ASSESSMENT ON BIODIVERSITY OF MEDICINAL PLANTS: CYTOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF A FEW MAJOR PLANTS

THESIS SUBMITED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
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DEDICATION

I DEDICATE THIS THESIS

TO

MY EVER LOVING FATHER AND MOTHER

LATE HARESWAR BURAGOHAIN

AND

LATE LILAWATI BURAGOHAIN

Abstract

The World Health Organization estimates that 3.5 billion people in developing countries rely on plant-based medicines for their primary health care. Medicinal practices of plants and plant products for the prevention and treatment of ailments among different ethnic groups have the potential of leading the discovery of new drugs. Searching for new drugs in plants implies screening of the extracts for the presence of novel compounds and an investigation of their biological activities. Genetic diversity of traditional medicinal plants is continuously under the threat of extinction as a result of growth related explosion, environment-unfriendly harvesting techniques, loss of habitats and unmonitored trade on medicinal plants.

The North Eastern region of India is recognized as one of the mega biodiversity centers of the world. The area lies in between 22° - 30° N latitude and 89° - 97° E longitude, and spreading over 2,62,379 sq km representing the transition zone between Indian, Indo-Malayan and Indo-Chinese biogeographic regions and a meeting place of the Himalayan Mountains and Peninsular India. Assam is a botanically rich state in North East India, which is situated in between 24°2' - 27°6' N latitudes and 88°8' - 96° E longitudes and covers an area of 78,523 sq. km. The people of this region have developed a rich ethnomedical tradition (Saikia *et al.*, 2006) and have an abundance of medicinal plants known to the native people (Asati and Yadav, 2004). Thus, there is ample scope to take up a major and systematic research on the medicinal plants of the region.

The present investigation was taken up for recording the medicinal plants of the region in respect of morphology and therapeutic uses, determination of chromosome number and karyotype, isolation and identification of a few active compounds and finally genomic DNA isolation, purification and genome size determination of the selected plants. Three important and potential plant species of the region were selected for cytological, biochemical and molecular investigation. The plant species were selected on the basis of their importance, potentiality and multiple therapeutic uses. Medicinal uses of these plants were known through discussion with traditional herbal practitioners and elderly men and women of different communities of the state. The selected plants were *Zanthoxylum*

oxyphyllum Edgew. (Rutaceae), *Rubus alceifolius* Poir. (Rosaceae) and *Meyna spinosa* Roxb. ex Link. (Rubiaceae). Tender shoots of *Z. oxyphyllum* are taken as vegetable and also useful against stomach trouble. If tender shoots are regularly eaten as vegetable, it is said to act as blood purifier and reduce the incidence of leucoderma. Fruits are used as spice and help in digestion. Root extract of *R. alceifolius* is given in piles and dysmenorrhoea. Decoction of tender shoots is prescribed for cough and pneumonia while unripe fruit is rubbed over the tongue to cure fungal infection. The mature fruits of *M. spinosa* are used to treat gastritis, piles and cracked heels. Seed paste is used as abortifacient and for curing pimples. In some parts, leaves are used as antidandruff shampoo. Ripe fruit has slightly sweet taste and is eaten raw, which is said to be useful for liver.

Data on medicinal plants were collected from primary and secondary sources. Primarily, the medicinal plant species were recorded with the help of local herbal practitioners and elderly men and women. Secondary sources included books written by local herbal healers, newspapers and unpublished thesis. In the investigation, information on 400 medicinal plants belonging to 128 plant families were recorded, verified and authenticated. Majority of the plant species was found to be used in skin diseases followed by stomach ailments and respiratory diseases. Herbs were found to be the dominant form, followed by trees and shrubs. The most cited plant family was Fabaceae followed by Asteraceae and Euphorbiaceae.

Leaf buds were used for the determination of chromosome number and karyotype of the selected plants by preparing semi-permanent slides with 2% acetocarmine stain. Based on this cytological investigation, the chromosome number of *Z. oxyphylum* was found to be $2n = 2x = 36$ with 12 pairs of median and 6 pairs of submedian chromosomes. The chromosome number of *R. alceifolius* was found to be $2n = 4x = 28$ with 10 pairs of median and 4 pairs of submedian chromosomes. On the other hand, chromosome number of *M. spinosa* was found to be $2n = 4x = 44$ with 12 pairs of median and 10 pairs of submedian chromosomes. The chromosome formulae indicated that the first species was a diploid while the last two were tetraploid.

A few compounds were isolated from the plants following the solvent extraction method of Tori *et al.* (2003) using column and thin layer chromatography. The tender shoots of *Z. oxyphylum* yielded a compound **ZO1**, which was a yellowish oil. The spectral data from IR, ^{1}H NMR and ^{13}C NMR suggested **ZO1** to be an alkaloid and its structure was determined to be 2-methylheptyl isonicotinate. The IR and NMR spectral data was in good agreement with the earlier report (Bordoloi *et al.*, 2001). The tender leaves of *R. alceifolius* yielded the compound **RA1**, which was also yellowish oil. The signals of ^{13}C NMR spectrum indicated the structure of the compound to be 2-methylheptyl isonicotinate. The mature fruit of M. spinosa yielded compounds **MS1** and **MS2**. **MS1** was a yellowish gum. The ^{1}H NMR and ^{13}C NMR spectral data indicated **MS1** to be an oleanane type triterpene having a carboxylic group and was identified as oleanolic acid. **MS2** was also a yellowish gum. The ^{1}H NMR spectral data clearly supported the olean structure of the compound. Based on the evidences from IR, MALDI TOF MS and ^{13}C NMR spectrum, **MS2** was identified as oleanol.

Antimicrobial potential of the isolated compounds was assessed against four bacterial strains such as *Bacillus subtilis* (MTCC 619), *Klebsiella pneumoniae* (MTCC 109), *Escheichia coli* (MTCC 739), *Staphylococcus aureus* (MTCC 737) and one yeast strain, *Candida albicans* (3017). Agar well diffusion method was employed to determine the antimicrobial potential of the compounds using Mueller-Hinton agar medium. Each test compound was dissolved in dimethylsulfoxide (DMSO) in the concentration of 250 $\mu g\ ml^{-1}$. The bioactivity was determined after 24 hours at 37°C for bacterial strains and 48 hours at 30°C for yeast strain by measuring the diameter of inhibition zone. **ZO1** showed the largest zone of inhibition (24.0 ± 0.14 mm) against *C. albicans* followed by **MS1** (23.0 ± 0.29 mm) against *S. aureus* revealing the presence of the highest antimicrobial activity. **ZO1** and **MS1** exhibited high antimicrobial activity against all the test organisms. **MS1** showed the highest activity against *S. aureus* and *E. coli.*

A simple, efficient and reliable CTAB method was standardized for the isolation of genomic DNA from the selected plants modifying some of the key steps of the earlier CTAB protocol of Khanuja *et al.* (1999). Key steps in the modified

procedure involved were addition of 4% PVP in the extraction buffer, additional chloroform: isoamyl alcohol (24:1 v/v) extraction and an overnight isopropanol precipitation at room temperature. The procedure yielded a high amount (38 - 46 μg g^{-1} fresh leaf tissue) of good quality DNA free from contaminants. The undigested DNA samples showed conspicuous bands of high molecular weight when resolved in 0.8% agarose gel. DNA from all the samples was effectively subjected to complete digestion with *Eco* RI, *Hin*d III and double digestion by *Eco* RI - *Hin*d III restriction enzymes after incubating at 37°C for 6 hours. Spectrophotometric measurement of the isolated DNA samples at 260 nm and 280 nm gave an absorbance ratio A_{260}/A_{280} of 1.76 - 1.86 indicating the presence of insignificant levels of contaminated proteins and polysaccharides.

The genome size of the plants was determined by flow cytometry using Otto I and Otto II buffer (Otto, 1991) and microscopy method (Konwar *et al.*, 2007). The genome size estimated by flow cytometry ranged from 2.84 - 3.93 pg or 2.0 x 10^9 - 3.93 x 10^9 bp indicating that the genome size of *M. spinosa* being bigger and smaller in the case of *R. alceifolius*. The genome size determined by the microscopy method ranged from 2.71 - 3.79 pg or 3.51 x 10^9 - 3.70 x 10^9 bp. The microscopy method was found to be comparable to the popular method of flow cytometry. The method was found to be new, innovative and less expensive.

DECLARATION

I hereby declare that the thesis entitled "ASSESSMENT ON BIODIVERSITY OF MEDICINAL PLANTS: CYTOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF A FEW MAJOR PLANTS" being submitted to the Department of Molecular Biology and Biotechnology, Tezpur University, is a record of original research work carried out by me. Any text, figure, method or result that are not of own devising are appropriately referenced in order to give credit to the original author(s). All sources of assistance have been assigned due acknowledgement. I also declare that neither this work as a whole nor a part of it has been submitted to any other university or Institute for any other degree, diploma or award.

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This is to certify that the thesis entitled 'ASSESSMENT ON BIODIVERSITY OF MEDICINAL PLANTS: CYTOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF A FEW MAJOR PLANTS' submitted to Tezpur University in the Department of Molecular Biology and Biotechnology, under the School of Science and Technology in partial fulfillment for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology is a record of research work carried out by Mr. Jitu Buragohain under my personal supervision and guidance.

All help received by him from various sources have been duly acknowledged.

No part of this thesis has been reproduced elsewhere for award of any other degree.

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CERTIFICATE OF THE EXTERNAL EXAMINER AND ODEC

This is to certify that the thesis entitled 'ASSESSMENT ON BIODIVERSITY OF MEDICINAL PLANTS: CYTOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF A FEW MAJOR PLANTS' submitted by Mr. Jitu Buragohain to Tezpur University in the Department of Molecular Biology and Biotechnology under the School of Science and Technology in partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology has been examined by us on 27th June, 08 and found to be satisfactory.

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Abbreviations and symbols

A_{240}	absorbance at 240 nm
ACN	acetonitrile
AFLP	amplified fragment length polmorphysm
C	Celsius
bp	base pair
cm	centimeter
$CDCl_3$	duterated chloroform
$CHCl_3$	chloroform
CTAB	cetyltrimethylammonium bromide
DIZ	diameter of inhibition zones
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
ds	double stranded
EDTA	ethylenediaminetetraacetic acid
et al.	et alia (and others)
etc.	et cetera (and so on)
EtOAc	ethyl acetate
FABMS	fast atom bombardment mass spectrometry
Fam.	family
Fig.	figure
g	gram
x *g*	gravity (multiples of, as in centrifugal field)
h	hour
HCl	hydrochloric acid
HR-MS	high-resolution mass spectrometry
IR	infrared
kb	kilobase
KBr	potassium bromide
l	liter
M	molar
MALDI TOF-MS	matrix assisted laser desorbtion/ionization time of flight mass spectrometry
Mbp	mega base pair
MeOH	methanol
m/z	mass to charge ratio
µg	microgram
mg	milligram
MH	Mueller-Hinton
µl	microliter
ml	milliliter
mm	millimeter
mM	milli molar
MTCC	microbial type culture collection

NA	Nutrient-Agar
NaCl	sodium chloride
nm	nanometer
NMR	nuclear magnetic resonance
OD	optical density
PDA	potato dextrose agar
p-DB	Paradichlorobenzene
%	percent
pg	picogram
PCR	polymerase chain reaction
PI	propidium iodide
PVP	polyvinylpyrrolydone
RAPD	rapidly amplified polymorphic DNA
RFLP	random fragment length polymorphysm
RNA	ribonucleic acid
rpm	revolution per minute
S N	Serial number
Syn.	Synonym
SNPs	single nucleotide polymorphisms
TAE	tris acetic acid ethylenediaminetetraacetic acid
TE	tris EDTA
TLC	thin layer chromatography
TMS	tetramethyl silane
tris	tris (hydroxymethyl)-aminomethane
UV	ultra violet
UV/VIS	ultra violet visible
v/v	volume/volume
w/v	weight/volume

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Chapter 1
Introduction

Chapter 1 Introduction

1.1 Plants as traditional medicine

The use of plants as mankind's chief method of healing is as old as human civilization. Over the centuries, people living in forests and inquisitive plainsmen have experimented and made use of several plants. It was basically a community-based knowledge that gradually led to the identification of plants that yielded drugs used in medical practices. Use of plants as a source of medicine has been verbally transferred and has become an important component of the health care system. Before the availability of synthetic drugs, humans were completely dependent on medicinal herbs for prevention and treatment of diseases. Human societies throughout the world accumulated a vast indigenous knowledge over the centuries on medicinal uses of plants, and for other related uses including as poison for fishing and hunting, purifying water, and for controlling pests and diseases of crops and livestock. The medicinal plants were well described in the earliest medical writings of 3,000 B.C. and their use might go back well before recorded history.

About 80% population of the developing countries still use traditional medicines derived from plants for treating human diseases (de Silva, 1997). China, India, Brazil, Sri Lanka, Cuba, Thailand and few other countries have endorsed the official use of traditional systems of medicine in their health care programmes. For example, the Indian systems of medicine 'Ayurveda', 'Siddha' and 'Unani' entirely, and homeopathy to some extent, depend on plant materials or other derivatives for treating human ailments (Prajapati *et al.,* 2003). Ayurvedic and other traditional healers in South Asia use at least 1,800 different plant species and are regularly consulted by some 800 million people. In China, where the use of medicinal plant goes back at least four millennia, healers employ more than 5,000 plant species (Tuxill, 1999). The Ayurvedic 'Materia Medica' includes 600 medicinal plants along with therapeutics.

1.2 Renewed interest in ethnomedicinal plants

The World Health Organization estimates that 3.5 billion people in developing countries rely on plant-based medicines for their primary health care. Evidence suggests that when economic woes and structural adjustment programmes restrict governments' abilities to provide health care, urban and even middle-class residents of developing countries also turn to more affordable traditional medicinal experts (Tuxill, 1999). Developed countries, in recent times, are turning to the use of traditional medical systems that involve the use of herbal drugs and remedies. Herbal preparations are popular and are of significance in primary health care in Belgium, France, Germany, and the Netherlands (Hoareau and daSilva, 1999). Communities have developed the knowledge of the plant, animal and mineral world to a mature and scientifically sound technology, which exists in several forms, the best known of which is perhaps Ayurveda (Penso, 1976).

Recent and renewed interest in medicinal plants coupled to developments in information technology has fuelled an explosion in the range and content of electronic information concerning medicinal plants as a re-emergent health aid. As a result of such developments, access to indigenous people and cultures concerning medicinal plants are greatly facilitated. Furthermore, the active participation of such natural custodians and practitioners of valuable knowledge is guaranteed in the generation of research focusing on screening programmes dealing with the isolation of bioactive principles and the development of new drugs (Hoareau and daSilva, 1999).

A number of drugs used by the people from time immemorial are still employed in much the same manner by present day's medical practitioners. Although, the extraction, separation, isolation and identification of the component constituents of plant drugs have occurred in relatively recent years, the purpose for which the medicinal substances are employed today parallels closely the use for which they were intended by our predecessors (Bhat *et al.*, 2005).

1.3 Ethonomedicinal plants – source of new potential drugs

Ethnomedicinal practices for the prevention and treatment of ailments among different ethnic groups have the potential of leading the discovery of new drugs, which are easily metabolized in the human body having less or no side effects. The work on *Terminalia chebula* (myrobalan) mentioned in Charaka Samhita is quite authentic and modern studies have revealed that the purgative activity mentioned in Ayurveda is justified by the isolation of chebulic acid, the active constituent of myrobalan. The Babylonians used *Allium sativum* (garlic) to treat disease as early as 3000 BC. In Egypt, a decoction of garlic in milk was given in hysteria, sciatica and heart diseases. During the last few decades the hypocholesterolemic properities of garlic have been widely reported. Sulphur containing compounds allinin and allicin have been shown to lower cholesterol levels in cholesterol fed as well as normal rats and other species. One of the most famous of the Chinese folk herbs is the ginseng (*Panax ginseng*) root, used for health maintenance and to treat various diseases. The biological effects are due to the synergy between saponins called ginsenosides and flavonoids. Another popular folk medicine mentioned in the 'Chinese Materia Medica' was the extract of *Ginkgo biloba*, which has an effect in improving memory and sharpening mental alertness. The main constituents responsible for biological activity are ginkgolides and flavonoids.

