytochemical methods described by [Horborne (1973)]. Presence of different chemical groups was identified as follows-

1 Detection of Phenolic compounds

Phenolic compounds are polyhydroxy organic compounds, which react with ferric chloride (FeCl_3) and give bluish green colour.

To 5.0 ml of 3% solution of extract or fraction in methyl alcohol 0.5 ml of 1% ferric chloride solution in 0.5N hydrochloric acid (HCl) was added. Development of bluish green colour indicated the presence of phenolic compounds.

2 Detection of Terpenoids

These compounds contain isoprene molecules $\text{CH}_2 = \text{C}(\text{CH}_3)\text{-CH=CH}_2$ i.e their carbon skeleton is made up of two or more isoprene units.

The presence of terpenoids was detected by Liebermann - Burchard reaction wherein 5.0 ml of 3 % hot methanolic solution of the extract or fraction was allowed to react with 0.5 ml of solution containing acetic anhydride and conc. sulphuric acid (20:1). Development of red or violet red colour showed the presence of terpenoids.

3 Detection of Organic acids

These include fatty acids and lipids. To 5.0 ml of 3% methanolic solution of extract or fraction 1% sodium bicarbonate solution was

slowly added. Development of effervescence in the tube indicated the presence of organic acids.

4 Detection of alkaloids

Alkaloids are basic substances, which contain one, or more nitrogen atoms joined in cyclic systems. These compounds show dramatic physiological action and are widely used in medicine.

Presence of alkaloids was detected by adding 5.0 ml of Mayer's reagent to equal volume of acidified hot 3% methanolic solution of the extract or fraction. Formation of the white precipitate at the interface showed the presence of alkaloids.

5 Detection of Glycosides

Glycosides are nitrogenous compounds associated with sugar. The presence of glycosides was detected by adding 3.0 ml. of 1% alcoholic naphthol solution and 2.0 ml concentrated sulphuric acid to 5.0 ml. of methanolic extract or fraction 3%. Development of red violet ring at the junction indicated presence of glycosides.

Results of phytochemical screening are presented in Table.

Synergistic activity of active extract and synthetic drug [Lorian V,1974]

There are three main reasons why to use more than one drug at the same time. Treatment with 2 drugs with different modes of action to both of which the infecting bacterium is originally sensitive prevents the outgrowth of mutant bacteria resistant to either drug. Two drugs may be given because the identity or sensitivity of the infecting bacterium is still unknown. Two drugs may be given because they are synergistic in their bactericidal action being strongly cidal in combination though not completely cidal when used separately.

Synergistic cidal effect is required mainly in treatment of subacute infections in which a complete bactericidal effect is essential for cure because any surviving bacteria are protected from the natural body defense mechanisms. Filter paper strips (Whatman No.1, 8 x 8 cms) were dipped in the antimicrobial solutions and left for 10 seconds and removed by drawing the strip against the side of the tube to remove excess liquid. The strips

were carefully placed onto the agar surface allowing the center of the strip to touch first and lowering the ends onto the agar ensuring whole strip is in good contact with the surface. The Muller Hinton agar plates were incubated at 37°C overnight. The plates were examined for signs of synergy by comparing the extent of inhibition of microbial growth surrounding the intersections of the paper strips with any inhibitions at a distance from the intersections.

The description of the flowers and plants used is as follows:-

[Akhtar H,1992 ; Dr Nadkarni]

1 *Hedychium coronarium* Koen.

Family : Zingiberaceae

Common name : Ginger-lily; garland flower

Habitat : Throughout India upto 2000 m and also grown in gardens of Assam and South India.

Use : Plant – in foetid nostrils; rhizome – febrifuge, tonic excitant, antirheumatic; base of stem- in swellings.

Biological activity : Hedychenone – anti-inflammatory, essential oil from rhizome – anthelmintic, mild tranquillizer. Cytotoxic diterpenes were found from the rhizomes of the flower.

Chemistry: Rhizomes gave furanoditerpene- hedychenone; essential oil contains α and β -pinene, limonene; Δ^3 -carene, β -phellandrene, p-cymene, 1:8 – cineole, β -caryophyllene and its oxide, linalyl and elemole in varying concentrations. The essential

oil also gave borneol, methyl salicylate, eugenol and methylantranilate.

2 Tagetes patula L

Family: Asteraceae

Common name: African marigold; Aztec

Vernacular name : Hindi: Genda

Habitat : Cultivated in Indian gardens

Use : Herb – antirheumatic, in cold, bronchitis; root- laxative; flower – depurative, febrifuge, astringent, carminative, in diseases of eyes bleeding piles, unhealthy ulcers, fits, scabies belching liver complaints teeth troubles; leaf- vermifuge, diuretic, in kidney troubles, muscular pain, erache, ophthalmia, applied to boils, carbuncles. Alpha T thiophene compounds isolated from *Tagetes patula* exhibited antifungal properties towards 5 dermatophytes [Romagnoli C, 1994]. Essential oil from this flower was evaluated for antifungal properties [Sagar V,2003]

Chemisty: Flowers gave lutein esters, dipalmitate, dimyristate and monomyristate. Fresh petals gave hydroxyflavonols Quercetagetin

and tagetiin; plant yields as essential oil with strong, sweet and lasting odour reminiscent of davana oil. The major components of oil are limonene, ocimene, linalyl acetate, linalool, tagetone and nonyl aldehyde.

3 *Polianthes tuberosa* L

Family : Agavaceae

Vernacular name : Hindi- Gulcheri B and S : Rajnigandha

Habitat : Native of Mexico or the Andes of South America; cultivated on large scale in Bangalore, Tumkur and Mysore district of Karnataka and as ornamentals in gardens in Dehra Dun.

Use : Bulb – antigonorrhoeic, in red pimples of infants; flower – diuretic, emetic. The compounds obtained from the aerial part of the plant were evaluated for their cytotoxic activity on leukemia cells [Khan K, 2003].

Chemistry: Leaves gave flavanoids - kaempferol and its 3-O-xyloside and 3,4-O-dixyloside; whole plant gave saponins - hecogenin and 9,11 – dehydrohecogenin; saponins – hecogenin –3-O-glucosyloside and 9,11- dehydrohecogenin- 3-O-

glucoside; absolute gave γ - lactones-(Z)- 5 –octen-4-olide (Z)-5-decen-4-olide, (Z)-6- nonen –4-olide,(Z)-6-dodecen –4-olide,(Z,Z)-6,9-octecadien-4-olide and tuberolide.

4 Michelia champaca L.

Family : Magnoliaceae

Vernacular name: Marathi- Kud champa, Pivala champa ; Hindi- champak

Habitat: Eastern Himalaya, lower hills of Assam and hills of South India up to 1000 m; cultivated in various parts of India.

Use: Bark- expectorant, astringent, abortifacient; root- contraceptive, purgative, emmenagogue, in abscesses; flower and fruit-antispasmodic, tonic, stomachic, antigonorrhoeic, carminative, in dyspepsia, nausea, vertigo; fruit and seed- for heal cracks in feet; oil from flower- antirheumatic, anticephalagic, in ophthalmia, gout; leaf- in colic; bark, flower and fruit-stimulant, diuretic, febrifuge

Biological Activity - Stem bark – hypoglycaemic, CVS active. The methanol extract of leaves stem seeds and rootbarks heartwoods of the plant and the obtained fractions exhibited a broad spectrum

of antibacterial activity [Khan M, 2002]. The essential oil from leaves showed significant broad spectrum antimicrobial activity against multiple drug resistant bacteria and fungi isolated from clinical specimen [Shind V,2003].