At least 130 drugs, all single chemical entities extracted from higher plants, are currently in use, though some of these are now produced synthetically for economic reasons. An analysis of the genesis of their induction in modern medicine would show that, invariably, the starting point has been references or clues of these medicinal plants in folklore, or traditional medicine. In fact, plant-derived compounds are showing promise in the treatment of cancer, malaria, diabetes, human immunodeficiency virus (HIV) etc. Plant products constitute approximately 25% of all prescribed medicines even in the most advanced countries like U.S.A. (Singh, 2000). The discovery of potential therapeutic compounds like reserpine, quinine, ephedrine, cocaine, emetin, khellin, colchicine, taxol, vinblastine, vincristine, artemisinine and gugulipid from medicinal plants with rich ethnobotanical lore gave impetus to ethnomedicinal plant research in the world. This

has marked a new era in the use of medicinal plants and the beginning of modern medicinal plant research. The recent discovery of bioactive compounds viz. artemisinin (antimalarial) from *Artemisia annua*, taxol (anticancer) from *Taxus brevifolia*, hypericin (antiviral) from *Hypericum perforatum*, gossypol (male contraceptive) from *Gossypium* spp., yuechukene (antifertility agent) from *Murraya paniculata* and digitalin (treating heart problems) from *Digitalis purpurea* raised expectations of scientists involved in medicinal plant research all over the world to discover potent herbal medicines.

1.4 Problems of research

Major areas in current research involving biologically active plant constituents are- treatment of cancer, antiinfective activity, control of tropical diseases, fertility regulation, inflammation and allergy, immunomodulation and adaptogenic activity, hepatoprotection etc (Hamburger and Hostettmann, 1991). Plants contain thousands of constituents and are a valuable source of new and biologically active molecules. But, among the estimated 3,50,000 plants on earth, only a small percentage has finally been investigated; the fraction subjectd to biological or pharmacological screening is even smaller. Moreover, a plant extract may contain several thousand different secondary metabolites but phytochemical analysis will reveal only a narrow spectrum of the constituents. Searching for new drugs in plants implies screening of the extracts for the presence of novel compounds and assessing their biological activity. Possible novel or bioactive compounds are generally isolated in order to elucidate the structure and to test their biological and toxicological value (Hostettmann *et al.*, 2001). The process to establish a pharmacologically active and pure constituent is very long and tedious that requires a multidisciplinary collaboration of botanists, pharmacologists, chemists and toxicologists (Hamburger and Hostettmann, 1991).

1.5 Indian scenario

India has 2.4% of world's area with 8% of global bio-diversity. It is one of the twelve mega-diversity hot spot regions of the world. Almost one fifth (about 20%)

of all the plants found in India is used for medicinal purpose. According to an all India ethnobotanical survey carried out by the Ministry of Environment and Forests, Government of India, there are over 8,000 plant species used by the people of India (Planning Commission Report, 2000). Forests are estimated to harbour 90% of India's medicinal plant diversity; only about 10% of the known medicinal plants are restricted to non-forest habitats. But according to Hamilton (2003) 44% of total flora in India is used medicinally. In the country there are currently about 2,50,000 registered medical practitioners of the Ayurvedic system against 7,00,000 of the modern medicine system. Seventy per cent of the rural population is dependent on the Ayurvedic system and most practitioners formulate and dispense own recipes, hence requires proper documentation and research (Seth and Sharma, 2004).

1.6 Medicinal plants and modern economy

Medicinal plants and herbal medicines play an important role in the modern economy throughout the world; phytochemicals, nutraceuticals, cosmetics and other herbal products have become a major sector of trade and commerce. It is estimated that the total number of medicinal and aromatic plants in the international trade is around 2,500 species (Schippmann *et al.*, 2002). A recent study indicates that the herbal drug market continues to grow at the rate of 15% annually. As per the estimate of the WHO, the global market of medicinal herbs and herbal products is about USD 62 billion and will hit the market by the year 2050 at the level of USD 5 trillion (Planning Commission Report, 2000). The socio-economic development of some countries like Kenya and Brazil is significantly dependent on the trade of medicinal plants. At present, India is exporting herbal materials and medicines to the tune of USD 1.4 billion. According to the data compiled by the International Trade Centre, Geneva; India ranks second amongst the exporting countries, after China. Besides meeting its domestic demand, China earns USD 5 billion per year from herbal trade.

1.7 Threat to medicinal plants

Genetic diversity of traditional medicinal herbs and plants is continuously under the threat of extinction as a result of growth-related exploitation, environment-unfriendly harvesting, loss of habitats and unmonitored trade. The increasing demand and expanding trade on medicinal and aromatic plants worldwide have serious implications on the survival of several plant species due to indiscriminate harvesting of natural flora (de Silva, 1997). Since the beginning of this century, more than half of the world's tropical forest area has been destroyed. It is estimated that worldwide about 24 ha of the rain forest disappear every minute. During the past 15 years, there has been a substantial loss of habitats, notably tropical forest, which are disappearing at a rate of about 1% per year (FAO, 2003), wetlands and other types of biome as a result of human action. According to an estimate prepared by the Threatened Plants Committee of the "International Union for Conservation of Nature and Natural Resources (IUCN)" about 10% (20,000-30,000) of the wild flowering plants are under threat. Indiscriminate felling of five of the top 12 medicinal trees in the Eastern Amazon region of Brazil for timber purpose has reduced the availability of barks and oils used otherwise for medicinal purposes (Shanley and Luz, 2003). Many other valuable species like '*yohimbe*' (*Pausinystalia johimbe*, bark of which is used to treat male impotence) in Central Africa (Sunderland *et al.*, 1999), '*goldenseal*' (*Hydrastis canadensis*) collected from hardwood forest in the Eastern part of North America (Hill and Buck, 2000) and '*African Cherry*' (*Prunus africana*, bark used to treat prostatitis) in Cameroon and Madagascar (Cunningham *et al.*, 2002) are becoming endangered due to over-exploitation. The situation is even more critical and serious in the Indian context. As the trade of herbs and herbal products has increased by many folds, the Indian herbal industry has touched the annual turnover of more than USD 4,000 million. It has been estimated that about 90% collection of medicinal plants is from wild sources and since 70% of collections involve destructive harvesting, many plants have become vulnerable or endangered or feral (Anon., 1982). Already 16 medicinal plants including high valued *Atropa acuminata, Dioscorea deltoidea* and *Rauvolfia*

serpentina are listed as endangered species in the North Western Himalayas (Gupta, 1986).

1.8 National Medicinal Plants Board

To look into the use and commercial aspects of medicinal plants, the Government of India has formed the National Medicinal Plants Board (NMPB) on November 9th, 2000 under the Ministry of Health and Family Welfare. So far, the Board has enlisted 32 medicinal plants for cultivation, characterization, improvement and conservation. These plants are *Amla, Ashok, Ashwagandha, Atees, Bael, Bhumi amlaki, Brahmi, Chandan, Chirata, Daruhaldi, Giloe, Gudmar, Guggal, Isapgol, Jatamansi, Kalihari, Kalmegh, Kesar, Kokum, Kuth, Kutki, Makoy, Mukti, Patterchur, Pipoli, Safed musli, Sarpagandha, Senna, Satavari, Tulsi, Vai-vidang* and *Vatsnabh.* As of now, out of 400 industrially useful medicinal species, only 20 are cultivated on commercial basis. In line with setting up of NMPB, 35 state Governments have formed State Medicinal Plant Boards.

1.9 Importance of North East India in terms of medicinal plant diversity

The North Eastern part of the country is recognized as one of the mega biodiversity centers of the world. The area is lying between 22° - 30° N latitude and 89° - 97° E longitude, and spreading over 2,62,379 sq km (Fig 1.1), represents the transition zone between Indian, Indo-Malayan and Indo-Chinese biogeographic regions and a meeting place of the Himalayan Mountains and Peninsular India. It was the part of the northward migrating 'Deccan Peninsula' that first touched Asian landmass after the break up of Gondwanaland. North Eastern region is endowed with high floristic richness and has been rightly called as the 'cradle of angiospermic plants'. The region has four micro endemic centers out of the 26 such centers in India. The region has 132 wild relatives of crop plants out of the 686 reported from Indian sub continent (Roy *et al.*, 2002). The region possesses more than 2,000 medicinal and aromatic plant species accounting for about 20% of the total plant diversity of the region. According to the recent 'Biodiversity Assessment in the North Bank Landscape' report, the North East India has the second richest

forest reserve in the world in terms of plant diversity (WWF report, 2005). The area surveyed by the WWF is called the North Bank Landscape, spanning 3,000 sq km of the Himalayan foothills, north of the Brahmaputra River in Assam and parts of Arunachal Pradesh, North Bengal and Bhutan. The richness of forests of the North Bank Landscape is higher than similar lowland forests in other biodiversity hotspots like Brazil, Cameroon, New Guinea and Peru. Because of exhaustive exploitation, the flora of North East India in general and medicinal and aromatic plants in particular are facing the threat of existence; many of which are already on the verge of extinction even without having a scientific glance. Assam is a botanically rich state in North East India, which is situated in between 24°2' - 27°6' N latitudes and 88°8' - 96° E longitudes (Fig. 1.2) and covers an area of 78,523 sq. kilometers. The state extends between foothills of eastern Himalayas and the Patkai and Naga Hills and is bordered by the nations Bhutan and Bangladesh. Assam has a humid subtropical climate with extremely heavy rainfall. A sizeable area of the state is covered with dense tropical forests of bamboo, and at large elevation evergreens. Assam experiences heavy rainfall between March and September with very high humidity in the summer months. Winter sets in from the month of November and lasts till the middle of March. The people of this region have developed a rich ethnomedical tradition (Saikia *et al.*, 2006) and have an abundance of medicinal plants known to the native people (Asati and Yadav, 2004).

1.10 Causes for the dwindling resources of medicinal plants in North East India

There are several causes for the dwindling resources of medicinal plants in the region:

- Shifting cultivation on the vast areas of forestland, steadily deplete natural resources including medicinal and other plants.
- Rapid deforestation gives rise to secondary forests, bushy hillocks, grassy or barren hillocks which ultimately lead to soil erosion, landslide and change of climate.
- Unwise and unsystematic deforestation on a rampant scale for the purpose of timber and firewood by the contractors, forest personnel and local people.

- Tourists, picnic parties and pilgrims to various wild habitats cause considerable damage to the forest flora of the region.
- Unsystematic, injudicious and unscrupulous collection of medicinal plants and their parts by the agents of profit-oriented business concerns.
- Lack of systematic cultivation of most of the medicinal and aromatic plants.

1.11 Investigation on medicinal plants of the region

In the North Eastern region as a whole and Assam in particular, there is a serious deficiency in conservation, establishment of natural reserves and to locate and protect the plant species of potential medicinal and aromatic use including unknown ones. Medicinal plants of the region so far have not been catalogued. A few books were written on locally available medicinal plants in Assamese language or other local languages, but these books fail to describe morpho-phenological and genetic characters, their chemical constituents and pharmacological activities. No systematic effort has so far been made to identify, characterize and conserve the medicinal plant diversity of the region. Confusion has prevailed and led to the situation where the same plant might be known by different names, or where widely differing species share the same names. This has serious implications. Physicians and manufacturers of Assamese traditional and Ayurvedic medicines have, therefore, had a critical interest in obtaining authentic descriptions of medicinal plants in currently valid taxonomic terms. It is thus necessary to document the local common names, use of specific parts having medicinal value and accurate botanical illustrations. A collaborative approach is necessary involving local medicine men, systematic botanists, biochemists, biotechnologists, physiologists and experts in modern structural elucidation techniques. So far, attempts in this direction are few and incomplete. Thus, there is ample scope to take up a major and systematic research on the medicinal plants of the region.

1.12 Medicinal plants selected for cytological, biochemical and molecular characterization

Three important and potential plant species of the region were selected from the list of recorded plants for cytological, biochemical and molecular characterization. The plant species were selected on the basis of their importance, potentiality and multiple therapeutic uses. They are found in wild conditions, very little work so far has been done and no cultivation practice has been adopted. Medicinal use of these plants is known through discussion with traditional herbal practitioners, elderly men and women of different communities of the state and local literature survey. The selected medicinal plants are:

1. *Zanthoxylum oxyphyllum* Edgew (local name-Mezenga; Family- Rutaceae) (Plate 1): The plant belongs to the family Rutaceae and is distributed in the temperate and subtropical Himalayas (Chopra *et al.*, 1956). Tender shoots of this plant are taken as vegetable, which are useful against stomach trouble. If tender shoots are regularly eaten as vegetables, it is said to act as blood purifier and reduce the incidence of leucoderma. Fruits are used as spice and help in digestion.

2. *Rubus alceifolius* Poir (syn. *R. moluccanus auc. non* L.) (local name-Jetulipoka; Family- Rosaceae) (Plate 2): The plant belongs to the family Rosaceae and is distributed throughout India, Australia and Malayasia (Bora and Kumar, 2003). It is considered as a serious invasive weed in South East Asia and Australia (Amsellem *et al.*, 2001). Root extract of the plant is given in piles and dysmenorrhoea. Decoction of tender shoots is prescribed for cough and pneumonia while unripe fruit is rubbed over the tongue to cure fungal infection.

3. *Meyna spinosa* Roxb ex Link (syn. *Vangueria spinosa* Roxb) (local name-Kutkura; Family- Rubiaceae) (Plate 3): The plant belongs to the family Rubiaceae, usually occurs in roadside areas and is distributed throughout India, Bangladesh, China, Malaya and Myanmar (Bora and Kumar, 2003). The mature fruits of the plant are used to treat gastritis, piles and cracked heels. Seed paste is used as abortifacient and for curing pimples. In some parts, leaves are used as antidandruff shampoo. Ripe fruits have slightly sweet in taste and are eaten raw, believing to be useful for liver.

1.13 Objectives

On the basis of the above facts, the present investigation has been taken up with the following objectives:

1. Morphological characterization of the medicinal plants with phenology and description of their therapeutic uses. The important plant species will be documented with the preparation of herbarium.

   A database on medicinal plants of the state and the region will provide keys for the correct identification of the plants and future phytochemical, pharmacological and molecular investigations. Such characterization will also help to take up systematic investigation of medicinal plants.

2. Cytogenetic study of the selected three medicinal plants with the determination of chromosome number and karyotype.

   Determination of chromosome number and karyotype will help to know the evolution pattern, reproduction behaviour, ploidy level and to identify the species from other related or allied species. Karyotype investigation will encourage future genetic improvement of medicinal plants through conventional breeding and modern genetic manipulations.

3. Biochemical analysis of the selected plants with isolation and identification of a few active compounds.

   Isolation and identification of compounds form the plants will provide scientific evidence in support of the ethnotherapeutic claims and might lead to the discovery of new potential drugs. Assessment of the isolated and purified compounds against pathogenic microorganisms will help to determine the antimicrobial potential of the compounds leading to their therapeutic applications. The elucidation of chemical structure of the potential therapeutic chemicals will help to identify them.

4. Genomic DNA isolation, purification and genome size determination of the selected plants.

   The isolation of quality genomic DNA from the medicinal plants and the determination of their genome size will provide a basis to plant breeders and

the plants like genetic transformation and gene copy number differentiation. The optimized protocol of genomic DNA isolation can be used to isolate quality DNA from other medicinal plants rich in secondary metabolites.

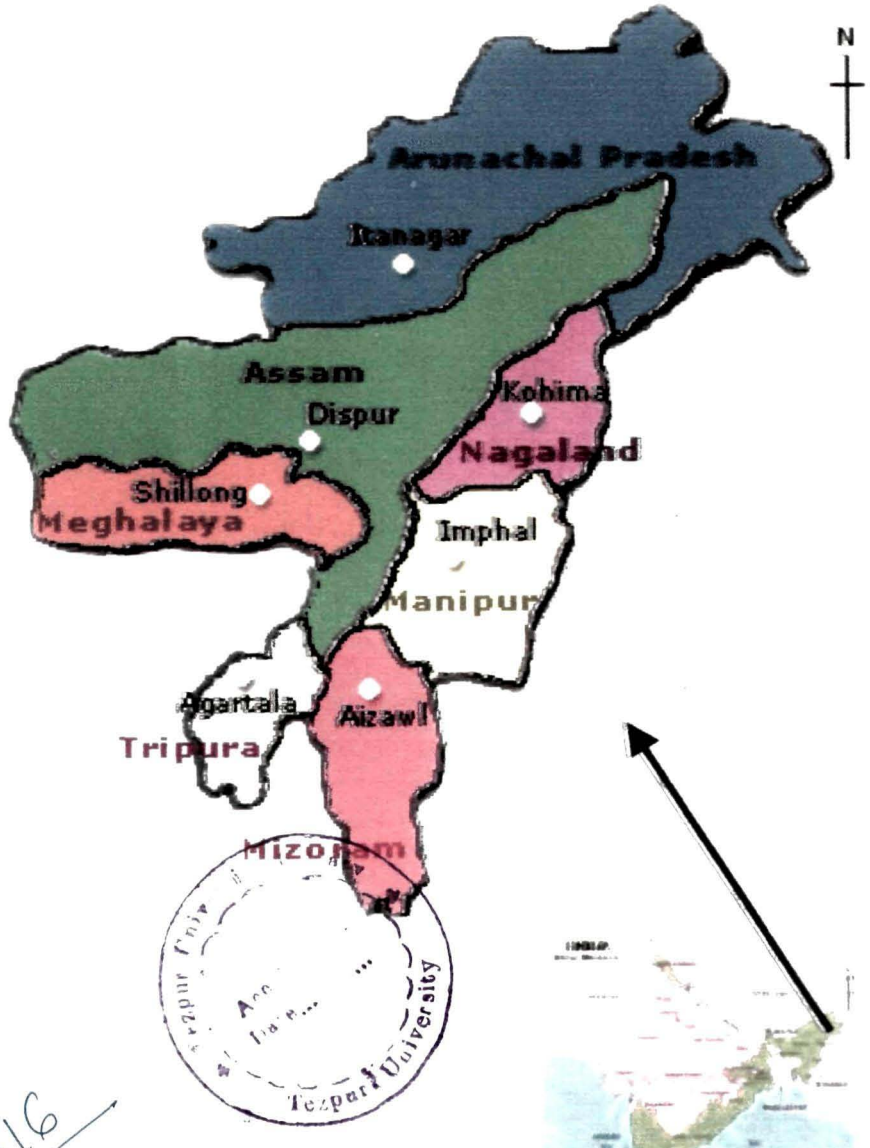

Fig. 1.1 Geographical map of North East India

Fig. 1.2 Geographical map of Assam

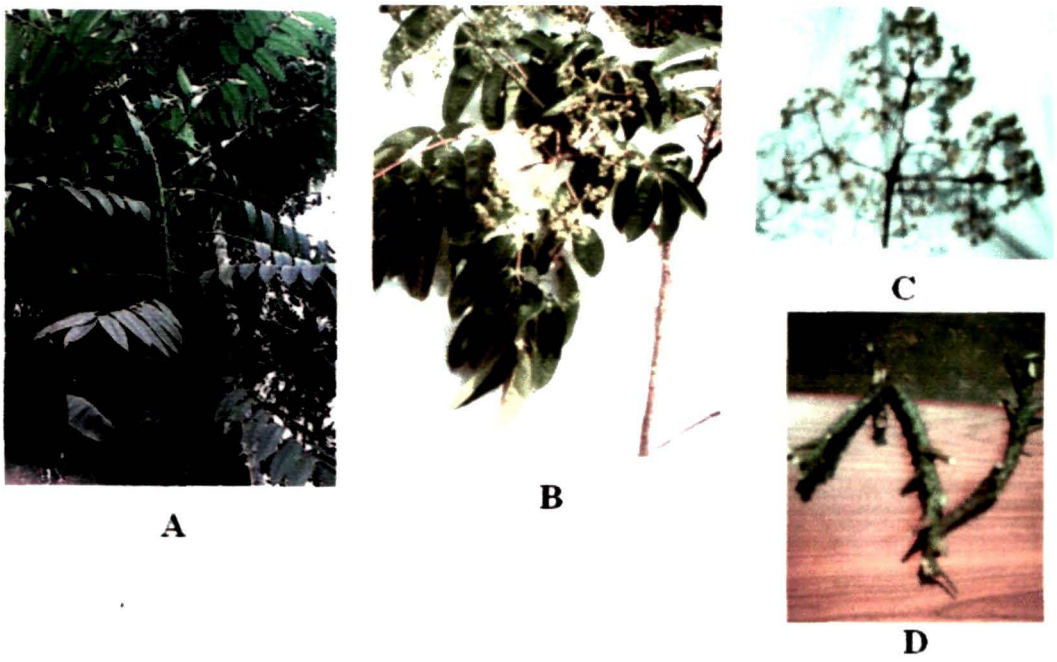

Plate 1 *Zanthoxylum oxyphyllum* **A.** Mature plant; **B.** Inflorescence bearing branch; **C.** Inflorescence; **D.** A spiny branch

Plate 2 *Rubus alceifolius* **A.** Mature plant; **B.** A part of the plant bearing flowers; **C.** A part of the plant bearing mature fruits

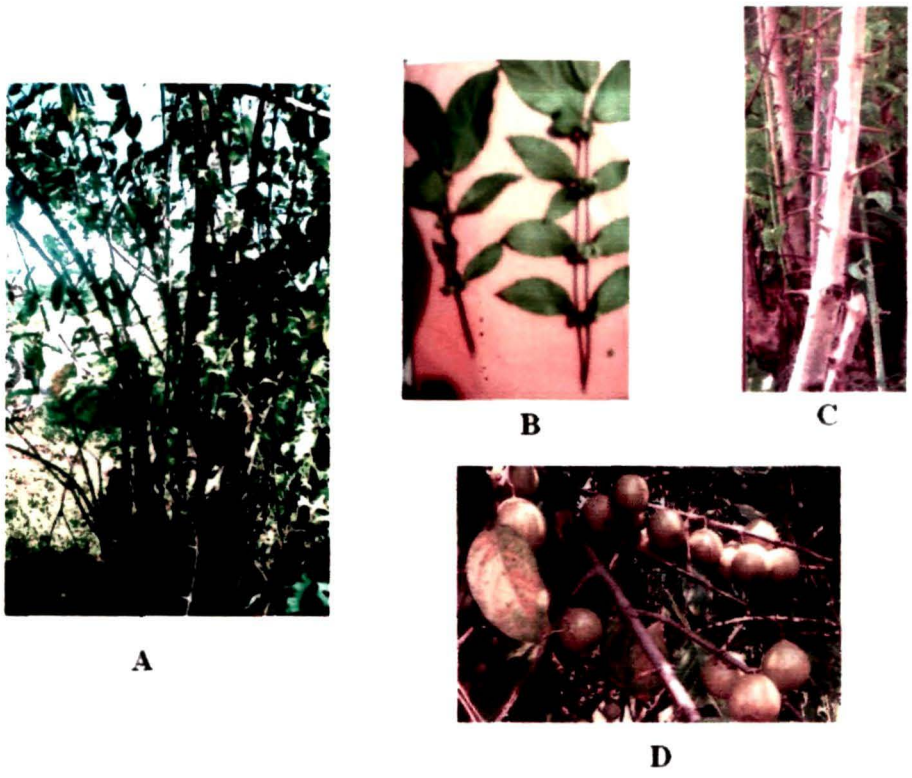

Plate 3 *Meyna spinosa* **A.** Mature plant; **B.** Inflorescence; **C.** Long axillary spines; **D.** A branch bearing mature fruits.

Chapter 2
Review of Literature

Chapter 2 Review of literature

2.1 Medicinal plant diversity

Different researchers from North East India and other parts of the country have reported ethnomedicinal plants in the past literature. The reports by different researchers in between 1996 and 2007 have been reviewed.

Pandey *et al.* (1996) reported 32 medicinal plants used by Tai Ahoms, Tai Khamyang, Tai Turung and Sonowal Kacharis in the Golaghat district of Assam. Borthakur (1997) documented 24 species used by Karbi people of Assam. Hajra and Baishya (1997) reported 32 species and Singh *et al.* (1997) 44 species used by Mishing people of Assam.

Jamir *et al.* (1999) documented 36 medicinal plants belonging to 35 genera and 28 families in Nagaland on the basis of an ethnobotanical survey conducted in 1993 - 1997. The study showed that the traditional herbal medication gained special importance in social life and culture of Nagaland.

Kattimani *et al.* (2000) described some important medicinal plants used by the people of Richur District of Karnataka. Arya (2001) studied the medicinal plant diversity in some hot spots of Almora and Bageswar District of Kumaon Himalayas. The observations were made to locate the richest bio-diversity areas of those plants, which have great demand in national and international markets.

Sharma *et al.* (2001) documented 135 medicinal plant species belonging to 122 genera and 68 families used by the native people of Mizoram, for which only limited literature is available. The study highlighted the diversity of plants and important knowledge of potential therapeutic applications of several plant species of Mizoram.

Singh *et al.* (2002) made ethnobotanical exploration to study the folk medicinal uses of certain plants by the tribes of Sonaghati of Sonbhadra District in Uttar Pradesh. A total of 125 plants from 57 families used therapeutically against different diseases were covered in the study.

Harsha *et al.* (2002) recorded 65 medicinal plants used by Kunabi tribe of Uttar Kanada District of Karnataka; 45 of them used to treat a wide range of ailments were described based on an ethnobotanical survey. In 2003, they described 52 herbal remedies against skin diseases from 31 plants belonging to 21 families in the same District.

Haridasan *et al.* (2002) surveyed extensively and reported 464 medicinal plant species from Arunachal Pradesh. The study highlighted the rich diversity of medicinal plants of the region

Kawata *et al.* (2004) conducted a floristic survey of ethnomedicinal plants occurring in the tribal areas of Rajasthan to assess the potentiality of plant resources for modern treatments. In the survey, 61 plant species belonging to 38 families were recorded.

Purkayastha *et al.* (2005) reported 55 plant species belonging to 34 families and 52 different genera used by the ethnic communities of Dibru-Saikhowa Biosphere Reserve in Upper Assam. For each species, ethnobotanical and pharmacognostic elements were provided in the report.

Chhetri *et al.* (2005) documented 281 plant species belonging to 229 genera and 108 families used in folk medicine in the Himalayan region of Darjeeling. Among the enumerated plants, about 58% showed hitherto unexplored ethnomedicinal uses. Darjeeling was not only rich in the medicinal plant diversity, but also in the traditions of folk medicine.

Kala (2005) investigated the wealth of medicinal plants used by the Apatani tribe settled in seven villages in Zero valley of Lower Subansiri district of Arunachal Pradesh. The study documented 158 medicinal plant species used by Apatanis; they belonged to 73 families and 124 genera. Almost 52 ailments were cured by using these plants.

Sajem and Gosai (2006) documented 39 medicinal plant species belonging to 27 families and 35 genera used by the Jaintia tribe in North Cachar district of Assam. Altogether, 30 ailments were reported to be cured by using these plants. The study underlined the potential of the ethnobotanical research and the need for the

documentation of traditional ecological knowledge pertaining to the medicinal plant utilization for the greater benefit of mankind.

Saikia *et al.* (2006) documented 85 plants belonging to 49 families used by Assamese people for curing different skin ailments and for cosmetics. The herbal medicines were prepared from various parts of the plant. In several cases, the pure herbal preparations are administered along with milk, ghee, honey, coconut oil, curd etc. Fourteen plants are known for their use to cure multiple skin diseases. The herbal cosmetic products used by the people of Assam include those applied for the enhancement of skin colour, hair care, removal of ugly spots, colouring of nails, palms and teeth. Herbal remedies were also available for skin burns, prickly heat and pimples.

Buragohain and Konwar (2007) documented 68 plant species belonging to 40 families used by five Indo-Mongoloid communities of Assam in various skin diseases. Majority of the plant species recorded was used in the treatment of abscesses, septic ulcers, scabies, ringworm, allergy and pimples. Most of the herbal remedies were taken in the form of paste.

2.2 Determination of chromosome number and karyotype

Chromosome data are fundamental characters of plant species and relevant to plant systematics and evolution (Stace, 2000). The most obvious morphological characters are chromosome size and position of the centromere. As very little or no report has been found in the past literature on karyological studies of the plants, the works done on other plant species have been reviewed.

Das *et al.* (1998) carried out extensive karyotype analysis including determination of somatic chromosome number, total chromosome length and volume and estimation of 4C DNA content on three species of *Pachypodium* namely *P. lamerei*, *P. namaquanum*, and *P. rosulatum* '*horombense*' for the first time. A significant variation in nuclear DNA amount was recorded at the interspecific level. The somatic chromosome number 2n = 18 was recorded in all species.

Meric and Dane (1999) investigated the chromosome number, karyotype and mitotic division of *Vicia sativa* L. subsp. *incisa* (Bieb.) Arc. var. *incisa*. The chromosome number was found to be 2n = 24 and counted for the first time. In the karyotype, one submedian and 6 subterminal chromosome pairs were found, in one of which SAT-chromosome was detected.

Blanchon *et al.* (2000) determined chromosome numbers in seven species of *Libertia* from New Zealand, Australia and South America. The result showed an extensive polyploid series, ranging from diploid with 2n = 2*x* = 38 through 2n = 12*x* = 228.

Sarma *et al.* (2000) studied karyomorphology of *Litsea cubeba* belonging to family Lauraceae using its leaf bud. The study revealed 2n = 24 with chromosome length 2.95 - 4.6 μm.

Oropeza *et al.* (2002) reported karyotype analyses of three Podostemaceae genera. Somatic chromosome numbers were 2n = 28 (*x* = 14) for *Oserya coulteriana* Tul. and *Vanoroyenella plumose* Novelo and Philbrick (sub-family Podostemoideae). The pantropical *Tristicha trifaria* (Bory ex Willdenow) Sprengel (subfamily Tristichoideae), with 2n = 20 (x = 10), had a karyotype of 10 median chromosome pairs.

Naruhashi *et al.* (2002) carried out cytological survey for 37 species of genus *Rubus* (Rosaceae) in Taiwan. The karyological studies revealed chromosome number ranging from 14 – 56 (2n) with basic chromosome number for all the species was *x* = 7.

Karadag and Buyukbure (2003) studied the morphology of chromosomes of some legume species (*Vicia noeana* Boiss., *Lathyrus sativus* L.) collected from the native vegetation in central province of Tokai in Turkey. The karyological study revealed chromosome number of *Vicia noeana* Boiss. and *Lathyrus sativus* L to be 2n = 12 and 2n = 24 respectively. All chromosomes were determined to be submedian. Out of 14 chromosomes in *Lathyrus sativus*, 4 were submedian and 10 median.

Agbagwa and Okoli (2005) carried out chromosome counts from natural populations of *Abrus pulchellus* in Nigeria. In all investigated samples tetraploid (2n

submedian. Out of 14 chromosomes in *Lathyrus sativus*, 4 were submedian and 10 median.

Agbagwa and Okoli (2005) carried out chromosome counts from natural populations of *Abrus pulchellus* in Nigeria. In all investigated samples tetraploid (2n = 44) was constant, which were divided into three cytomorphological categories: 8 metacentric and 8 submetacentric and 6 acrocentric pairs having chromosome length ranging from 0.5 - 1.4 μm. The polyploid cytotype was reported for the first time in this taxon.

Chowdhury and Konwar (2007) studied the karyotype of *Schumanianths dichotomus* (Rox.) Gagenep of Marantaceae. The study revealed chromosome number 2n = 20 (n = x = 10) with the range of chromosome length from 0.8 - 2.2 μm and predominance of metacentric and submetacentric behaviour.

2.3 Isolation and identification of biochemical compounds

The active phyto-compounds are pure molecules and some of them are even more pharmacologically active than their synthetic counterparts. The sequence of development of pharmaceuticals begins with the identification of active lead molecules followed by detailed biological assay. As few reports have been found in the past literature elsewhere on biochemical analysis of the present plant species, the works done on the related and other allied species have been reviewed.

Gopinath *et al.* (1963) isolated a neutral compound, $C_{18}H_{18}O_3$ (m.p. 119-120^0) from the petroleum ether-extract of the bark of *Zanthoxylum hamiltonianum*. The petrol exhausted bark on extraction with ethanol yielded an alkaloid, which was identified as nitidine.

Deshpande *et al.* (1979) made chemical investigation of three *Zanthoxylum* species, *Z. alatum*, *Z. oxyphyllum* and *Z. acconthopodium*. Four lignans, sesamin, fargesin, eudesmin and epieudesmin; three furoquinoline alkaloids, dictamnine, 8-hydroxydictamnine and γ-fagarine and a neutral lactone pluviatide were isolated from *Z. alatum*. Sesamin, eudesmin, epieudesmin, syringaresinol, γ-fagarine, β-sitosterol and lupeol from *Z. oxyphyllum*. *Z. acconthopodium* afforded the same four lignans from *Z. oxyphyllum*. Masood *et al.* (1980) isolated a lactone from the

Ghosh *et al.* (1983) isolated oleanolic acid from the seeds of the plant *Randia dumetoum* (Rubiaceae). The compound showed significant anti-inflammatory activity in the exudative and proliferative phases of inflammation in the doses of 25 and 100 mg kg^{-1}. Significant analgesia was observed only on thermal stimulus. It did not show any antipyretic activity against Brewer's yeast induced pyrexia in rats. The approximate oral LD_{50} were found to be 3,600 mg kg^{-1} and 1,500 mg kg^{-1} in mice and rats respectively.