Chemistry –Stem bark and roots yielded in alkaloid – lirodenine (Micheline B) Roots bark yielded sesquiterpene lactonescostnelide, parthenolide, dihydroparthenolide and micheliolide; leaves gave polyisoprenolic and β -sitosteroland its glucoside and linelenine essential oil from the fruit rind gave phenyl ethyl alcohol ,pinocamphone, phenyl ethyl acetate, 1,8-cineole, linalool, pinocampheol, α -phellandrene and ester pinocampheol, that from flowers gave sesquiterpene hydrocarbon, champacene, the leaves gave linallol, linalylacetate, methylheptenone and geraniol. Seeds gave fatty acids-myristic, palmitic, hexadecadiene, stearic, oleic, linoleic, arachidic, and elcosenta and hexadecanoic acids.

5 *Jasminum sambac*(L) Ait

Family - Oleaceae

Vernacular name- Hindi- Mogra

Habitat- Throughout India

Use- Plant – in insanity, mouth affections, emmenagogue and for nasal diseases.

Biological Activity- Aerial part – CVS and CNS active, in vitiated conditions of pitta, in Ayurvedic practice.

Chemistry – Leaves contain jasminin, quer-iso-quercitrin, rutin, quercitrin-3-dirhamnoglycoside, kaempferol-3-rhamnoglycoside, mannitol, α -amyrin, β -sitosterol, and an iridoidglycoside named sambacin. The absolute contains several pyridine and nicotinate derivatives including methyl –5-ethyl-4-methyl nicotinate as a major one.

FLOWERS SELECTED FOR THE STUDY



Michelia champaca L.



Jasminum sambac(L) Ait

FLOWERS SELECTED FOR THE STUDY



Hedychium coronarium Koen.

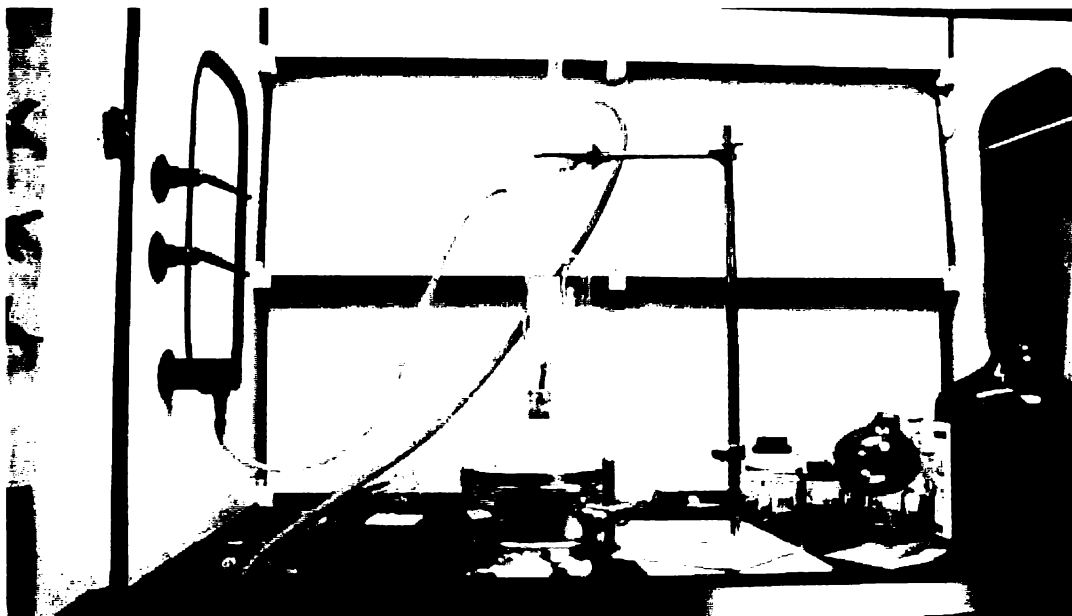


Tagetes patula L

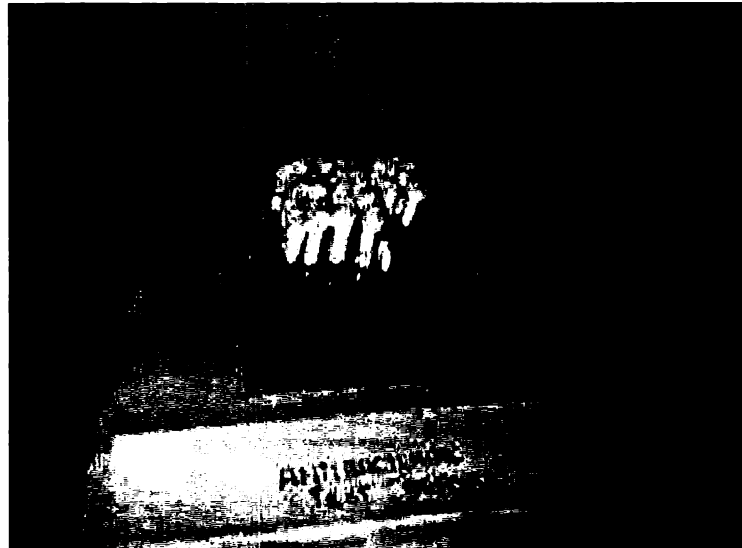
FLOWERS SELECTED FOR THE STUDY



Polianthes tuberosa L



EXTRACTION BY SOXHLET APPARATUS



ANTIBACTERIAL TEST BY TUBE DILUTION METHOD



COLUMN CHROMATOGRAPHY

Chapter 5
Results
and
Discussion

CHAPTER FIVE : RESULTS AND DISCUSSION

The antibacterial activity of five flower extracts was evaluated against different bacterial species (3 Gram - positive and 3 Gram - negative) as they possess one or the other biological activity. Some of the flowers were not tested for the evaluation of antibacterial activity. It was, therefore, of interest to determine if the crude flower extracts would show any promising antibacterial activity. Different extracts were, therefore, prepared using method Harborne and Davis etal (1950) and then further purified using silica gel columns. All these crude extracts as well as purified fractions were further investigated for their possible active chemical constituents.

For the preparation of crude extracts, flower powder was extracted sequentially with solvents of increasing polarity viz petroleum ether (60-80°C) (direct), methanol (in succession), acetone (direct) and distilled water. The yields of different extracts obtained are reported in table 2.

All crude flower extracts were screened for their antibacterial activity using broth dilution method. All the flowers except *Michelia champaca* L. did not show any significant antibacterial activity even at the highest concentration tested and did not give promising lead for further work.

It was observed that the petroleum ether extract of the flowers of *Michelia champaca* L. was active at 192 µg/ml concentration for S.aureus standard, 131 µg/ml for S.aureus clinical isolate and 310 µg/ml for S.pyogenes clinical isolate. The acetone extract of the plant was active at 150 µg/ml concentration for S.aureus standard, 160 µg/ml for S.aureus clinical isolate and 300 µg/ml for S.pyogenes clinical isolate. The extracts were active against Gram-positive organisms but did not exhibit activity against E.coli standard and clinical strain as well as S.typhi standard strain, Gram-negative organisms under study. The methanol extract though yield being considerably higher and aqueous extract did not exhibit any antibacterial activity even when tested at 500 µg/ml concentration, and hence could not be used to pursue further work. The methanol extracts of leaves, seeds, stem and root barks, stem and root heartwoods of *Michelia champaca* L. and their eluted fractions exhibited a broad spectrum of antibacterial activity. This is

contrary to the results of the plant got. This is because of two reasons change in protocol of extraction as well as different part of the plant is under consideration. The present study of extraction was carried out sequentially that is direct solvents followed by in successive solvents (less polar solvent followed by solvent with increase in polarity). Starting with less polar solvent petroleum ether the active principle responsible for inhibition is extracted in the solvent. However, treatment with more polar solvent there is probably no active principle left in the residue after extraction with alcohol. Whereas extracts of *Michelia champaca* L. flowers obtained from acetone yielded active material. The extracts obtained from distilled water did not yield any active material.