Mendez *et al.* (1995) reported the isolation and identification of flavonoids and triterpenoids from the dried leaves of *Licania pittieri*, which yielded nine compounds: ursolic and oleanolic acids, catechin, epicatechin, quercetin, isoquercetin, hyperin and 3-arabinopyranoside.

Hao *et al.* (1996) isolated a new triterpenoid, 3β, 13β-dihydroxy-urs-11-en-28-oic acid, along with four known triterpenoids, oleanolic acid, ursolic acid, 2α-hydroxyursolic acid and 2α, 19α-dihydroxyursolic acid from the aerial parts of *Isodon loxothyrus*. Two known diterpenoids were also isolated and characterized from the leaves of the plant. The structures of these compounds were elucidated by spectral analyses.

Gogoi and Sarma (1997) isolated a new triterpenoid saponin from the fresh leaves of *Meyna laxiflora* (syn. *Vangueria spinosa*) of Rubiaceae. The structure of the new triterpenoid was established as oleanolic acid – 3 – O – [- rhamnopyranosyl – (1 2)] – D – xylopyranoside based on spectral analysis.

Kumar and Muller (1999) screened 11 methanol extracts obtained from four different species of *Zanthoxylum* species for their antiproliferative activity against the growth of human keratinocytes. The extract obtained from *Z. armatum* bark was highly active with an IC_{50} value of 11 μg ml^{-1}. Also, the extracts obtained from *Z. oxyphyllum* bark and roots with IC_{50} values of 53 and 57 μg ml^{-1} respectively, showed potent activity.

Rabe and Staden (2000) isolated a sesquiterpenoid muzigadial for the first time from the stem bark of *Warburgia salutaris*, a South African endangered medicinal plant. The isolated compound showed antimicrobial activity against gram-positive bacteria with MIC values ranging from 12.5 - 100 μg ml^{-1}.

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Lu *et al.* (2000) isolated three olean-12-ene type triterpenoid saponin as methyl esters from the roots of *Camellia sinensis* var. *assamica* after treatment with diazomethane by extensive 1D and 2D NMR as well as FABMS and HR-MS analyses.

Bedir *et al.* (2000) isolated six triterpene saponins, including two new compounds from the fruit of *Hedera helix* (Araliaceae). The structure of the new compounds were established as 3-O-β-D-glucopyranosyl- (1 2)- β -D glucopyranosyl hedeagenin 28-O- β-glucopyranosyl-(1 6)- β –D-glucopyranosyl ester, and 3-O-β-D-glucopyranosyl- (1 2)- β -D-glucopyranosyl oleanolic acid 28-O- β-glucopyranosyl-(1 6)- β –D-glucopyranosyl ester, respectively, on the basis of chemical and spectral data.

Tori *et al.* (2003) isolated (12E, 14E, 17E)-11-Hydroxy-12, 14, 17-dodecatrienoic acid from the stems of *Illex integra* and its structure was determined on the basis of spectroscopic data as methyl ester.

Ahmad *et al.* (2003) isolated pseudophrynamine, lunacridine and a new compound 2-(2', 4', 6'-trimethyl-heptenyl)-4-quinozolone from the leaves of *Zanthoxylum budrunga* on the basis of spectral data.

Gustavo *et al.* (2006) isolated oleanolic acid as the major wound healing constituents after acid hydrolysis of an ethanolic extract followed by *in vivo* activity guided fractionation from the fresh leaves and stems of *Anredera diffusa*. The wound healing activity was equivalent to 42.9% ($p < 0.01$) above the control at a concentration of 12.5 mg ml^{-1}. The highest cicatrizant activity was obtained by applying 40 μg of oleanolic acid per gram of body weight in mice.

2.4 Antimicrobial assay

Plant-based antimicrobials are vast untapped source for medicines and have enormous potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. As few reports have been found in the literature regarding the antimicrobial activity of the phytochemicals isolated from the selected plant species, the works done on other species have been reviewed.

Samy *et al.* (1998) assayed 34 ethnomedicinal plant species belonging to 18 different families for antibacterial activity against *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonus aeruginosa* (gram-negative) at 1,000-5,000 ppm using the disc diffusion method. Of these, 16 plants showed activity; among them *Cassia fistula*, *Terminalia arjuna* and *Vitex negundo* showed significant antibacterial activity which confirmed the traditional therapeutic claims for these herbs.

Ahmad *et al.* (1998) subjected 82 Indian traditional medicinal plants to preliminary anti-bacterial screening. Aqueous, hexane and alcoholic extracts of each plant were tested for their antibacterial activity. The results indicated that out of 82 plants, 56 exhibited antibacterial activity against one or more test pathogens. On the whole, the alcoholic extracts showed greater activity than their corresponding aqueous and hexane extracts.

Nascimento *et al.* (2000) evaluated antimicrobial activity of plant extracts and phytochemicals with antibiotic susceptible and resistant microorganisms. In addition, the possible synergistic effects when associated with antibiotics were studied. Extracts of *Achillea millifolium* (yarrow), *Caryophyllus aromaticus* (clove), *Melissa officinalis* (lemon-balm), *Ocimum basillicum* (basil), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Syzygium joabalanam* (jambolan) and *Thymus vulgaris* (thyme) were investigated. The phytochemicals benzoic acid, cinnamic acid, eugenol and farnesol were also utilized. The highest antimicrobial potentials were observed for the extracts of clove and jambolan, which inhibited 64.2 and 57.1% of the tested microorganisms, respectively, with higher activity against

bacteria. Association of antibiotics and plus extracts showed synergistic antibacterial activity against antibiotic-resistant bacteria. The results obtained with *Pseudomonus aeruginosa* was particularly interesting, since it was inhibited by clove, jambolan, pomegranate and thyme extracts.

Ahmad and Beg (2001) studied the ethanolic extracts of 45 Indian traditional medicinal plants for their antimicrobial activity against certain drug resistant bacteria and a yeast *Candida albicans* of clinical origin. Of these, 40 plant extracts showed varied levels of antimicrobial activity against one or more test bacteria. Overall, broad-spectrum antimicrobial activity was observed in 12 plants. No correlation was observed between susceptibility of test strains with plant extracts and antibiotic resistance behaviour of the microbial strains (*Staphylococcus aureus*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*). Qualitative phytochemical tests, thin layer chromatography and TLC bioautography of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as major active constituents.

Erturk *et al.* (2003) determined antimicrobial activity of *Viscum album* subsp. *abietis* against six pathogenic bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonus aeruginosa*, *Enterobacter cloaceae* and *Proteus vulgaris* and one yeast *Candida albicans* using agar well diffusion method with different concentrations. The fractions 6 and 7 of n-Hexane extract showed antimicrobial activity against the test organisms.

Hamill *et al.* (2003) performed antimicrobial susceptibility assay of two chemical reactions of the crude extracts of *Rubus apetalus*. Fractionation of one of the crude fractions led to the isolation of a mixture of related compounds that exhibit antimicrobaial activity against *Staphylococus aureus* (MIC = 62 $\mu g\ ml^{-1}$), *Streptococcus faecalis* (16 $\mu g\ ml^{-1}$) and *Candida albicans* (32 $\mu g\ ml^{-1}$).

Hichri *et al.* (2003) prepared oxidizing, phosphorus, ester and amide derivatives of oleanolic acid (1) and tested the antibacterial activity of compound (1), isolated from the fruit skin of *Periploca laevigata* (Ascleipiadaceae) and its derivatives using Tween-80 as complex agent to form water-soluble triterpenes.

The same activity of maslinic acid acetate (2), β-amyrin (3) and its acetylated derivatives, isolated from the same source as that oleanolic acid (1), was also investigated.

Bonjar (2004) screened 50 methanolic plant extracts belonging to 44 plant species of 33 families used in Iranian folklore medicine for antibacterial activity. A total of 30 samples, including 28 species in 20 families, expressed antibacterial activity against at least one bacterial species.

Bonjar and Nik (2004) determined antibacterial activity of methanol extract of Iranian traditional medicinal plants by *in vitro* bioassay using agar-diffusion method against strains of *Pseudomonus aeruginosa* and *Pseudomonus fluorescens* at 20 mg ml^{-1}. From 160 plant species tested only 13 showed anti-*Pseudomonas* activity. The most active extracts were from the plants *Dianthus caryophyllus*, *Terminalia arjuna* and *Myrtus communis* with the MIC of 3.75, 1.87 and 7.5 mg ml^{-1} against *P. aeruginosa*; 0.46, 0.93 and 1.87 mg ml^{-1} against *P. fluorescens*, respectively.

Adebolu *et al.* (2005) evaluated the antibacterial potentiality of different extracts from the leaves of *Ocimum gratissimum* against the pathogenic bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Salmonella typhimurium* that cause diarrhoea. The extracts evaluated included cold-water extract, hot water extract and steam distillation extract. Only steam distillation extract had inhibitory effect on the selected bacteria and the minimum inhibitory concentration (MIC) ranged from 0.1% for *Staphylococcus aureus*, 0.01% for *Escherichia coli* and *Salmonella typhimurium*, and 0.001% for *Salmonella typhi*.

Venkatesan *et al.* (2005) tested aqueous (room temperature, boiled and autoclaved) and various solvents (methanol, chloroform and hexane) extracts of the leaves of *Suregada angustifolia* against 12 human pathogenic bacteria by agar well-diffusion method. Aqueous extracts did not show any activity. Antibacterial activity was recoded in the order of methanol, hexane and chloroform extracts. Maximum activity was recorded against *Staphylococcus aureus* (skin infections) in methanol and hexane extracts and moderate activity against *Vibrio vulnificus* (hexane extract) and *Vibrio cholerae* (chloroform extract).

Buragohain and Konwar (2006) performed antimicrobial assay of two compounds isolated from fruits of *Meyna spinosa* (Rubiaceae) against five standard microbial strains *Escherichia coli*, *Klebsiella pneumonieae*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*. Both compounds showed activity against all the test microorganisms.

Erdemoglu *et al.* (2007) tested antibacterial and antifungal activities of alkaloid extract of *Lupinus angustifolius* (Fabaceae) against standard microbial speciess of *Escherichia coli*, *Pseudomonus aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans* and *C. krusei*. The alkaloid extract showed significant activity against *B. subtilis*, *S. aureus* and *P. aeruginosa*. On the other hand, the extract possessed moderate activity against *C. albicans* and *C. krusei*.

2.5 Genomic DNA isolation

The problem of DNA extraction was reported to be an important issue in the field of plant molecular biology (Csaikl *et al.* 1998). Over the last decade, there has been an increased requirement for the isolation of pure genomic DNA that performs well in any downstream application such as PCR, genotyping of single nucleotide polymorphism (SNPs), southern hybridization, AFLP, RFLP and microsatellite analysis. Poor quality DNA can lead to sub-optimal result and DNA that is impure or contaminated will not perform well in downstream applications. Many standard protocols are available for the isolation of plant genomic DNA, but problems are invariably encountered when DNA is first isolated from a plant species (Sharma *et al.*, 2000). Molecular biological studies require high quality genomic DNA. Isolation of good quality genomic DNA from plant is complicated because of the presence of exceptionally high amounts of polysaccharides, polyphenols and other secondary metabolites. Polyphenols and polysaccharides bind firmly to nucleic acids during DNA isolation and interfere with subsequent reactions (Pirtilla *et al.*, 2001). As there is no report of DNA isolation from the present plant species, the literature found on the related works on allied and different plant species have been reviewed.

Lodhi *et al.* (1994) developed a quick, simple and reliable DNA extraction method modified from Doyle and Doyle (1990) for grapevine species, hybrids and *Ampelopsis* (*Vitaceae*). It was a CTAB-based extraction procedure modified by the use of NaCl to remove polysaccharides and PVP to eliminate polyphenols during DNA purification. The method was successfully used for the extraction of total DNA from other fruit trees such as apple (*Malus domestica*), apricot (*Prunus armeniaca*), cherry (*Prunus avium*), peach (*Prunus persica*), plum (*Prunus domestica*) and raspberry (*Rubus idaeus*). DNA was completely digestible with restriction endonuclease and was suitable for PCR amplification.

Kim *et al.* (1997) demonstrated an easy DNA extraction process by modifying several existing protocols. Using the modified protocol, they isolated DNA from four fruit trees, grape (*Vitis* spp.), apple (*Malus* spp.), pear (*Pyrus* spp.) and persimmon (*Diospyros* spp.) and four species of conifers, *Pinus densiflora*, *P. koraiensis*, *Taxus cuspidate* and *Juniperus chinensis* within a few hours. As compared to the existing methods they could isolate high quality DNA (260/280 = 1.8 - 2.0) routinely yielding 250 - 500 ng/μl (total 7.5 - 15 μg DNA from 4 - 5 tissue discs).

Csaikl *et al.* (1998) compared four DNA extraction protocols for their ability to produce DNA from the leaves or needles of several species such as oak, elm, pine, fir, poplar and maize (fresh materials) and rhododendron (silica dried or frozen materials). With the exception of maize and poplar, the other species were known to be difficult for DNA extraction. Two protocols represented classical procedures for lysis and purification, and the other two were a combination of classical lysis followed by anion exchange chromatography. Test results indicated superiority of one of the protocols; a combination of CTAB lysis followed by anion exchange chromatography enabled DNA extraction from all seven species.

Khanuja *et al.* (1999) described a rapid DNA isolation protocol that can be used for diverse medicinal and aromatic plants, which produce essential oils and secondary metabolites. The procedure was applicable to dry as well as fresh plant tissues. The protocol permitted isolation of DNA from tissues of diverse plant

species and produced fairly good yields. The isolated DNA proved amenable to PCR amplification and restriction digestion.

Chaudhury *et al.* (1999) developed a procedure to isolate nuclear DNA from local cotton leaves (*Gossypium hirsutum*). The method consisted of rapid isolation of stable nuclei, which hinders covalent interactions with phenols, followed by DNA extraction. The yield and quality of the resulting DNA was satisfactory and the protocol could be scaled up or down according to sample size. It was suitable for PCR and restriction enzyme digestion necessary for Southern blotting and RFLP analysis.

Pirttila *et al.* (2001) developed two DNA isolation protocols for sundew and tarragon that produce DNA suitable for molecular biological applications. One of the methods was also suitable for milfoil and Siberian ginseng.

Rout *et al.* (2002) developed an alternative protocol for rapid isolation of genomic DNA from cashew plant with reproducible PCR amplification. The protocol described the use of extraction buffer (boric acid, CTAB, EDTA, NaCl and β-mercaptoethanol) and subsequently two steps of chloroform extraction to bind chlorophyll and proteins. Addition of 5 M NaCl and ethanol increased the solubility of polysaccharides, thereby effectively decreasing co-precipitation of the polysaccharides and DNA. DNA purification was made by using sodium iodide and washing buffer. The purified DNA was fairly reproducible in all varieties of cashew.

Keb-Llanes *et al.* (2002) described a rapid procedure for simultaneous extraction of clean DNA from many samples with little reagent waste. The procedure permitted the processing of 80 - 100 samples per day. They analyzed naturally propagated and micropropagated populations of henequen and other *Agavaceae* species using AFLP. The protocol involved precipitation and resuspension of DNA three times at the end of the preparations that increased DNA digestibility and the sharpness of AFLP bands.

Kawata *et al.* (2003) developed a simple protocol that could be used to isolate PCR-quality DNA adaptable to detect single and few copy-number DNA fragments from plant tissue. The method was fast and effective for screening various transformed rice tissues and other plant species. In designing the protocol, they

investigated several factors influencing the release of nucleic acid from the tissue, including the incubation time and buffer composition.

Michiels *et al.* (2003) optimized a CTAB protocol for the isolation of genomic DNA from latex-containing plants. Key steps in the modified protocols were the use of etiolated leaf tissue for extraction and an overnight 25°C isopropanol precipitation step. The purified DNA had excellent spectral qualities, was efficiently digested by restriction endonuclease and suitable for long fragment PCR amplification.

Since the current DNA isolation method had limitations in their ability to obtain quality and/or quantity DNA from *Terminalia arjuna*, *T. belerica* and *T. chebula*, which had low pH and high amounts of secondary metabolites in tissue extracts, Warude *et al.* (2003) developed a modified DNA isolation protocol to address the problem. The procedure yielded good quality, high-molecular weight DNA free from contaminants and coloured pigments and was suitable for PCR amplification. The method was also useful for isolating DNA from dry powders.

Sarwat *et al.* (2006) combined CTAB-based DNA isolation and a column-based purification step to isolate DNA from *Terminalia arjuna*. The DNA isolated using the standardized protocol was high in quality and suitable for restriction digestion and generation of RAPD and AFLP.

Buragohain and Konwar (2006) standardized a CTAB protocol based on Khanuja *et al.* (1999) for the isolation of genomic DNA from fresh leaves of *Zanthoxylum oxyphyllum*. The DNA isolated was of good quality and suitable for restriction digestion with *Eco*RI and *Hin*d III enzymes.

Khan *et al.* (2007) developed a simple and efficient protocol for isolating genomic DNA from fresh and dry roots of medicinal plants. The method involved a modified CTAB procedure using 3% CTAB, 4% β-mercaptoethanol, 2 M NaCl and 5% PVP. The extraction was carried out at 70°C. The DNA samples were found suitable for analysis with restriction digestion and RAPD. The yield and purity of isolated DNA was higher when compared with DNA extracted by the methods of Delaporta *et al.* (1983) and Doyle and Doyle (1990).

2.6 Genome size determination

The genome size of an organism is the amount of nuclear DNA in its unreplicated gametic nucleus, irrespective of the ploidy level of the taxon (Singh, 2003). The amount of DNA in an unreplicated gametic nucleus is referred to as 1C value. The 1C value is loosely referred to as genome size, but strictly speaking, genome size is the amount of DNA in an unreplicated, basic, gametic chromosome set (Soltis *et al.*, 2003). The genome size or nuclear DNA content is measured by weight or number of base pairs; where, 1 picogram (pg) = 978 megabases (Mb). As there is no literature found on the genome size of the present plant species, genome size of a few related and other species determined by flow cytometery has been reviewed.

Chung *et al.* (1998) attempted to determine the relationship between genome size, seed size and length in soybean (*Glycine max*). Flow cytometry was used to estimate the 2C nuclear DNA among 12 soybean strains, representing three distinct seed size groups. Variation of 2C nuclear DNA content was 14%, ranging from 2.37 pg for a small seed strain to 2.48 pg for a large seed strain. The results indicated that there was a significant correlation between genome size and leaf and seed size in soybean.

Arumuganathan *et al.* (1999) attempted to determine the genome size of 12 turfgrass species and an interspecific hybrid by means of flow cytometry and to compare genome size of warm and cool season grasses. The seven species and an interspecific hybrid of warm-season turfgrsses had genome size ranging from 0.86 - 1.95 pg/2C, while the genome size of five cool season grasses ranged from 5.65 - 15.59 pg/2C. The observation of the distinct genome size of the warm and cool season turfgrasses agreed with previous reports regarding genome sizes of tropical and temperate species in certain angiosperm families in Gramineae.

Johnson *et al.* (1999) attempted to compare 14 potential reference standards for plant DNA content determination by using flow cytometry. In their study, both chicken and plant internal standards were used, as were propidium iodide (PI) and 4'-6'-diamidino-2-phenylindole (DAPI) as fluorochromes. They recommended five species of plants as the initial set of international standards for future plant DNA

content determination. PI was recommended as fluorochrome of choice for flow cytometric determination of nuclear DNA content instead of DAPI, which should be used only if the estimated DNA value is corroborated by using a second stain that has no bias for AT- or GC-rich sequences within genomes.

Obermayer and Greilhuber (1999) investigated 10 Chinese accessions of *Glycine max* (five allegedly ranking high and five low) for genome size using PI flow cytometry and Feulgen densitometry. Using flow cytometry, the maximum difference between accessions was 1.018-fold (non-significant); the difference between the means of the high ranking and low ranking group was 1.002-fold (non-significant). With Feulgen densitometry, the maximum difference between accessions was 1.034-fold (non-significant). The data suggested genome size consistency, in terms of cytometric evidence, for the Chinese soybean accessions in question. Likewise, no reasonable evidence was obtained for a difference between Chinese and American soybeans.

Lysak *et al.* (2000) studied the intraspecific genome size variation in a perennial grass *Sesleria albicans* Kit. ex. Schult (Poaceae). Flow cytometry was used for the analysis of nuclear DNA content in 10 geographically isolated populations of *S. albicans*. Despite long-term isolation and lack of gene flow between the populations, only negligible inter-population differences were observed. Although the differences between the populations were statistically significant, the maximum inter-population difference reached only 1.6% of the mean 2C value (9.78 ± 0.04 pg). The study demonstrated that the species *S. albicans* belongs to the plant taxa with highly stable genome size.

Amsellem (2001) compared the ploidy level of *Rubus alceifolius* in its Asian native range and in Indian Ocean islands where it was introduced. The nuclear DNA content of other individuals from the native range and the areas of introduction were estimated using the flow cytometry method.

Suda (2004) attempted to establish flow cytometry technique into plant systematics and to identify new promising domains where flow cytometry should be particularly beneficial. In the study, he estimated ploidy level and nuclear DNA content in a few species of plants.

Greilhuber *et al.* (2005) discussed the currently unstable usage of the terms 'genome size' and 'C-value' and proposed a new unified terminology, which can describe nuclear DNA contents with ease and ambiguity. According to them, there is a need to maintain the term genome size in a broad sense as a covering term, because, it is widely understood, short and phonetically pleasing.

Dolezel *et al.* (2005) reviewed the current procedures for estimation of absolute DNA amounts in plants using flow cytometery, with special emphasis on preparation of nuclear suspensions, stoichiometric DNA staining and the use of DNA reference standards. In addition, methodological pitfalls encountered in estimation of intraspecific variation in genome size were discussed as well as problems linked to the use of DNA flow cytometry for fieldwork.

Hendrix and Stewart (2005) examined the standardization procedures used for DNA content determination with flow cytometry as applied to *Gossypium*, and reviewed DNA content estimates for all available *Gossypium* species using best standard practices. Both external and internal standardization with *Oryza sativa* 'IR36' yielded statistically similar DNA content estimates for *Gosypium*. Nuclear DNA content estimates were generated for 37 *Gossypium* species using external standardization. Due to unknown factors, internal standardization with *Hordeum vulgare* 'Sultan' may not be appropriate for DNA content determination of *Gossypium*.

Konwar *et al.* (2007) developed a new, simple and cost effective method for the determination of nuclear DNA content or genome size. This method of genome size determination involved several steps including measurement of average volume of single cell of leaf tissue, measurement of the volume of intercellular space in the leaf tissue, cell number and DNA present per g of leaf tissue and finally DNA present in a single cell of the leaf tissue of the plant. The genome size of *Streblus asper*, *Spondius pinnata*, *Zanthoxylum oxyphyllum*, *Rubus alceifolius* and *Meyna spinosa* were determined by this method and flow cytometry. The results were found comparable from the accuracy point of view.

Chapter 3
Materials and Methods

Chapter 3 Materials and methods

3.1 Medicinal plant diversity

3.1.1 Data collection

Data on medicinal plants were collected from primary and secondary sources. Regular field trips were conducted during the period from January 2002 to January 2004 in many areas of Assam. In the trips contacts were made with village heads, elderly people or herbal practitioners of different communities. Prior informed consent was obtained from the village heads and from the participants in the study. Information was collected from local herbal practitioners, elderly men and women through normal conversation, interview and discussion. A questionnaire was designed with regard to name of the plant, plant parts used and therapeutic uses for interviewing the participants of different communities. All discussions were conducted in Assamese, the official language of Assam. Only specific and reliable information, crosschecked with different informants at different places were incorporated. Data on medicinal plants were also collected from secondary sources, which included books written by local herbal practitioners (Sharma, 1978; Nath, 2001; Khanikar, 2002), newspapers and unpublished thesis (Gogoi, 1997).

3.1.2 Identification of plant species

The plant species collected were identified with the help of local floras (Bora and Kumar, 2003; Dutta, 1983, Kanjilal *et al.*, 1940), herbarium species of Dibrugarh University, Dibrugarh and Gauhati University, Guwahati and bulletin (Rao and Rabha, 1967; Rao, 1972) and herbarium species of Botanical Survey of India, Shillong. The information gathered was also crosschecked with some published literature on Indian medicinal plants (Annonymous, 1986, 1993, 1995; Chopra *et al.*, 1956; Kirtikar and Basu 1984; Kurien, 1995).

3.1.3 Morphology and phenology

The important morphological characters such as leaf shape, arrangement; type of inflorescence; flower colour and type of fruit were studied in the field and recorded in the notebook following the descriptions given by Lawrence (1967), Sharma (1993) and Subrahmanyam (1997). In some cases, plant species were collected and characters were studied in the laboratory. Plant height of the selected plants was measured with the help of a bamboo pole and a measuring tape. In other cases, a scale was used to measure length and breadth of leaves, length of petiole, diameter of flowers etc. For the exact colour of flower and floral parts, a colour chart was used, compared and recorded in the notebook. All data were recorded taking at least ten samples of the plants from different localities. The phenological data such as flowering and fruiting periods were studied and recorded at monthly intervals.

3.1.4 Preparation of herbarium

Herbarium of a number of important plant species were prepared by following the routine herbarium practice recommended by Jain and Rao (1997) and Sharma (1993). The plant parts bearing flowers or fruits or both were selected as the representative herbarium specimens. In some cases, along with flowers and fruits root systems were also selected for herbarium. After the collection, specimens were pressed in between two blotters in such a way that the entire bundle became uniformly thick in the middle and sideways. Special care was taken to keep some upper and lower leaves well exposed. After all such arrangements, pressing was done with the help of a field press made of thick and hard ply board. The press was tightly bound with ropes to prevent the wrinkling of the specimens. The blotters were changed at regular interval till the plant samples became dry. The specimens were then transferred to ordinary newspapers and then preserved by dipping the specimens in a saturated solution of laurylpentachlorophenate in ethyl alcohol to prevent fungal infection, insects attack etc. The specimens were then mounted on 28.75 x 41.25 cm herbarium sheets using quality glue. Data from field notebook were presented in the permanent label pasted on the lower right side of each

herbarium sheet. The label contained accession number, local name, English name, Botanical name, family name, place from where collected, collector's name, and a few distinguished features such as, flower colour, flowering and fruiting period etc. The voucher specimens were deposited in the Herbarium, Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Tezpur – 784028, Assam.

3.2 Plant species selected for cytological, biochemical and molecular characterization

After assessing the biodiversity of medicinal plants, a few major plants were selected for cytological, biochemical and molecular characterization. Three important and potential plant species were selected for the present investigation on the basis of their importance, potentiality and multiple therapeutic uses. The selected plant species were *Zanthoxylum oxyphyllum*, *Rubus alceifolius* and *Meyna spinosa*.

3.3 Determination of chromosome number and karyotype of the plants

Leaf buds were used for the determination of chromosome number and karyotype of the plants.

3.3.1 Chemicals and solutions

Carmine powder
Glacial acetic acid
Distilled water
Saturated paradichlorobenzene (*p*-DB)
Carnoy's fluid

3.3.2 Preparation of acetocarmine stain

1. In a 250 ml conical flask, a volume of 45 ml glacial acetic acid was added to 55 ml distilled water to prepare 100 ml of 45% acetic acid solution.
2. The mouth of the flask was loosely closed with cotton plug and the solution heated until boiling. Carmine powder (2 g for 2% and 1 g for 1% solution) was

weighed and added to the boiling acetic acid solution slowly and stirred gently with a glass rod.

3. Gentle boiling was continued till the dye dissolved properly in the solution.
4. The solution was allowed to cool down to room temperature and then filtered using two layers of Whatman (size 42) filter paper fitted in a glass funnel.

The filtrate was transferred into dark coloured bottles and stored at 4°C for subsequent uses.

3.3.3 Preparation of Carnoy's fluid

For fixing the material, Carnoy's fluid was used. It was prepared by mixing one part of glacial acetic acid with six parts of absolute ethanol and three parts of chloroform.

3.3.4 Collection, pre-treatment and fixation

1. Leaf buds were collected over a period of 10 h from 6.15 am to 4.15 pm at half-hourly interval. Timely collection of leaf buds was done to find out the proper time of cell division of the plant species.
2. The leaf buds were transferred into a conical flask containing the aqueous-saturated pretreatment solution of *p*-DB and kept for 4 h at 12°C.
3. After pretreatment for 4 h, leaf buds were washed thoroughly with distilled water to remove the chemical.
4. The leaf buds were then transferred to a conical flask containing the Carnoy's fluid (fixative) and then kept at 12°C for 24 h.
5. After fixing for 24 h, the leaf buds were taken out of the Carnoy's fluid and washed with distilled water.
6. The material was stored in 70% ethanol at 12°C for subsequent studies.

3.3.5 Tissue hydrolysis and staining

1. After the fixation, leaf buds were taken out from storage solution (70% ethanol) and washed thoroughly with distilled water.
2. Hydrolysis of leaf buds and simultaneous staining of chromosomes were done by transferring the leaf buds into 5 - 8 ml of 2% aceto-carmine stain and then kept at room temperature for 4 h, followed by heating on a heating mantel at 60°C for 10 min.

3.3.6 Squashing and slide preparation

1. The meristematic parts of the leaf buds were excised and transferred into a small drop of 1% aceto-carmine stain on a clean slide.
2. The meristematic tissue was then pressed by using needle and scalpel to convert into a thin layer of cell mass.
3. The cell mass was covered with a clean cover-slip and squashed carefully by applying uniform pressure on the cover-slip with the thumb. The thumb pressure was applied by placing a piece of blotting paper over the cover slip.
4. Enough care was taken not to allow air bubbles while putting the cover slip on the cell mass.
5. Finally good sides having the mounted cover slips were sealed with euperol to make airtight.

3.3.7 Viewing of slides

1. The semi-permanent slides thus prepared were observed under a compound microscope (Leica ATC 2000 Model) using the low magnification of 100*x*.
2. Slides revealing distinct cells were viewed under the high power of 400*x* for tracking the nucleus and chromosomes.
3. Finally cells were viewed under the oil emersion with 1000*x* for individual chromosome studies.
4. A total of 10 well-scattered and perfectly stained cells having the chromosomes in the late prophase and metaphase were marked for karyomorphological analysis of

the chromosomes following the standard method described by Levan *et al.* (1964).

5. Microphotographs of the well-illustrated cells were taken by using a C-5060 Wide Zoom Digital Camera attached on the Olympus – B x 41 Microscope at 1000*x* magnification.
6. Measurement of chromosomes was taken using an ocular micrometer.

3.3.8 Parameters recorded to describe karyomorphological traits

Total length of the individual chromosome –

Individual chromosome length was measured in μm by adding the length of the long arm and the short arm.

Relative length of chromosome (percentage length of individual chromosome) –

It is the ratio between the length of the individual chromosome and the total chromosome length of the haploid set expressed in percentage.

Chromosome arm ratio:

The arm ratio of each chromosome was calculated as –

R = L/S where, L is the length of the long arm and S is the length of the short arm

Centromeric index (I) –

The location of the centromere on the chromosome was expressed as the percentage of ratio between the short arm to the total length of the chromosome and was calculated as the centromeric index or I (Levan *et al.,* 1964). Individual chromosomes were named as metacentric and submetacentric based on centromeric index.

3.4 Isolation of biochemical compounds from the plants

Tender shoots of *Z. oxyphyllum*, tender leaves of *R. alceifolius* and mature fruits of *M. spinosa* were used for the isolation of active compounds.

3.4.1 (A) Preparation of crude extract from *Z. oxyphyllum*

Collected tender shoots of *Z. oxyphyllum* were air-dried and chopped. Chopped material (250 g) was extracted with 400 ml of methanol in a Soxhlet apparatus for 6 h and then the solvent was evaporated to dryness in a rotary evaporator. The procedure yielded 3.04 g of dark green methanol extract. The crude methanol extract was partitioned between equal volumes of ethyl acetate and water following the procedure described by Tori *et al.* (2003). The ethyl acetate soluble part was concentrated and the extract (1.02 g) was taken for further analysis.

3.4.1 (B) Fractionation of crude extract

The crude ethyl acetate extract was chromatographed over silica gel (60-120 mesh size) with column size 45 cm x 20 mm and flow rate 1ml/min, and eluted successfully in the order of increasing polarity with mixtures of n-Hexane and ethyl acetate and chloroform and methanol. The fraction eluted with n-Hexane and ethyl acetate (90:10, v/v) afforded the compound. **ZO1** and the fraction eluted with chloroform - methanol (95:5, v/v) afforded the compound **ZO2**. TLC was visualized by iodine spray followed by heating. Further analysis of **ZO2** could not be done due to poor yield and quality and discarded later on.