Since acetone and petroleum ether extracts of flowers of *Michelia champaca* L. exhibited antibacterial activity only these two were subjected to purification. The petroleum ether and acetone extracts of *Michelia champaca* L. were subjected to thin layer chromatography using various solvent systems in order to detect and isolate the constituents that can be obtained from each crude extract and to establish a suitable solvent system for purification of crude extracts on a silica gel column (fig of table). Profiles of the best system were petroleum ether and methanol in ratio 70:30

respectively which gave maximum separation and 3 bands hence could be visualized.

The solvent systems used for column were petroleum ether (60-80°C), chloroform and ethanol. 2 g of each crude extract was subjected to fractionation on the silica gel column. The adsorption chromatography was used in which elutes according to their color were separated. Three bands yellow, orange and brown from three different respective solvents were eluted and then were tested for their antibacterial activity of the fractions.

After chromatographic fractionation of petroleum ether and acetone extract of *Michelia champaca* L. and evaluating all elutes for antibacterial activity by broth dilution method, it was observed that petroleum ether fraction of petroleum ether extract and chloroform elute of acetone crude extract of the flowers showed prominent activity. Remaining elutes did not show any significant results. The yields of fractions were also obtained. The petroleum ether fraction of petroleum ether extract exhibited antibacterial activity at 101 µg/ml concentration for S.aureus standard, 110 µg/ml for S.aureus clinical isolate and 209 µg/ml for S.pyogenes clinical isolate. The chloroform fraction of crude

acetone extract exhibited antibacterial activity at concentration 80 µg/ml concentration for S.aureus standard, 100 µg/ml for S.aureus clinical isolate and 220 µg/ml for S.pyogenes clinical isolate against these Gram-positive bacterial species.

These fractions were also subjected to thin layer chromatography to determine the most efficient solvent system to achieve further separation, where 3 bands (sub fractions) were obtained using petroleum ether and methanol in ratio 70:30. An increase in antibacterial activity obtained through extraction and fractionation of the plant extracts was observed in the above experiments.

Attempts were made to detect functional groups or chemical nature of active components in the extracts and their fractions. These chemical constituents either individually or in combination have the ability to exert inhibitory action on bacteria. Different chemical groups present in the active extracts and fractions are shown in table. It was found that alkaloids appear to be common chemical moieties exhibiting antibacterial activity, although the role of glycosides and organic acids present in the

extract is not clear, these may also contribute to the observed activity.

There is no synergistic activity found between the synthetic drugs and the active plant as well as between the acetone extract of *Michelia champaca* L. and the petroleum ether extract of the flowers. They both are indifferent that is they both do not have an increasing activity in combination.

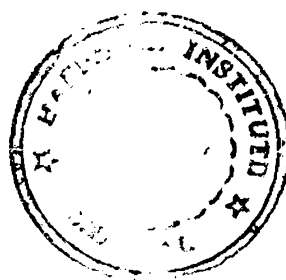


TABLE 1—

BOTANICAL INFORMATION ON FLOWERS USED IN THE STUDY –

Sr no	Plant (Botanical name)	Vernacular name	Family
1	<i>Hedychium coronarium</i> Koen.	Butterfly ginger or garland flower	<i>Zingiberaceae</i>
2	<i>Polianthes tuberosa</i> L.	Tuberose or Rajnigandha	<i>Agavaceae</i>
3	<i>Michelia champaca</i> L.	Champak or Golden champa	<i>Magnoliaceae</i>
4	<i>Tagetes patula</i> L.	French Marigold	<i>Asteraceae</i> / <i>Compositae</i>
5	<i>Jasminum sambac</i> L (Ait)	Arabian Jasmine or Moghra	<i>Oleaceae</i>

**TABLE 2—
DETERMINATION OF YIELDS OF VARIOUS FLOWER EXTRACTS**

Powdered flower (500 g)

+

(250 ml x10) of respective solvent

↓

Soxhlet extraction

% Yield

Sr.No.	Plant	Petroleum ether extract(60- 80°C) Direct	Methanol extract (in succ.)	Acetone Extract (Direct)	Aqueus Extract (Hot decoction method)
1	<i>Hedychium coronarium</i>	2.98 %	24.23%	12.08%	2.7%
2	<i>Jasminum sambac</i>	2.41%	21.25%	5.96%	1.45%
3	<i>Michelia champaca</i>	6.50%	37.80%	22.85%	2.4%
4	<i>Polianthes tuberosa</i>	3.89%	64.57%	5.65%	3.4%
5	<i>Tagetes patula</i>	5.90%	25.88%	10.31%	2.4%

TABLE 3 –
DETERMINATION OF ANTIBACTERIAL ACTIVITY OF SELECTED FLOWERS –
A) NAME OF THE FLOWER – *Hedychium coronarium* Koen. Family: *Zingiberaceae*

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S aureus</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>Ecoli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

B) NAME OF THE FLOWER– *Jasminum sambac* L Family : *Oleaceae*

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S aureus</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>Ecoli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : growth (no antibacterial activity)
- : no growth (antibacterial activity)
C : solvent control

C) NAME OF THE FLOWER – *Polianthes tuberosa* L Family: *Agavaceae*

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S aureus</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

D) NAME OF THE FLOWER—*Tagetes patula* L Family- *Compositae*

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S aureus</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : growth (no antibacterial activity)

- : no growth (antibacterial activity)

C : solvent control

E)_NAME OF THE FLOWER – *Michelia champaca L* Family- *Magnoliaceae*

Solvents	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
Test Organisms	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+
<u>S aureus</u> clinical	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
<u>Ecoli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : growth (no antibacterial activity)

- : no growth (antibacterial activity)

C : solvent control

NAME OF THE FLOWER : *Michelia champaca* L. Family- *Magnoliaceae*

Solvents	Petroleum ether (60-80 C) µg/ml				Acetone extract (direct) µg/ml			
Test Organisms	130	150	190	200	130	150	190	200
<u>S aureus</u> standard	+	+	+	-	+	-	-	-
<u>S aureus</u> clinical	+	-	-	-	+	+	-	-

Solvents	Petroleum ether (60-80 C) µg/ml			Acetone extract (direct) µg/ml		
Test Organisms	300	400	500	300	400	500
<u>S pyogenes</u> clinical	+	-	-	-	-	-

Solvents	Petroleum ether (60-80 C) µg/ml	Acetone extract (direct) µg/ml
Test Organisms		
<u>S aureus</u> standard	192	150
<u>S aureus</u> clinical	131	160
<u>S pyogenes</u> clinical	310	300

**TABLE 4 --
DETERMINATION OF YIELD OF THE FRACTIONS FROM FLOWER
EXTRACTS OF *Michelia champaca* L. Family- *Magnoliaceae* --**

Solvents	Petroleum ether extract (direct) (60-80 °C)		Acetone extract (direct)	
Length of the column	37.5 cm		37.2 cm	
Diameter of the column	3.1cm		3.1cm	
Silica gel	127.4g		125.3g	
Extract Loaded	2.1g		2.5g	
[in succession]	g	Yield %	G	Yield %
Petroleum ether Elution	0.07	3.33	0.12	4.76
Chloroform elution	0.46	21.9	0.63	25.04
Ethanol Elution	1.51	71.9	1.57	62.60
Total Recovery	97.1			92.4

TABLE 5 --
MINIMUM INHIBITORY CONCENTRATIONS OF THE FRACTIONS of
***Michelia champaca* L. Flower Family- *Magnoliaceae* --**

Solvents	Petroleum Ether Extract (direct) (60-80 °C)			Acetone extract (direct)		
	Petroleum ether fraction µg/ml	Chloroform Fraction µg/ml	Ethanol Fraction µg/ml	Petroleum ether fraction µg/ml	Chloroform Fraction µg/ml	Ethanol Fraction µg/ml
<u>S aureus</u> standard	101	111	131	100	80	97
<u>S aureus</u> clinical	110	120	120	120	100	101
<u>S</u> <u>pyogenes</u> Clinical	209	210	220	240	220	222

TABLE 6 --
PHYTOCHEMICAL SCREENING OF THE EXTRACTS OF
Michelia champaca L. Flower Family- *Magnoliaceae* –

	Petroleum Ether Extract	Petroleum ether Fraction
Phenols	-	-
Terpenoids	-	-
Organic Acids	+	+
Alkaloids	+	+
Glycosides	-	-
Flavonoids	-	-
	Acetone Extract	Chloroform Fraction
Phenols	-	-
Terpenoids	-	-
Organic Acids	+	+
Alkaloids	+	+
Glycosides	+	+
Flavonoids	-	-

Note:

+ Chemical moiety present

- Chemical moiety absent

Chapter 6

Conclusions

CHAPTER SIX :- CONCLUSIONS

Our results therefore offer a scientific basis for the traditional use of both petroleum ether and acetone extracts of the flowers of *Michelia champaca* L., which possess remarkable antibacterial activity against Gram-positive organisms. However *in vivo* studies on this medicinal plant are necessary and should seek to determine toxicity of the active constituents, their side effects, serum-attainable levels, pharmacokinetic properties and diffusion in different body sites. The antimicrobial activities could be enhanced if the active components are purified and adequate dosage determined for proper administration. This may go a long way in curbing administration of inappropriate concentration; a common practice among many traditional medical practitioners. This study represents an exploratory report on antibacterial activity of the crude extracts of the flowers of *Michelia champaca* L., an Indian medicinal plant. The identification of this potential antibacterial plant from local flora will definitely open the field for further research on this plant.

Chapter 7

Summary

CHAPTER SEVEN: - SUMMARY

The realization that many infectious pathogenic organisms have fast developing resistance(s) against the prevailing drugs has necessitated a search for new sources of antibacterial compounds. In the course of their life cycle, plants encounter infection by a variety of viruses, bacteria, fungi and parasites specific to them.

They are expected to synthesize a variety of secondary metabolites capable of providing them protection against the infectious agent(s). Antibacterial activities have been detected in chemicals extracted from root, stem, leaves, flowers, fruits and seeds of diverse species of plants.

The petals of the flowers are variously shaped coloured and aromatic to attract pollinating insects. They enclose the androecium and gynoecium and facilitate pollination. The petal tissues may possess antibacterial activity as a natural protection system for reproduction and further perpetuation through seed formation. Here the flowers of some plants have been studied for differential activity potential and were found to be active against a spectrum of the

Gram-positive and Gram-negative bacteria with differences in activity profiles.

Most of the indigenous plants studied in the present investigation is perennially available in large quantities. The cost involved in the preparation of these extracts is very minimal. Moreover, solvents used for extraction could be recovered and recycled. It was observed that only one fifth of the solvent used for extraction is lost during the concentration of the extract. The extract of the natural products and their use in control operation appear to be more economical and less polluting to the environment.

The present study has been carried out to determine the antibacterial activity of five indigenous plants against the Gram-positive as well as Gram-negative organisms. Petroleum ether (60-80 °C, methanol (in succession), aqueous and acetone extracts of the plant material were prepared and were screened for possible antibacterial activity. Among the crude extracts screened petroleum ether and acetone extracts of *Michelia champaca* L. showed possible antibacterial activity against Gram - positive organisms. It

was observed that the petroleum ether extract of the flowers of *Michelia champaca* L. was active at 192 µg/ml concentration for S.aureus standard, 131 µg/ml for S.aureus clinical isolate and 310 µg/ml for S.pyogenes clinical isolate. The acetone extract of the plant was active at 150 µg/ml concentration for S.aureus standard, 160 µg/ml for S.aureus clinical isolate and 300 µg/ml for S.pyogenes clinical isolate.

The petroleum ether extract and acetone extract of *Michelia champaca* L. were further subjected to fractionation. After chromatographic fractionation of pet ether and acetone extract it was observed that petroleum ether fraction of petroleum ether extract and chloroform elute of acetone crude extract showed prominent activity. Remaining elutes didn't show any significant results. The petroleum ether fraction of petroleum ether extract exhibited antibacterial activity at 101 µg/ml concentration for S.aureus standard, 110 µg/ml for S.aureus clinical isolate and 209 µg/ml for S.pyogenes clinical isolate. The chloroform fraction of crude acetone extract exhibited antibacterial activity at concentration 80 µg/ml concentration for S.aureus standard, 100 µg/ml for S.aureus clinical isolate and 220 µg/ml for S.pyogenes clinical isolate against these Gram-positive bacterial species.

Studies on chemical characterization of the active crude extracts revealed alkaloids appear to be common chemical moieties exhibiting antibacterial activity, although the role of glycosides and organic acids present in the extract is not clear, these may also contribute to the observed activity.

The identification of this potential antibacterial plant from local flora will definitely open the field for further research on this plant.

Chapter 8

References

Chapter eight : REFRANCES:-

- ◆ Pandit H A, (2003) "Studies on immunomodulatory activity of certain indigenous plants." Master of Science (Technology) U.I.C.T., Mumbai. Pp-1
- ◆ Stuart C A, (1911) "Quoted from Q Chinese Materia Medica Shanghai (Cited from Florey et al 1949 Antibiotics Vol I and II)
- ◆ Nadkarni A K ,(1976) " Indian Material Medica "Bomday Popular Prakashan Vol 1, XXXVII – XXXIX Pp- 961-964
- ◆ Fabricant D et al, (2001) " The value of plants used in Traditional Medicine for drug discovery" Environmental Health Perspectives Supplements Vol 109, NoS1. Pp-69-75
- ◆ Suffredini I B et al, (2004) " Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic forest." Brazilian Journal Med. Biol. Vol 37(3) Pp-379-384
- ◆ Khanum A et al, (2005) " Role of Biotechnology in Medicinal and Aromatic plants " Hyderabad Jilkaaz Pub VII Vol-XII Pp-377

- ♦ Iwu M W et al, (1999) “ New antimicrobials of plant origin”
Perspectives on new crops and new uses ASHS Press Alexandria
VA J JANIK (ed) Pp-457-462

- ♦ Handa S S, (1993) “ Emerging frontiers in the area of
hepatoprotective antiulcer and adaptogenic herbal drugs.” A
National Seminar Organised by Indian Society of Pharmacognosy
and JSS College of Pharmacy Ootacamund.

- ♦ Sci. Comm., (1995) Silymarin clinical update,1.