3.4.2 (A) Preparation of crude extract from *R. alceifolius*

The crude extract was prepared in the same way as described in the section 3.4.1. The procedure yielded 2.52 g of dark green methanol extract. The crude methanol extract was partitioned between equal volumes of ethyl acetate and water and the crude ethyl acetate extract (760.20 mg) was taken for further analysis.

3.4.2 (B) Fractionation of crude extract

The ethyl acetate extract was chromatographed over silica gel (60-120 mesh size) with column size 45 cm x 20 mm and flow rate 1ml/min and eluted in the order of increasing polarity with mixtures of chloroform - methanol. Each fraction was monitored by TLC and visualized by iodine spray followed by heating. Five fractions collected after elution with mixtures of chloroform - methanol (80:20 and

60:40 v/v) were combined according to similar TLC pattern. The combined fractions were rechromatographed over silica gel (60-120 mesh size) in the order of increasing polarity with mixtures of chloroform - methanol. The fraction eluted with chloroform - methanol (95:5 v/v) afforded the compound **RA1** (25.2 mg).

3.4.3 (A) Preparation of the crude extract from *M. spinosa*

The mature fruits of *M. spinosa* were shade dried, grounded and the crude extract was prepared in the same way as described in the section 3.4.1. The procedure yielded 4.42 g dark brown methanol extract. The crude methanol extract was partitioned between ethyl acetate and water in 1:1 proportion and the crude ethyl acetate extract (2.54 g) was taken for further analysis.

3.4.3 (B) Fractionation of crude extract

The ethyl acetate extract was subjected to silica gel (60-120 mesh size) column chromatography with column size 45 cm x 20 mm and flow rate 1ml/min and eluted in the order of increasing polarity with mixtures of chloroform - methanol starting from 90:10, 80:20 and so on. The eluate from mixtures of chloroform - methanol (90:10, v/v) yielded two fractions, of which the first fraction afforded the compound **MS1** (54.0 mg). The second fraction showed two spots in TLC and was further subjected to preparative TLC.

3.4.3 (C) Preparative TLC

The preparative TLC was performed on the TLC plate having size 30 cm x 20 cm and coated with silica gel. (60-120 mesh size). The TLC plate was put in the chromatographic chamber containing the mixtures of chloroform - methanol - water (95:5:1, v/v). After elution of the solvent the TLC plate was visualized by iodine spray followed by heating. The iodine visible portions were scraped, then dissolved in the same solvent system and eluted with a column where the silica gel retained and the dissolved compound passed through. After evaporation of the solvent the first fraction afforded the compound **MS2** (45.5 mg). The second fraction was discarded for further analysis because of poor quality and yield.

3.4.4 Identification of the isolated compounds

The isolated compounds **ZO1, RA1, MS1** and **MS2** were subjected to infrared (IR) spectroscopy, matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF MS), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy for their identification and characterization.

3.4.4.1 IR spectroscopy

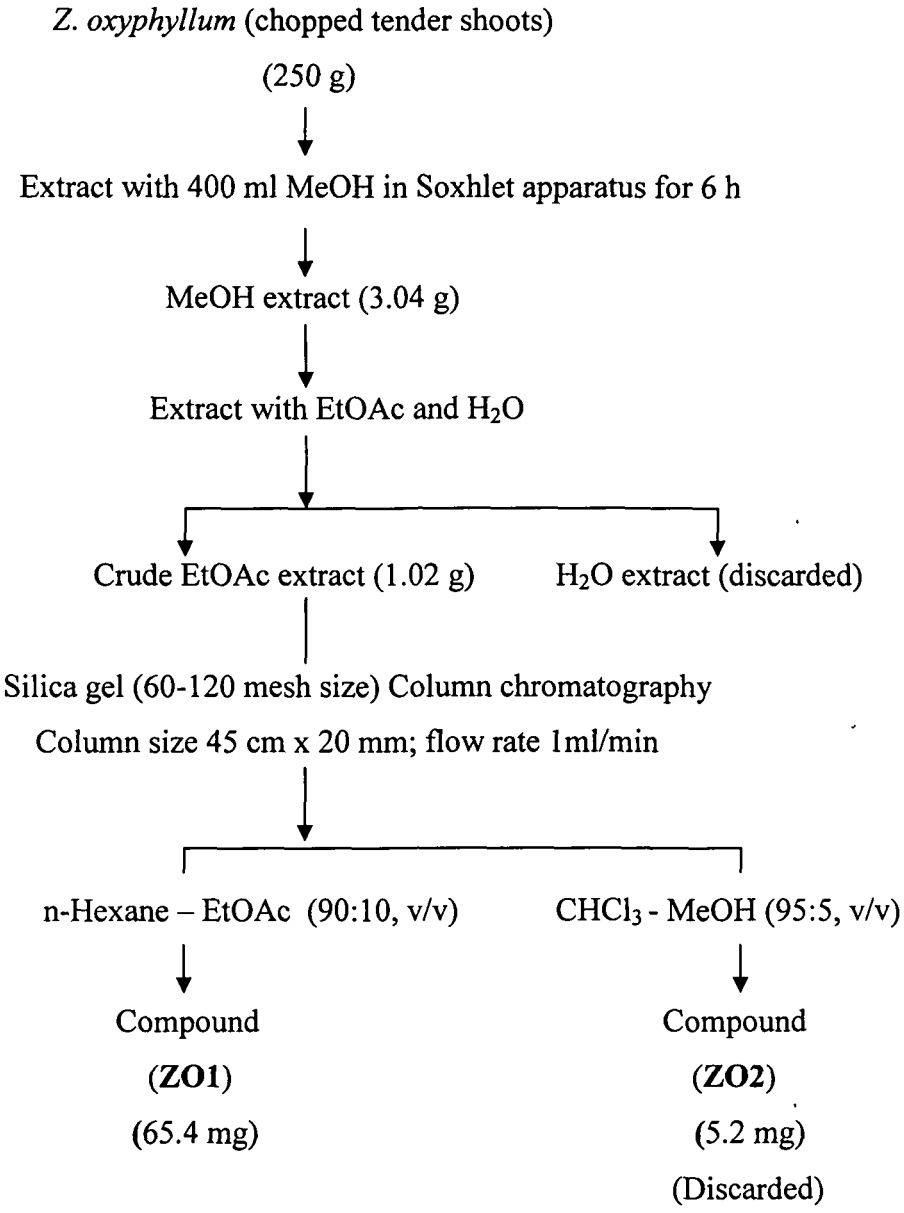
The IR spectra of the isolated compounds were recorded using KBr pellet in Nicolet Impact 410 FT-IR Spectrometer. From each sample, 5 mg was prepared by dispersing the sample uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. The spectra showing functional groups were used to study the composition of the compounds. IR spectra were collected from 500 – 4,000 wave numbers (cm^{-1}).

3.4.4.2 MALDI TOF MS

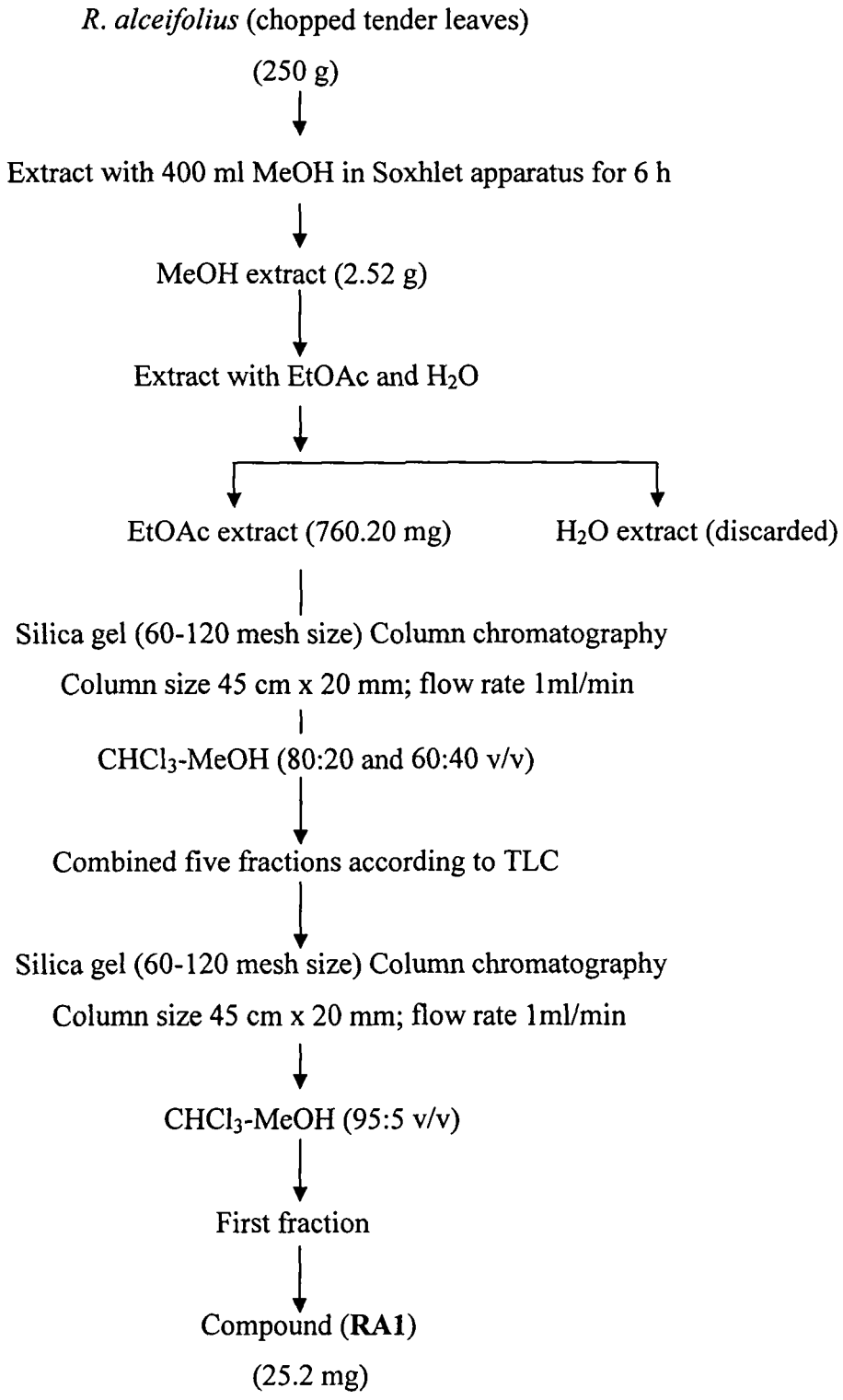
The mass spectra of the compounds were recorded in a Micromass TofSpec 2E instrument using a nitrogen 337 nm laser with 4-nanosecond pulse. α-cyano-4 hydroxycinnamic acid dissolved in acetonitrile/methanol (ACN/MeOH) was used as the matrix. Sample (1-2 mg) and matrix solutions were mixed together and 1 µl was spotted on MALDI target.

3.4.4.3 NMR spectroscopy

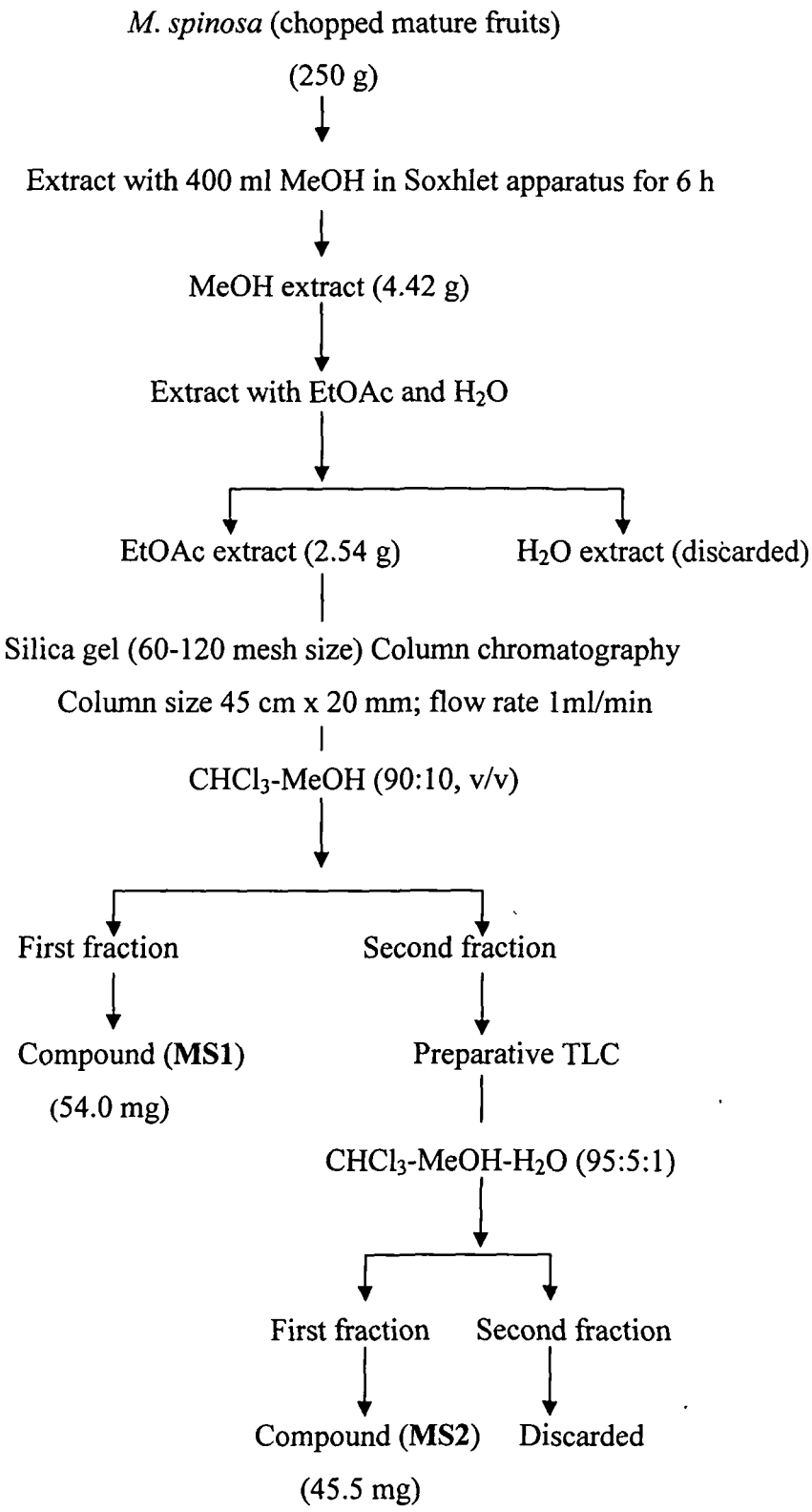
NMR spectra of the compounds were scanned on Varian Mercury 400 Spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei respectively. The duterated chloroform ($CDCl_3$) was used as solvent and tetramethyl silane (TMS) was used as internal standard. For ^{1}H NMR spectra 5 mg of sample and for ^{13}C NMR spectra 15-25 mg sample were taken for analysis.



Scheme 1.1 Isolation of compounds from *Z. oxyphyllum* (Tori *et al.*, 2003)



Scheme 1.2 Isolation of compound from *R. alceifolius* (Tori *et al.*, 2003)



Scheme 1.3 Isolation of compound from *M. spinosa* (Tori *et al.*, 2003)

3.5 Antimicrobial assay of the compounds

The isolated compounds were subjected to antimicrobial assay to determine the antimicrobial potential using four bacteria and one yeast.

3.5.1 Test organisms

The standard strains of Microbial Type Culture Collection (MTCC) procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India were used to assess the antimicrobial potential of the compounds. Bacterial strains of *Bacillus subtilis* (MTCC 619), *Klebsiella pneumoniae* (MTCC 109), *Escherichia coli* (MTCC 739), *Staphylococcus aureus* (MTCC 737) and yeast strain of *Candida albicans* (MTCC 3017) were used as test organisms.