- ♦ Fleming A,(1932) Proc R Soc Med” Vol-26 Pp-71

- ♦ Chain E et al, (1940) Lancet Vol-2 Pp-226

- ♦ Abrahm E.P. et al, (1941) Lancet Vol-2 Pp-177

- ♦ Osaborne E M (1943) Brit J Exptl Patho Vol-24 Pp- 227

- ♦ Rao P R et al, (1946) Nature Vol-158 Pp- 746

- ♦ Nai Chu Chang et al (1950) Inter Congr Microbiol Abstracts of papers 5 th Congress ,Rio de Janeiro (Quoted from Chem Abst 1954 48 :1478) Pp- 47-48
- ♦ Bishop C J et al, (1951) Conad J Bot Vol-29 Pp-260.
- ♦ Strik WA, Schwall A N, Ligh M E (2003)South African Journal of Botany, vol.69 (4) "Potential medicinal values of some South African seaweed", Pp – 462-468.
- ♦ Bren Linda ;(2002) FDA Consumer Magazine; "Battle Of The Bugs :- Fighting Antibiotic Resistance" Report
- ♦ Institute of Science in society report (25 June 2001) "Radical solutions needed for antibiotic resistance."
www.i-sis.org.uk/antibiotic_resistance.php
- ♦ CNN Report, (2000) " Improper drug use to blame. Antibiotic resistance a growing threat, WHO reports."
www.cnn.com/2000/Health/06/12/antibiotic.resistance/-49k

- ◆ Thatte U et al, (2002) " A Drug is born" Popular Science National book trust India Pp-2,23
- ◆ Harding A, (2002) " Chinese herb may be tough to beat antibiotic" Salt lake city Reuters Health Cross road Institute
- ◆ Snow G A et al, (1998) "Biochemistry of antimicrobial action " 5 th edition Pp-2
- ◆ Joshi B R, (1985) " Study of an ayurvedic preparation against dermatophytes" PhD Microbiology HITRT Mumbai
- ◆ Arber A, (1938) " History of Botany 1470-1670" Catab U Press (Quoted from Florey et al 1948) Antibiotics Vol 1 Oxford University Press London Pp-1-2
- ◆ Sereitii MRAI et al, (1999) "Pharmacology of Rosemary (Rosemarinus officinalis) and its therapeutic potential" Indian Journal Of Experimental Biology Vol-37 Pp-124

- ◆ Desai S T, (2002) " Potency of larvicidal properties of plant extract against mosquito larvae under laboratory conditions" MSc Applied Biology HITRT Mumbai Pp-2
- ◆ Magama S et al, (2003) "Antimicrobial properties of extracts from *Euclea crispa* (Ebenaceae) towards human pathogens" South African Journal Of Botany Vol 69(2) Pp-193-196
- ◆ Biodiversity Profile Of India.
www.wcmc.org.uk/igcmc/main.html
- ◆ No.1 Global Herbal Exporters
www.unaniherbalist.com/
- ◆ Davis, H Patridge M.W., Robinson A.1 (1950), Bentley's by Balliere, Tindall and Co (London) 5th Ed. Pp 248.
- ◆ Vahanwala S J etal (2000) "Antimicrobial activity of *Couropita Guianensis Aubl*" Indian Drugs 377, ' Pp-343-345.
- ◆ Indian Herbal Pharmacopoeia, (1998)" A Joint Publication Of RRL Jammu Tawi And IDMA Mumbai Vol-1 Pp-180

- ◆ Colin, C.H. and Lyne (1965), Cruikshank. "Antibiotics and antibacterial substances" , P.M.(Eds) In Microbiological methods, Pp 414-428.
- ◆ Deshmukh S, (1993) " Synthesis and study of Quinoxaline / Alloxazine derivatives as antimalarial / antibacterial agents" Ph D HITRT Mumbai Pp-203-204
- ◆ Nostro A et al, (2000) "Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity" Letters in Applied Microbiology Volume 30 Issue 5 Pp- 379
- ◆ Bobbit J M, (1963) TLC Reinhold Book Corp. Pp-67
- ◆ Lattu R G, (1994) "Studies on antibacterial activity of extracts of Anantmool (Root)" M.Sc., HITRT Mumbai Pp– 81.
- ◆ Lederer E et al, (1957) Chromatography " A review of principles and application" Elsevier Publishing Comp, second edition

- ♦ Rane J et al, (2001) "Chemical examination of flowers of *Couropita guainensis* Aubl." Indian Journal Of Pharmaceutical Sciences Pp- 72-73

- ♦ Frank J Welcher, (1963) PH. D. Editor, Standard Methods Of Chemical Analysis 6 th Edition Vol-2, D Van Nostrand Company London Pp-216-219.

- ♦ Horborne, J.B. (1973)/(1984) Phytochemical methods-"A guide to modern tech of plant analysis", Chapman and hall publishers, 2nd Ed. Pp- 1-29

- ♦ Lorian V et al, (1974) "Technique for determining the bactericidal effect of drug combination" Antimicrobial Agents Chemotherapy Vol- 5(6) Pp-630-633

- ♦ Hussain Akhtar et al; (1992) Dictionary of Indian medicinal plants Central Institute of medicinal and aromatic plants, Lucknow. Pp-

- ♦ Dr. Nadkarni .K.M. Indian Materia medica Vol 1 Bombay Popular Pharmacy Pp- 704,795,997 and 1191

- ◆ Romagnoli C et al, (1994) " Antifungal effects of alphaterthienyl from *Tagetes patula* on 5 dermatophytes" *Phytotherapy research* Vol-8(6) Pp- 332-336
- ◆ Sagar V et al, (2002) "Antifungal Activity of *Tagetes patula* essential oil" *Indian perfumer* Vol-46(3) Pp-269-272
- ◆ Khan K et al, (2003) " Isolation and structure elucidation of 3 glycosides of along chain alcohol from *Polianthes tuberosa* Linn " *Nat Prod Lett.* Vol- 16(4) Pp-283-290.
- ◆ Khan MR, (2002) "Antimicrobial activity of *Michelia champaca*." *Fitoterapia.* Vol- 73(7-8) Pp-744-748
- ◆ Shind V M et al, (2003) " Antibacterial and antifungal activity of essential oil and decoction of *Michelia champaca* leaves" *MAPA* No2 2003-02-1014
- ◆ Rios J L et al, (1988) *Journal of Ethanopharmacol* Vol-23 Pp-127-129
- ◆

- ◆ Mitscher L A et al, (1972) Antimicrobial agents from higher plants
IV. Z anthoxylum elephantiasis Isolation and identification of
canthin-6-one Lloydia Vol-35 Pp-177-180

- ◆ Taninguchi M et al, (1993) Ethnobotanical drugs discovered
based on medicine men's trials in the African Savanna : Screening
Of East African plants for antimicrobial activity II J. Nat. Prod. Vol
56 Pp-1539-1546.

- ◆ Grien E et al, (1994) " Antibacterial activity of plant extracts used
externally in traditional medicine. J. Ethanopharm. Vol-44 Pp-35-
40.

- ◆ Chhabra S C et al, (1991) "Antibacterial activity of some
Tanzanian plants used in traditional medicine." Fitoterapia LXII pp-
499-501

- ◆ Ampel N M et al, (1994) " Comparison of tube dilution and
microtitre methods for the detection of antibiotic tolerance in
strains of Staphylococcus aureus" J Antimicrob. Chemother. Vol-
13 Pp-417-421.

- ◆ James P A et al, (1990) " Comparison of four methods for determination of MIC and MBC of penicillin for viridans Streptococci and implication for penicillin tolerance J Antimicrob Chemother Vol-25 Pp-209-216

- ◆ Okemo P O et al, (1998) "Antibacterial activity of plant extracts – a comparison of agar dilution and microtitre broth dilution methods " Discov. Innov., Vol-10 (1/2) Pp- 106-110.