3.5.2 Media

The bacterial test strains were maintained on nutrient agar (NA) medium. The yeast (*C. albicans*) was grown on potato dextrose agar (PDA) medium. For antimicrobial activity test, Mueller-Hinton (MH) agar medium was used. The compositions of the media are given below:

3.5.2.1 NA medium

Component	g/l
Peptone	10.0
Beef extract	10.0
Sodium chloride	5.0
Agar	12.0

pH = 7.3

3.5.2.2 PDA medium

Component	g/l
Potato infusion	4.0
(Infusion from 200 g potatoes)	
Dextrose	20.0
Agar	15.0

pH = 5.6

3.5.2.3 MH agar medium

Component	g/l
Beef infusion	300.0
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17.0

pH = 7.2

3.5.3 Determination of antimicrobial potential

Agar well diffusion method was followed to determine the antimicrobial potential of the compounds using Mueller-Hinton (MH) agar medium. Suspension of 1.5 x 10^8 cells ml^{-1} in normal saline was prepared and about 1.5 ml of each strain was uniformly swabed on MH medium in 9 x 2.5 cm sterile glass petri dishes. The plates were left aside for 15 min at room temperature and excess of suspension was drained and discarded properly. Wells of 6 mm in diameter were prepared in the culture medium using sterile cork borers. Each test compound was dissolved in DMSO (dimethylsulfoxide) and administered to fullness in each well at the concentration of 250 µg ml^{-1}. The culture plates were incubated at 37°C and 30°C for bacteria and yeast respectively. The bioactivity was determined after 24 h for bacterial and 48 h for yeast strains by measuring the diameter of the inhibition zone (DIZ) in mm. Controls included the DMSO solvent without test compounds.

3.6 Genomic DNA isolation

Fresh young leaves were used for the isolation of genomic DNA from the plant species. The leaves were collected in the morning, placed in between moist tissue papers and then stored in darkness at room temperature.

3.6.1 Equipment

Autoclave

Mortar and pestle

Mettler electronic balance

Micropipettes – (2-20 µl, 20-200 µl and 200-1000 µl)

Microtips

Refrigerator

Polypropylene tube (25 ml)

Microcentrifuge tube (1.5 ml)

Microwave oven

Incubator (37°C)

Sorvall RC 5*B* Plus centrifuge

Bench top centrifuge (Hettich Zentrifugen, MIKRO 12-24)

Magnetic stirrer

Shaking hot water bath

Speed vacuum (Maxi dry plus, Hoefer Pharmacia Biotech Inc., USA)

Gel Doc system (BIO RAD Gel Doc 1000)

Vertical Gel Apparatus

UV/VIS Spectrophotometer (Beckman DU® 530 Life Sciences)

3.6.2 Reagents and chemicals

Tris-Cl pH 8.0 (1.0 M)-

Tris base 121.1 g was dissolved in 800 ml of dH_2O. The pH was adjusted to 8.0 by adding concentrated HCl. The solution was allowed to cool to room temperature. The volume was adjusted to 1 liter and sterilized by autoclaving. The solution was stored at room temperature.

EDTA pH 8.0 (0.5 M)-

Na_2 EDTA.$2H_2O$ 186.1 g was dissolved in 700 ml of dH_2O. The pH was adjusted to 8.0 with 10 M NaOH (~ 50 ml). The volume was adjusted to 1 liter and sterilized by autoclaving. The solution was stored at the room temperature.

NaCl (5.0 M)-

NaCl 292 g was added to 900 ml of dH_2O and the volume was adjusted to1.0 liter

CTAB (20%)

Chloroform: Isoamyl alcohol (24:1 v/v)

PVP

β -mercaptoethanol

Liquid Nitrogen

Bromophenol blue (Loading dye)

Ethidium bromide (Fluorochrome)

Isopropanol

RNase

Agarose

Hind III digested λ DNA molecular weight marker (Banglore Genei, India)

Eco RI and *Hind* III restriction enzymes (Banglore Genei, India)

3.6.3 Buffers

DNA extraction was performed using extraction buffer, high salt TE buffer and TAE buffer.

3.6.3.1 Extraction buffer

100 mM Tris-Cl (pH 8.0)

25 mM EDTA

1.5 M NaCl

2.5% CTAB

0.2% β -mercaptoethanol (v/v) – added immediately before use

4% PVP (w/v) – added immediately before use

3.6.3.2 High salt TE buffer

1 M NaCl

10 mM Tris-Cl (pH 8.0)

1 mM EDTA

3.6.3.3 TAE buffer

For 100 ml stock solution

24.2 g Tris-Cl

57.1 ml Glacial Acetic Acid

10.0 ml EDTA (pH 8.0)

3.6.4 Loading dye and fluorochrome

Bromophenol blue and ethidium bromide were used as loading dye and fluorochrome, respectively for DNA visualization during and after electrophoresis.

3.6.4.1 Bromophenol blue (6x, 4.0 ml)

Bromophenol blue 10 mg

Xylene cyanol 10 mg

Glycerol 1.2 ml (autoclaved)

3.6.4.2 Ethidium bromide (10 mg/ml)

Ehidium bromide 100 mg

Sterile dH_20 10 ml.

Stored at 4°C in darkness

3.6.5 DNA extraction protocol

A CTAB based DNA isolation protocol was standardized based on the earlier protocol described by Khanuja *et al.* (1999).

1. Fresh leaves weighing 3 g of the selected plant was ground into fine powder in liquid nitrogen in a chilled mortar.
2. The powder was transferred directly to a 25 ml polypropylene tube and 6 ml of freshly prepared preheated extraction buffer were added and mixed by gentle inversion to slurry.
3. The sample was incubated at 60°C in a shaking waterbath (100 rpm) for 2 h with occasional mixing to avoid aggregation of the homogenate.
4. Chloroform: isoamyl alcohol (24:1) 6 ml was added to the extract and mixed by inversion for 10 min.
5. The extract was centrifuged at 7,650 x *g* (8,000 rpm, SS 34 rotor) in a Sorvall RC-5B Plus centrifuge for 10 min at 25°C. The upper phase was transferred to a

clean 25 ml polypropylene tube and the process was repeated twice to clear the aqueous phase.

6. An aliquot of 3 ml of 5 M NaCl was added to the aqueous phase and mixed properly by gentle inversion without vortexing.
7. Isopropanol (0.6 volumes) was added to the mixture and was mixed by inversion. The mixture was then incubated at room temperature (25-30°C) overnight to precipitate the nucleic acid.
8. The sample was centrifuged at 11,952 x *g* (10,000 rpm) in the Sorvall RC 5*B* Plus centrifuge (SS 34 rotor) for 10 min at 25°C. The supernatant was poured off, the pellet was washed with 80% ethanol and carefully transferred to a clean microcentrifuge tube. The pellet was again washed with 80% ethanol.
9. The pellet was dried in the speed vacuum (Maxi dry plus, Hoefer Pharmacia Biotech Inc., USA) for 15 min and dissolved in 0.5 ml of high salt TE buffer. 5 µl of RNase was added to the sample and incubated at 37°C for 1.5 h.
10. After incubation, the sample was extracted with equal volume of chloroform: isoamyl alcohol (24:1). The aqueous layer was transferred to a fresh 1.5 ml microcentrifuge tube and added 2 volumes of pre-cooled ethanol.
11. The sample was then centrifuged at 7,500 x *g* (10,000 rpm) for 10 min in a bench top centrifuge (Hettich Zentrifugen, MIKRO 12-24) at room temperature (25-30°C) to precipitate the DNA.
12. The pellet was rinsed with 80% ethanol, dried in speed vacuum (Maxi dry plus, Hoefer Pharmacia Biotech Inc., USA) and resuspended in 200 µl of high salt TE buffer.

3.6.6 Modifications of the protocol

To purge the polyphenolic contents of leaf tissues of the plants, the concentration of PVP was increased from 1% to 4% in the extraction buffer. The chloroform: isoamyl alcohol (24:1) washing step was performed twice to clear the aqueous phase of the extract. Different isopropanol incubation time from 1 h to overnight and temperature from 4°C to room temperature (25-30°C) was tried to

precipitate the DNA. These modifications were also checked for DNA yield and purity.

3.6.7 Spectrophotometric quantification of isolated DNA

The concentration and the purity of the isolated DNA were measured by taking the reading at 260 nm and 280 nm in a UV/VIS spectrophotometer (Beckman DU® 530 Life Sciences) against blank and diluted sample. Isolated DNA sample (5 µl) was taken in a quartz cuvette and made up the volume to 1 ml by adding double distilled water. Since 1 OD (optical density) corresponds to 50 µg of double stranded (ds) DNA/ml, the following calculation was done to determine the concentration of DNA:

DNA concentration (µg/ml) =

(OD_{260}) x (dilution factor) x (50 µg/ml)

The ratio of absorbance of DNA solution at 260 nm/280 nm is a measure of the purity of DNA sample and it should be in between 1.75 to 2.00.

3.6.8 Restriction digestion of isolated DNA

The extracted DNA samples were subjected to single digestion with *Eco* RI, *Hind* III (Bangalore Genei, Banglore, India) and double digestion with *Eco* RI and *Hind* III restriction endonuclease enzymes. For the purpose, 1.5 ml capacity eppendorf tubes were serially labeled for the restriction enzymes. DNA sample (5 µl) was aliquoted into each labeled tube for digestion and the final volume of each sample was made up to 25 µl. The following were the reaction mixtures.

3.6.8.1 Single digestion

Eco RI/*Hind* III	10 units
Buffer	2.0 µl
Sterile water	18.0 µl
DNA sample	5.0 µl (8-10 µg)

3.6.8.2 Double digestion

Eco RI	10 units
Hind III	10 units
Buffer	4.0 µl
Sterile water	14.0 µl
DNA sample	5.0 µl (8-10 µg)

* 1 µl of enzyme is equivalent to 10 units of enzymes.

The reaction mixtures were incubated at 37°C for 6 h. The digested DNA samples were supplied with 5 µl of loading dye and then incubated for another 15 min.

3.6.9 Agarose gel electrophoresis

1. Agarose 0.8% solution was prepared in a 200 ml conical flask by adding 49 ml of distilled water, 1ml of TAE buffer (1x) and 0.4 g of agarose powder.
2. The solution was put in a microwave oven for 1-3 minutes until the agarose was fully dissolved and the solution becoming clear. Prior to the solidification of the agarose gel, 2 µl of ethidium bromide (final concentration 0.5 µg ml^{-1}) was added to the solution.
3. The pre-cleaned gel tray was cello-tapped in both end and the comb placed in the tray approximately 1 inch from one end. The comb was positioned vertically in such a way that the teeth were about 1 - 2 mm above the bottom of the tray.
4. The gel solution was poured into the tray to a depth of approximately 5 mm and then allowed to solidify for about 30 min at room temperature.
5. After the solidification of the gel, the comb and cello-tapes were removed. The gel was then put in the electrophoresis tank and then covered with about 250 ml of 1 x TAE buffer or until the wells of the gels were submerged.
6. From each digestion mixture, DNA sample (5 µl) was taken and mixed with 5 µl of bromophenol blue. All samples were then loaded into the wells of the gel.

7. Marker DNA (3 µl λ *Hin*d III digested DNA) was loaded on the extreme left side of the gel or on both sides of the gel. The gel was electrophoresed at 150 V for 1 h until the dye marker migrated to two third distance of the gel.
8. After electrophoresis, the DNA was visualized in a trans-illuminator and documented by taking photographs in a Gel Doc system (BIO RAD Gel Doc 1,000).

3.7 Genome size determination

Genome size of the plants was determined by using flow cytometry and microscopy methods.

3.7.1 Flow cytometry

A one-step DNA flow cytometry procedure (Otto, 1990) with minor modifications was followed for the determination of genome size using Otto I and Otto II buffer.

3.7.1.1 Otto I buffer

0.1 M citric acid monohydrate 4.2, g
0.5% (v/v) Tween 20 1.0 ml.
Volume adjusted to 200 ml and kept at 4°C

3.7.1.2 Otto II buffer

0.4 M Na2HPO4.12H2O 28.65 g
Volume adjusted to 200 ml and kept at room temperature

3.7.1.3 Stain or flurochrome

Propidium Iodide 50 µg/ml
RNase 50 µg/ml

3.7.1.4 Procedure

1. About 20 mg of fully grown young leaves were collected from a selected plant, washed thoroughly and chopped with a new razor blade in 0.5 ml of ice cold Otto I buffer in a Petri dish.
2. Then added another 0.5 ml of ice cold Otto I buffer and mixed thoroughly with a pipette.
3. The suspension was filtered through a 42 μm nylon mesh and the sample was incubated for 5 min with occasional shaking.
4. Otto II buffer 2 ml was added to the sample along with the stained solution (200 μl of stock solution) and stored at room temperature for about 15 min.
5. The sample was analyzed in a FACS Calibur flow cytometer (Becton Dickinson, USA) for relative DNA content of isolated nuclei. The instrument was calibrated using FACS COMP software.
6. Garden pea (*Pisum sativum*) was used as the external reference standard. The use of an internal reference standard gave poor reading of results in peak quantities, probably resulting from interference between the staining solutions and the genome of pea and the selected species. For this reason external reference standard was used and controlled every 3 samples to check the calibration of the flow cytometer.
7. The gain of the instrument was adjusted so that G_0/G_1 peak of pea (reference standard) was positioned at channel 200.
8. The nuclear DNA content of the plant samples was estimated according to the equation:

   2C nuclear DNA content of the sample =

   (9.09 x G_0/G_1 peak mean of the sample) / G_0/G_1 peak mean of pea
9. The means of nuclear DNA content were calculated for each sample and analyzed as a single value.

3.7.2 Microscopy method

Konwar *et al.* (2007) developed a new and easy method for the determination of genome size of the plants by measuring the DNA content of a single somatic cell

nucleus. In this method, the average volume of a cell from the leaf tissue was calculated. The number of cells present in a particular leaf tissue with known volume and weight [less 7 – 70% cells as intercellular space depending upon the species and the habitat of the particular plant (Turrell, 1934)] was calculated. If, the amount of DNA per g fresh tissue, number of cells per g tissue are known, the amount of DNA in a single cell can be calculated easily by dividing the amount of DNA present in that tissue by the total number of cells in the same tissue. Thus, the genome size of a particular plant can be worked out as the amount of DNA present in the haploid set of its chromosomes.

3.7.2.1 Cell volume determination in leaf tissue

Fine transverse and longitudinal cross sections of the pre-weighed leaf tissue of 1 cm^2 size were obtained with a sharp razor blade and observed under a microscope (Leica ATC 2000) at 400*x* magnification. The volume of the whole tissue section (1 cm^2) was determined by the formula: length x breath x thickness. The length, breath and thickness of rectangular cells, length and radius of cylindrical cells and radius of spherical cells were measured with a micro scale having 400x magnification. Data generated from five randomly selected cells of five random sections as well as cell volumes were recorded with specific formula. The intercellular space of the leaf tissue of the plant was measured in five small leaf sections of known dimensions by measuring with a microscale at 400*x* magnification.

3.7.2.2 Mathematical deduction of cell number in the leaf tissue

Average volume of a single cell (l x b x t) = $x\ \mu^3$ where, l = length

b =breath

t =thickness

Volume of the tissue (l x b x t) = $t\ \mu^3$

Volume of the intercellular space = $s\ \mu^3$

Actual cell mass	$= (t - s) = v\ \mu^3$
Total number of cells in the cell mass	$= v\ \mu^3 / x\ \mu^3 = y$
Now, weight of the tissue section	$= w$ g
w g tissue contains	$= y$ cells
So, 1 g tissue will contain cells	$= y/w = d$
DNA yield per gram of leaf tissue	$= d\ \mu$g
	$= d \times 10^6$ pg
So, one cells contains	$= (d \times 10^6) / (y/w)$ pg
	$= (d \times 10^6) / (y/w) \times 978$ Mbp

Chapter 4
Results

Chapter 4 Results

The investigation was carried out with a view to explore the uses of the medicinal plants of the region. The selected plant species were scientifically assessed with respect to karyological study, isolation of compounds, antimicrobial potential of the isolated compounds, isolation of genomic DNA and determination of genome size. The investigation generated interesting results with respect to the above-mentioned parameters, which are presented below.

4.1 Medicinal plant diversity

In the present investigation, information on 400 medicinal plants belonging to 128 plant families were collected, verified and authenticated, which are presented in Annexure I. Life forms of the recorded plant species, most cited plant families, most widely used plant parts and most cited ailments cured by the recorded medicinal plants are presented in the Fig. 4.1, 4.2, 4.3 and 4.4.