- ◆ Chakraborty et al, (1998) " Preliminary phytochemical and antimicrobial studies of *Plumbago zeylanica* Linn " Indian Journal Of Natural Products Vol- 13(2) Pp-3-6

- ◆ Gehlot D et al, (1998) " Antimicrobial activity of various plant part extracts of *Aerva persica*." Advances in plant Sciences Vol- 11(1) Pp-109-111

- ◆ Eloff J N et al, (2002) " Antibacterial activity of *Marula* (*Sclerocarya birrea*) (A. rich) Hochst subsp *caffra* (Sond Kokwarp) (Anacardiaceae)bark and leaves." Journal of Ethanopharmacology Vol- 76(3) Pp-305-308

- ♦ Perez G S et al, (2001) " Antimicrobial study of bark from 5 tree species." Phototherapy Research Vol-15 (4) Pp-356-359
- ♦ Alyelaagbe O O et al, (2001) "Antibacterial activity of *Jatropha multifida* roots." Fitoterapia Vol-72(5) Pp-544-546
- ♦ Ofokansi K C et al, (2003) "Antibacterial activity of the leaf extract of *Ocimum gratissimum* (Fam. Labiatae)" J. of Bio. Res. and Biotech. Vol-1(1) Pp-35-42
- ♦ Ahmad I et al, (2003) "Broad spectrum antibacterial and antifungal activities and potency of crude alcoholic extract and fraction of *Delonix regia* fowers ." 2 nd World Congress on Biotechnological Developments of Herbal Medicine Pp-74
- ♦ Kianbakht S et al, (2003) " Evaluation of antibacterial activity of *Tribulus terrestris* L.growing in Iran" Iranian Journal of Pharmacology and Therapeutics Vol-2 Pp-22-24
- ♦ Naqvi B S et al, (1994) "Studies of Antibacterial Activity of Ethanolic Extracts from *Nerium Indicum* and *Habiscus Rosasinensis*." Journal of Islamic Academy of Sciences Vol-7 No-3
- ♦ Cavallito C J Bailey J H (1944) J. Am. Chem. Soc. 66:1950

Chapter 9

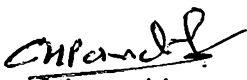
Synopsis

A Synopsis
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The Degree Of Master Of Science (Research) In Applied Biology

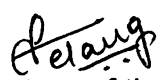
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STUDIES ON ANTIBACTERIAL ACTIVITY OF SOME SELECTED FLOWERS.

Ever since the antibiotics became widely available about 50 years ago, these have been hailed as miracle drugs – magic bullets, able to destroy disease-causing bacteria. But with each passing decade, antibiotic - resistant infectious diseases created a public health crisis worldwide. In industrialized nations, despite the progress made in understanding of microbiology and their control, incidences of epidemics caused by drug resistant microorganisms are on the increase and the emergence of diseases caused by drug resistant microbes is of public health concern [1]. It is one of the major problems faced by the world in public health sector all over the world this has been the subject of a series of high-level international conference 'The Microbial Threat' held in 1998.

Infectious diseases are responsible for one quarter of all deaths in the world, second only to cardiovascular diseases during the past twenty- five years. These have been associated with the new strains of bacteria and viruses that are highly resistant

to available drugs and antibiotics. Antibiotics have been reported to inhibit the Gram positive and Gram-negative organisms through varied mechanisms of actions. However, the development of bacterial resistance to presently available antibiotics has necessitated search for newer antibacterial drugs. Though antibiotic resistance is not a new phenomenon, resistance to new drugs is appearing with increasing rapidity, often within a year of its introduction. Few years after the introduction of penicillin, penicillin resistant infection caused by S. aureus emerged. At present S.aureus has also developed resistance even to methicillin (strongest arsenal of drugs) and vancomycin (a potent drug against resistant pathogens). E.coli presents with resistance against sulfonamides, ampicillin, chloramphenicol and cephalothin. S.pyogenes has been reported to be resistant to clindamycin and erythromycin. It means little or no returns to drug companies that manufacture these novel life saving antibiotics. Furthermore, drugs that are toxic to bacteria are often toxic to human beings and the side –effects pose a great problem during their clinical use. China began integrating traditional medicine in their national health care system in 1950's. Health officials trained in modern medicine sought compatibility between traditional and modern medicine by western science based approach emphasizing empirical research [2].

India has one of the oldest, richest and the most diverse cultural traditions associated with the use of medicinal plants. There are many species of indigenous plants, which are known for their antibacterial activity. After an extensive use of totally or partially synthesized molecules isolated from nature, there is a gradual global shift from the use of synthetic medicines to natural medicine. It is believed that India presently has a share of Rs.600 crores in the global herbal market and has a potential to increase it to Rs.3000 to Rs.4000 crores during the next decades. Keeping in mind all these forthcoming possibilities, things have been rapidly moving not only in India but also in other countries all over the world [3].

Plant products are fairly cheaper and are claimed to be safe. These, as compared to modern synthetic drugs, may also provide leads for developing new synthetic agents. Historically, plants have provided an important source of anti-infective therapeutic agents like quinine, emetine, and berberine and have remained highly effective moieties in fight against microbial infection. The use of plant preparations as foodstuffs, flavoring agents, dyes, insecticides, cardioactive, hypolipidaemic, antimicrobial and antitumour agents are some of the examples of immense chemical

diversity in plants. A variety of drugs exhibiting moderate to powerful antibacterial action have been grouped together and are now designated as non-antibiotics [4]. During the last decade, infectious diseases have been treated with plant extracts / isolates. Today these are still used extensively for primary health care in developing countries where traditional medicine plays an important role.

Plants are still the exclusive source of drugs for a majority of the world's population. Major pharmaceutical companies have shown renewed interest in higher plants like *Azadirachta indica*, *Nycanthes arbor tritis*, *Hibiscus rosasinensis* etc. as a source of possible novel active substances [5]. The World Health Organization (WHO) has estimated that almost 65% of the world's population have incorporated herbal remedies into primary health care [6]. India possesses a distinct identity due to its varied geographical conditions resulting in a great diversity of its natural ecosystem [7]. The Himalayas have given India a wealth of plant life, in the form of herbs, fruits & flowers with incredible properties, yielding to us some of the most precious active principles, with powerful healing and rejuvenating qualities. Their virtues have been extolled in our ancient Vedic texts and these have become a part of the Indian herbal traditions of Unani and Ayurveda [8].

India boasts of a rich heritage of medicinal plants and over centuries, a variety of medicinal plants have been used as "Ayurvedic medicines" to treat certain chronic ailments, that have been reported in the literature [9]. Antibacterial activities have been detected in chemical constituents extracted from roots, stem, leaves, flowers, fruits and seeds of diverse species of plants. Though several studies regarding drugs from the plants are recorded in the past, there is much more still left to be explored in the vast plant kingdom. Considering the wealth of floral and plant based resources available in India, it was envisaged to select and screen some of the indigenous plants for antibacterial properties.

Flowers of plant species may possibly possess antibacterial activity as a natural protection mechanism for the reproductive organs of the plants to help perpetuation of the species through seed formation. The present study has, therefore, been carried out to determine the antibacterial activity of flowers of five selected indigenous plants against both the Gram-positive and Gram-negative organisms under standard laboratory conditions.

RATIONALE:

India has the necessary infrastructure, expertise, trained manpower, heritage and natural resources to become a major player in global market for natural products for commercial and therapeutic utilities. However, in India rather limited investigations have been reported to achieve a suitable alternative antibacterial drug of plant origin. Hence, an attempt was made here to screen some of the plants for possible antibacterial activity.

AIMS AND OBJECTIVES: -

- Collection, botanical identification, drying and powdering the flowers of the plants under study.
- Extraction of powdered plant material using polar to non-polar solvents.
- Preparation of aqueous extracts of plant material.
- Evaluation of these extracts for their antibacterial activity.
- Fractional purification of the active extracts.
- Chemical characterizations of fractions— Functional group identification.

MATERIALS AND METHODS: -

♦ Preparation of Flower extracts: -

Powdered flower material (500g) was extracted in Soxhlet apparatus employing 2.5 L of solvent. The solvents used were Petroleum ether (60 – 80 ° C) and methanol (in succession) and direct extractions of flower powder with acetone. Extraction with distilled water was also carried out using conventional procedures [10].

A brief description of the plants studied is given below [11 and 12].

The plants used are very common in occurrence throughout India. These or the members of their families have been reported to possess a variety of biological activities /medicinal properties.

- Botanical name :- *Hedychium coronarium Koen.*

Family :- *Zingiberaceae*

Common name :- Ginger lily / Garland Flower.

Use :- This plant is used in foetid nostrils. Rhizomes are used as febrifuge, tonic , excitant, antirheumatic. Base of the stem is used for treatment of swollen joints.

Biological activity :- Hedychenone derived from the plant has been shown to possess anti-inflammatory activity. Essential oil from rhizome is antihelmintic, mild tranquilizer. Cytotoxic diterpenes were found from the rhizomes of the flower.

- Botanical name :- *Jasminum sambac* (L). Ait

Family :- *Oleaceae*

Common name :- Arabian Jasmine

Use :- This plant is used to treat insanity and mouth affections.

Roots are emmenagogue and used for nasal diseases

Biological Activity :- Constituents of aerial parts are CVS and CNS active, useful in vitiated conditions of pitta, in Ayurveda practice.

- Botanical name :- *Michelia champaca* (L)

Family :- *Magnoliaceae*

Common name :- Champak

Use :- Bark is used as astringent and expectorant. Roots are used as purgative and used in abscesses. Flowers and Fruits are antispasmodic, antigonorrhoeic used for treatment of nausea. Oil from flowers is antirheumatic and anticephalagic used in gout. Leaves are used in colic and bark, flowers, and fruits are used as stimulant, diuretic, and febrifuge.

Biological active : - Stem bark is hypoglycemic and also has CVS activity. The methanol extract of leaves, seeds, stem and rootbarks, stem and heart woods of the plant and the obtained fractions exhibited a broad spectrum of antibacterial activity.

- Botanical name :- *Polianthes tuberosa L*

Family :- *Agavaceae*

Common name :- Tuberose

Use :- Bulb is antigonorrhoeic and used in red pimples in pediatric medicines. Flowers have diuretic and emetic properties.

Biological activity:- The compounds obtained from aerial parts of the plant were evaluated for their cytotoxic activity on leukemia cells.

- Botanical name :- *Tagetes patula (L)*

Family :- *Asteraceae / Compositae*

Common name:- French marigold

Use:-The herb is aromatic, digestive and is used as a diuretic and sedative in indigestion and colic and for severe constipation, cough and dysentery.

♦ Bacterial cultures employed in the study: -

The following bacterial cultures were employed to study antibacterial activity of crude extracts and their fractions.

- 1 Staphylococcus aureus standard [NCTC 3750]
- 2 Staphylococcus aureus clinical isolate [pus sample]
- 3 Streptococcus pyogenes clinical isolate [pus sample]
- 4 Escherichia coli standard [NCTC10148]
- 5 Escherichia coli clinical isolate [water isolate]
- 6 Salmonella typhi standard [NCTC T786]

♦ Evaluation of flower extracts for their antibacterial activity: -

Agar dilution technique was employed to evaluate the antibacterial activity of the extracts. Test substance (10 mg) was dissolved in 0.1 ml Tween 80 and then diluted to 10 ml with DMSO. The solution was sterilized by autoclaving and was added aseptically to the medium (M-H broth) in such an amount as to give final concentrations of 500,200,100,40,20,10 µg/ml of the medium.

To each test tube 0.1 ml of standardized inoculums (24 hr old cultures of the test organism diluted to 10^{-2}) were added. After mixing the contents, the tubes were incubated at 37 °C and examined at the end of 48 hr. for the presence or absence of the

growth of the test organism. The lowest concentration of the test compound, which showed no visible growth, was taken as the minimum inhibitory concentration (MIC). The compounds showing inhibition up to the concentration of 500 µg/ml and below were considered to be active [13 &14]. Ampicillin and trimethoprim were taken as standard drugs.

♦ Fractionation of active extracts: -

Only those extracts, which showed promising activity, were subjected to fractionation using Thin layer chromatography (TLC) and column chromatography (silica gel 60-120 mesh) employing a standard method. The eluted fractions were collected to isolate and identify the active constituents [15]. The active fractions were subjected to chemical analysis for detection of alkaloids, glycosides, tannins, etc. as per routine phytochemical methods [16].

Observations:

Out of the five plants under investigation, the petroleum ether and acetone extracts of the flowers of *Michelia champaca* L. showed significant antibacterial activity. This led to the undertaking of chemical investigations on the plant to isolate the active constituent

responsible for antibacterial activity. Other plant extracts did not show any appreciable antibacterial activity.

Results and discussion:

The antibacterial activities of five plant extracts were tested or evaluated against different bacterial species (3 gram positive and 3 gram negative). Except *Michelia champaca* L. all other plants did not show any significant antibacterial activity even at the highest concentration tested and did not give promising leads for further work.

It was observed that the petroleum ether extract of the flowers of *Michelia champaca* L. was active at 192 µg/ml concentration for S.aureus standard, 131 µg/ml for S.aureus clinical isolate and 310 µg/ml for S.pyogenes clinical isolate. The acetone extract of the plant was active at 150 µg/ml concentration for S.aureus standard, 160 µg/ml for S.aureus clinical isolate and 300 µg/ml for S.pyogenes clinical isolate. The extracts were active against Gram-positive organisms but did not exhibit activity against E.coli standard and clinical strain as well as S.typhi standard strain, Gram-negative organisms under study. The methanol and aqueous extract did not exhibit any antibacterial activity even when tested at

500 µg/ml concentration, and hence could not be used to pursue further work.

The petroleum ether and acetone extracts of *Michelia champaca* L. were subjected to thin layer chromatography using various solvent systems in order to detect the constituents. Profiles of the best system were petroleum ether and methanol in ratio 70:30 respectively which gave maximum separation and 3 bands hence could be visualized.

After chromatographic fractionation of petroleum ether and acetone extract of *Michelia champaca* L. and evaluating various elutes for their activity, it was observed that petroleum ether fraction of petroleum ether extract and chloroform elute of acetone crude extract showed prominent activity. Remaining elutes did not show any significant results. The yields of fractions were also obtained. Each of these fractions was again tested for antibacterial activity. The petroleum ether fraction of petroleum ether extract exhibited antibacterial activity at 101 µg/ml concentration for S.aureus standard, 110 µg/ml for S.aureus clinical isolate and 209 µg/ml for S.pyogenes clinical isolate. The chloroform fraction of crude acetone extract exhibited antibacterial activity at concentration 80 µg/ml concentration for S.aureus standard, 100

µg/ml for S.aureus clinical isolate and 220 µg/ml for S.pyogenes clinical isolate against these Gram-positive bacterial species.

These fractions were also subjected to thin layer chromatography to determine the most efficient solvent system to achieve further separation, where 3 bands (sub fractions) were obtained using petroleum ether and methanol in ratio 70:30. An increase in antibacterial activity obtained through extraction and fractionation of the plant extracts was observed in the above experiments.

Attempts were made to detect functional group or chemical nature of active components in the extracts and their fractions. It was found that alkaloids appear to be common chemical moieties exhibiting antibacterial activity, although the role of glycosides and organic acids present in the extract is not clear, these may also contribute to the observed activity.

The findings of the present investigations reveal that the flowers of *Michelia champaca* possess remarkable antibacterial activity against Gram-positive organisms. The identification of this potential antibacterial plant from local flora will definitely open the field for further research on this plant.

TABLE 1—
FLOWERS USED IN THE STUDY --
Part Of The Plant: Flower

Sr no	Plant (Botanical name)	Vernacular name	Family
1	<i>Hedychium coronarium</i> Koen.	Butterfly ginger or garland flower	<i>Zingiberaceae</i>
2	<i>Polianthes tuberosa</i> L.	Tuberose or Rajnigandha	<i>Agavaceae</i>
3	<i>Michelia champaca</i> L.	Champak or Golden champa	<i>Magnoliaceae</i>
4	<i>Tagetes patula</i> L.	French Marigold	<i>Asteraceae</i> / <i>Compositae</i>
5	<i>Jasminum sambac</i> L (Ait)	Arabian Jasmine or Moghra	<i>Oleaceae</i>

TABLE 2—
DETERMINATION OF YIELDS OF VARIOUS FLOWER EXTRACTS

Powdered flower (500 g)

+

2.5 L of respective solvent

↓

Soxhlet extraction

% Yield

Sr.No.	Plant	Petroleum ether extract(60- 80°C) Direct	Methanol extract (in succession)	Acetone Extract (Direct)	Aqueous Extract (Hot decoction method)
1	<i>Hedychium coronarium</i>	2.98 %	24.23%	12.08%	2.7%
2	<i>Jasminum sambac</i>	2.41%	21.25%	5.96%	1.45%
3	<i>Michelia champaca</i>	6.50%	37.80%	22.85%	2.4%
4	<i>Polianthes tuberosa</i>	3.89%	64.57%	5.65%	3.4%
5	<i>Tagetes patula</i>	5.90%	25.88%	10.31%	2.4%

TABLE 3 –
DETERMINATION OF ANTIBACTERIAL ACTIVITY OF SELECTED FLOWERS –
A) NAME OF THE FLOWER – *Hedychium coronarium* Koen. Family: *Zingiberaceae*

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S aureus</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>Ecoli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

B) NAME OF THE FLOWER-- *Jasminum sambac* L Family : *Oleaceae*

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S aureus</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>Ecoli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : growth (no antibacterial activity)
- : no growth (antibacterial activity)
C : solvent control

C) NAME OF THE FLOWER – *Polianthes tuberosa* L Family: *Agavaceae*

Solvents	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
Test Organisms	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S aureus</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

D) NAME OF THE FLOWER—*Tagetes patula* L Family- *Compositae*

Solvents	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
Test Organisms	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S aureus</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : growth (no antibacterial activity)

- : no growth (antibacterial activity)

C : solvent control

E) NAME OF THE FLOWER – *Michelia champaca* L Family- *Magnoliaceae*

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml				Methanol extract (in succession) µg/ml				Acetone Extract (Direct) µg/ml				Aqueous Extract µg/ml				C
	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	
<u>S aureus</u> standard	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+
<u>S aureus</u> clinical	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+
<u>S pyogenes</u> clinical	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
<u>E coli</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>E coli</u> Clinical	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<u>S typhi</u> Standard	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

NAME OF THE FLOWER : *Michelia champaca* L Family- *Magnoliaceae*

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml				Acetone extract (direct) µg/ml			
	130	150	190	200	130	150	190	200
<u>S aureus</u> standard	+	+	+	-	+	-	-	-
<u>S aureus</u> clinical	+	-	-	-	+	+	-	-

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml			Acetone extract (direct) µg/ml		
	300	400	500	300	400	500
<u>S pyogenes</u> clinical	+	-	-	-	-	-

Solvents Test Organisms	Petroleum ether (60-80 C) µg/ml		Acetone extract (direct) µg/ml	
<u>S aureus</u> standard	192		150	
<u>S aureus</u> clinical	131		160	
<u>S pyogenes</u> clinical	310		300	

**TABLE 4 --
DETERMINATION OF YIELD OF THE FRACTIONS FROM FLOWER
EXTRACTS OF *Michelia champaca* L Family- *Magnoliaceae* --**

Solvents	Petroleum ether extract (60-80 °C)		Acetone Fraction (direct)	
Length of the column	37.5 cm		37.2 cm	
Diameter of the column	3.1cm		3.1cm	
Silica gel	127.4g		125.3g	
Extract Loaded	2.1g		2.5g	
[in succession]	g	Yield %	g	Yield %
Petroleum ether	0.07	3.33	0.12	4.76
Chloroform	0.46	21.9	0.63	25.04
Ethanol [Alcohol]	1.51	71.9	1.57	62.60
Total Recovery	97.1			92.4

TABLE 5 --

MINIMUM INHIBITORY CONCENTRATIONS OF THE FRACTIONS of *Michelia champaca* L. Family- *Magnoliaceae* Flower --

Solvents	Petroleum Ether Extract (60-80 °C)			Acetone extract (direct)		
	Petroleum ether fraction µg/ml	Chloroform Fraction µg/ml	Ethanol Fraction µg/ml	Petroleum ether fraction µg/ml	Chloroform Fraction µg/ml	Ethanol Fraction µg/ml
<u>S aureus</u> standard	101	111	131	100	80	97
<u>S aureus</u> clinical	110	120	120	120	100	101
<u>S pyogenes</u> Clinical	209	210	220	240	220	222

TABLE 6 --

PHYTOCHEMICAL SCREENING OF THE EXTRACTS OF *Michelia champaca* L. Flower Family- *Magnoliaceae* --

	Petroleum Ether Extract	Petroleum ether Fraction
Phenols	-	-
Terpenoids	-	-
Organic Acids	+	+
Alkaloids	+	+
Glycosides	-	-
Flavonoids	-	-
	Acetone Extract	Chloroform Fraction
Phenols	-	-
Terpenoids	-	-
Organic Acids	+	+
Alkaloids	+	+
Glycosides	+	+
Flavonoids	-	-

Note:

+ Chemical moiety present

- Chemical moiety absent

Bibliography: -

- 1] Bren Linda ;(2002); "Battle Of The Bugs :- Fighting Antibiotic Resistance" FDA Consumer Magazine
- 2] Institute of Science in society report (25 June 2001) "Radical solutions needed for antibiotic resistance."
www.i-sis.org.uk/antibiotic_resistance.php
- 3] Desai S T, (2002) " Potency of larvicidal properties of plant extracts against mosquito larvae under laboratory conditions." M.Sc. HITRT Mumbai Pp-2.
- 4] Seretii MRAI, et al. (1999), "Pharmacology of rosemary (*Rosemarinus officinalis*) and its therapeutic potential" Indian Journal of Experimental Biology vol. 37 Pp – 124.
- 5] Magama S, et al (2003) "Antimicrobial properties of extracts from *Euclea crispa* (Ebenaceae) towards human pathogens." South African Journal of Botany, vol. 69(2) Pp– 193-198.
- 6] Strik WA, et al (2003) "Potential medicinal values of some South African seaweed", South African Journal of Botany, vol.69 (4) Pp – 462-468.
- 7] Biodiversity Profile Of India.
www.wcmc.org.uk/igcmc/main.html
- 8] No.1 Global Herbal Exporters
www.unaniherbalist.com/
- 9] Lattu R G, (1994)"Studies on antibacterial activity of extracts of Anantmool (Root)" M. Sc. HITRT Mumbai Pp– 81.
- 10] Davis, H Patridge M.W., Robinson A.1 (1950), Bentley's by Balliere, Tindall and Co (London) 5th Ed. Pp 248.
- 11] Hussain Akhtar etal; (1992) Dictionary of Indian medicinal plants Central Institute of medicinal and aromatic plants, Lucknow. 
- 12] Nadkarni .K.M. Indian Materia medica Vol 1 Bombay Popular Pharmacy Pp- 704,795,997 and1191
- 13] Colin,C.H. and Lyne , (1965), "Antibiotics and antibacterial substances" Cruikshank. P.M.(Eds) In Microbiological methods, Pp 414-428.
- 14] Vahanwala S J etal (2000), "Antimicrobial activity of *Couropita Guianensis Aubl*" Indian Drugs 377 Pp-343-345.

15] Horborne, J.B. (1984) "A guide to modern tech of plant analysis", Phytochemical methods-Chapman and hall publishers, 2nd Ed.

16] Frank J Welcher PH. D. Editor ,Standard Methods Of Chemical Analaysis 6th Edition Vol-2 ,D Van Nostrand Company London Pp-216-219.