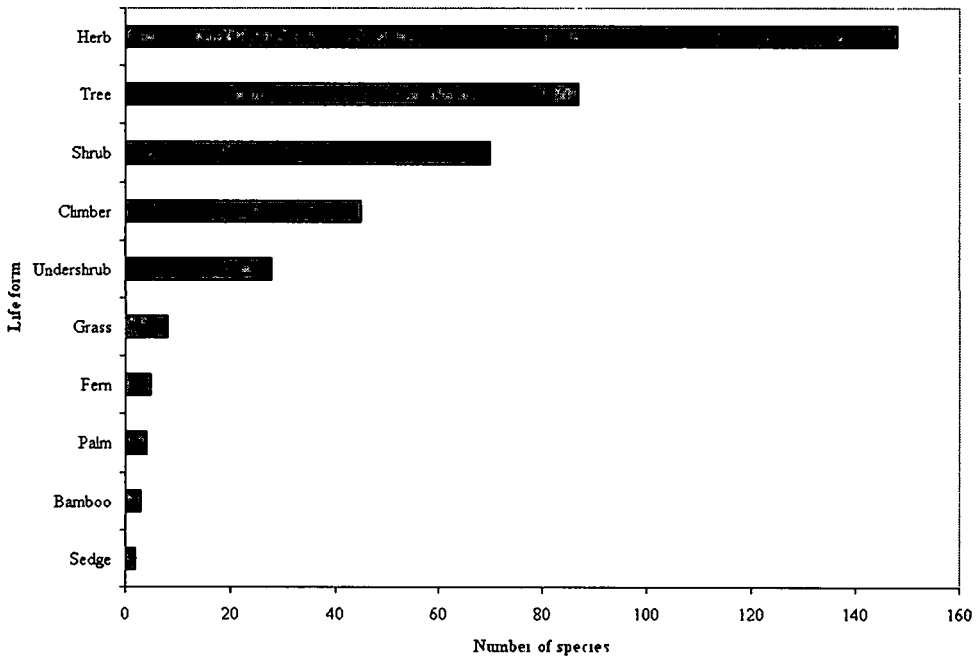


Fig. 4.1 Life forms of the recorded medicinal plant species

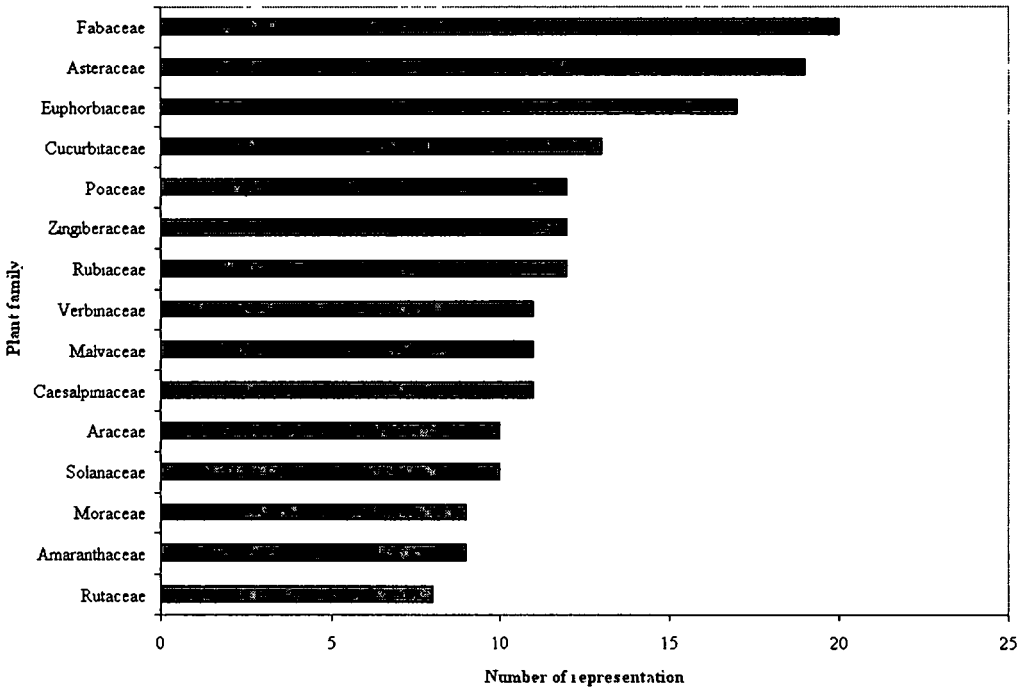


Fig. 4.2 Most cited families incorporating the recorded plant species

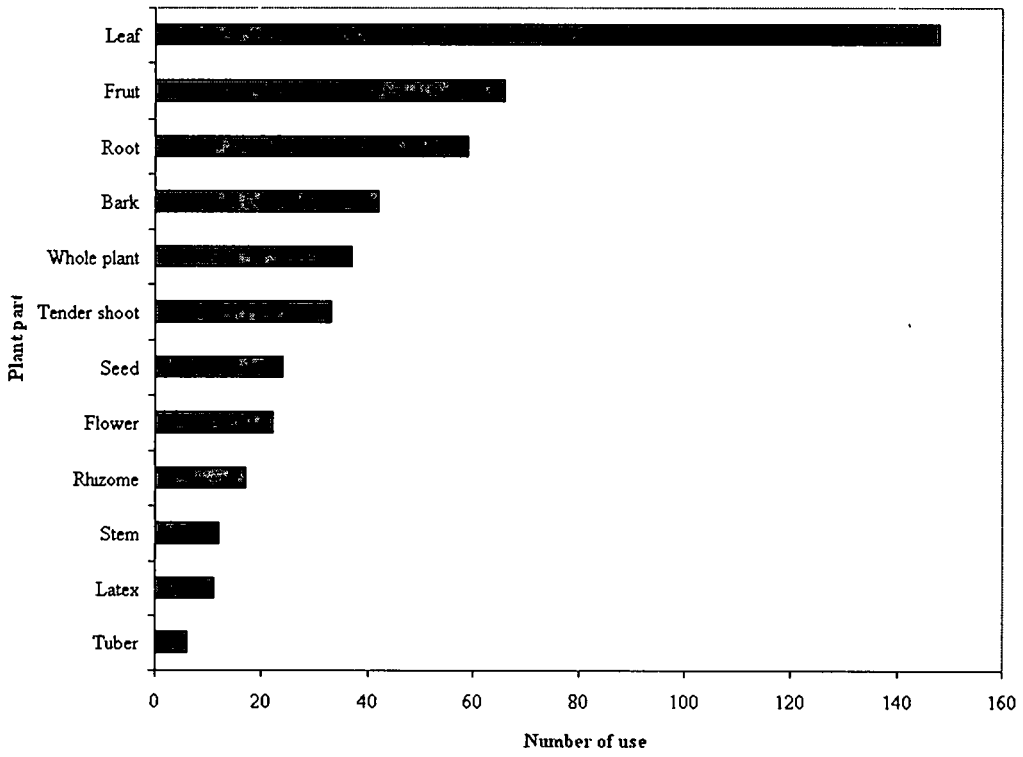


Fig. 4.3 Most widely used plant parts

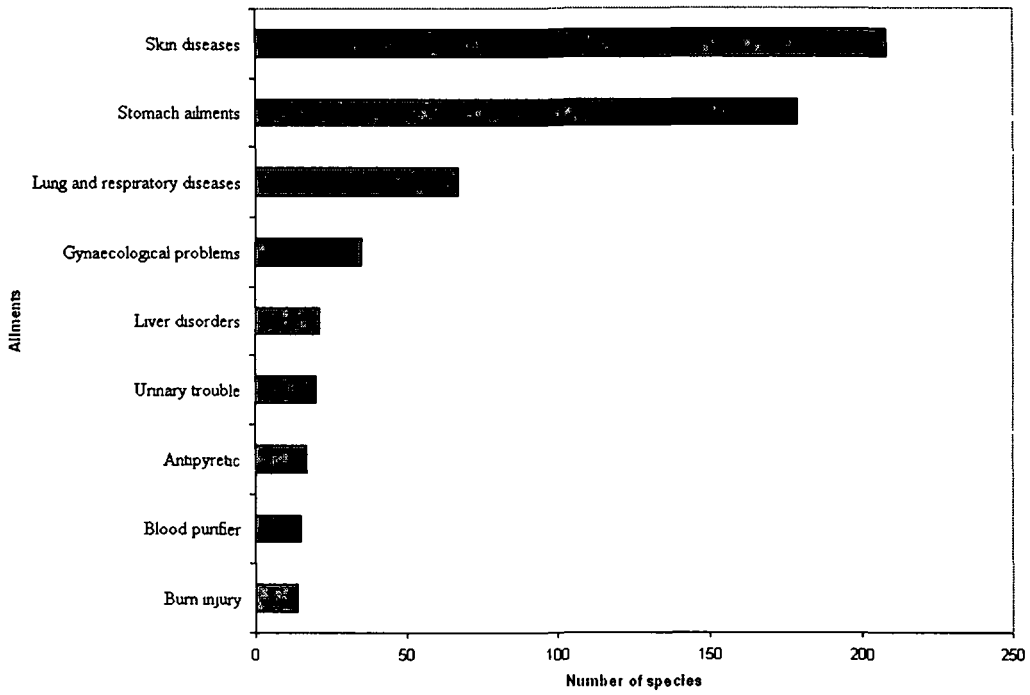


Fig 4.4 Most cited ailments claimed to be cured by the recorded medicinal plants

4.2 Morpho-phenological characters of the selected plants

4.2.1 *Z. oxyphyllum*

The plant is a slender, erect, aromatic, glabrous, 3.50 - 8.0 m tall, large shrub or scrambling tree with hooked prickles having large and septate pith. Leaves are 15.2 - 35.5 cm long and the rachis is armed with prickles beneath and with two faint longitudinal ridges above. The leaflets are strongly aromatic, widely variable in size and shapes, opposite and alternate, about 2.5 - 7.5 cm long and 1.25 - 2.8 cm broad, coriaceous, crenate or crenulate with a large translucent gland at each sinus. 10 - 12 lateral nerves are present on either side of the midrib of the leaf. The bases of the leaflets are acute with 0.15 - 0.5 cm long petioles. Flowers are pale yellow, polygamous, 0.38 - 0.75 cm across with slender pedicels and umbelled in much branched, panicled, axillary cymes. Petals and sepals are 4 in number, obtuse and imbricate. Fruits are one-seeded cocci, aromatic; seeds 0.35 cm across, globose, blue-black and shining. The plant flowers in the month of early March and continues up to April, whereas fruiting occurs in May and matures after one month.

4.2.2 *R. alceifolius*

The plant is 2.5 - 3.0 m tall straggling or subscandent shrub with hooked prickles. Initially young shoots appear to be whitish or fulvous and silky but later on become tomentose, often mixed with longer hairs. The stems are robust, 10 - 50 cm in diameter at the base. Prickles are 0.65 cm long, scattered over the branches, petioles, midribs and sometimes on the veins. Leaves are pubescent, 5 - 15 cm long, breath is slightly less than the length, broadly ovate or suborbicular, 3 - 7 lobed; lobes rounded to acute or acuminate, unequally serrate-dentate, base cordate, usually 5-nerved, nearly smooth, thinly pubescent, sometimes hirsute or glabrous above, undersurface greyish tomentose and with spreading hairs on the veins or hairy along with the veins. Petioles are 1.5 - 10 cm long, greyish tomentose, hirsute or pubescent. Stipules are leafy and ovate-lanceolate or oblong with incised margins, 0.75 - 2.5 cm long, pubescent or tomentose. Flowers are white, 1.25 - 2.0 cm across, occurring in short, few flowered axillary racemes or in contracted terminal panicles. Pedicels are 0.75 - 1.25 cm long, bracts 0.5 - 2.5 cm long and resemble stipules or elliptic to orbicular or sometimes almost entire. Calyx is densely fulvous or grey silky, lobes 0.5 - 1.25 cm long, ovate-lanceolate, entire or cleft at the apex, or with a few teeth on the upper half and more or less spreading on fruits. Petals are shorter than the calyx lobes, white and obovate. Stamens and carpels are numerous and glabrous. Fruits are globose, 1.0 - 1.5 cm across, sometimes longer than the calyx, scarlet when ripe and succulent. Flowering appears from early July and continues up to late August. Fruiting occurs in late August and continues till September.

4.2.3 *M. spinosa*

The plant is 2.8 - 5.5 m tall, spiny deciduous shrub or small tree with vertically cracked brown or deep grey barks. The spines are straight, about 1.75 cm long and somewhat supra axillary. Leaves are opposite, 3.8 - 12.5 cm long and 2.5 - 6.85 cm broad, entire, ovate-elliptic or elliptic-ovate, acute or bluntly acuminate, membranous and more or less glabrous. Lateral nerves are 6 - 9 on both half and curving upwards near the margin. Nerve axils are with tufts of hairs. Petioles are 0.5

- 1.25 cm long; stipules are connate and cuspidate from a broad base. Flowers are greenish white and appear on short peduncled cymes, which are axillary or supra axillary. Calyx is 5-toothed. Corolla tubes are short, sub-globose, throat wooly and lobes spreading. Stamens are 5 in number, sub-sessile and anthers exserted. Ovary is 5-celled, style long, stigma lobed, ovules pendulous and solitary. Fruits are fleshy, globose or obovoid drupes, about 2.5 - 3.75 cm across, yellowish when ripe with 3 - 5 woody seeds. The plant flowers in late March, continues till April while fruiting appears form late April and matures in the month of October to early December.

4.3 Chromosome number and karyotype of the plants

4.3.1 *Z. oxyphyllum*

The somatic chromosome number of *Z. oxyphyylum* was shown in Fig. 4.5 and the karyomorphology was presented in Table 4.1. Number of chromosomes was observed to be 2n = 36. The total length of haploid chromosome was measured to be 22.6 μm. The length of the chromosome varies from 0.8 - 2.0 μm. Based on the position of the centromere, chromosomes were divided into two groups, group I consisted of 12 chromosome pairs having median centromere and group II consisted of 6 chromosome pairs having submedian centromere.

4.3.2 *R. alceifolius*

The somatic chromosome number of *R. alceifolius* was given in Fig. 4.6 and the karyomorphology was presented in Table 4.2. A study of 10 randomly distributed cells undergoing metaphase stage revealed chromosome number of *R. alceifolius* to be 2n = 28. The total length of haploid chromosomes was measured to be 17.3 μm. The length of chromosome varies from 0.7 - 1.8 μm. Based on the position of the centromere, chromosomes were divided into two groups, group I consisted of 10 chromosome pairs having median centromere and group II consisted of 4 chromosome pairs having submedian centromere.

4.3.3 *M. spinosa*

The study of 10 metaphase cells revealed chromosome number of *M. spinosa* was 2n = 44. The somatic chromosome number was shown in Fig. 4.7 and the karyomorphology was presented in Table 4.3. The total length of haploid chromosomes was 30.8 μm. The length of chromosome varies from 0.8 - 2.4 μm. Based on the position of the centromere, chromosomes were divided into two groups, group I consisted of 12 chromosome pairs having median centromere and group II consisted of 10 chromosome pairs having submedian centromere.

Table 4.1 Karyomorphology of *Z. oxyphyllum* (M-Metacentric; SM-Submetacentric)

Chromosome pair	Long arm (μm)	Short arm (μm)	Total length (μm)	Arm ratio	Centromeric index	Centromeric position	Group
1	0.9	0.9.	1.8	1.0	50.0	M	I
2	1.0	1.0	2.0	1.0	50.0	M	I
3	0.8	0.8	1.6	1.0	50.0	M	I
4	0.8	0.8	1.6	1.0	50.0	M	I
5	0.5	0.5	1.0	1.0	50.0	M	I
6	0.6	0.6	1.2	1.0	50.0	M	I
7	0.6	0.6	1.2	1.0	50.0	M	I
8	0.6	0.6	1.2	1.0	50.0	M	I
9	0.9	0.3	1.2	3.0	25.0	SM	II
10	1.0	0.4	1.4	2.5	29.0	SM	II
11	1.0	0.4	1.4	2.5	29.0	SM	II
12	0.6	0.3	1.0	2.0	30.0	SM	II
13	0.8	0.2	1.0	4.0	20.0	SM	II
14	0.8	0.2	1.0	4.0	20.0	SM	II
15	0.6	0.6	1.2	1.0	50.0	M	I
16	0.6	0.6	1.0	1.0	50.0	M	I
17	0.4	0.4	0.8	1.0	50.0	M	I
18	0.4	0.4	0.8	1.0	50.0	M	I

Table 4.2 Karyomorphology of *R. alceifolius* (M-Metacentric; SM- ubmetacentric)

Chromosome pair	Long arm (μm)	Short arm (μm)	Total length (μm)	Arm ratio	Centromeric index	Centromeric position	Group
1	0.6	0.6	1.2	1.0	50.0	M	I
2	0.6	0.6	1.2	1.0	50.0	M	I
3	0.7	0.7	1.4	1.0	50.0	M	I
4	0.8	0.8	1.6	1.0	50.0	M	I
5	0.9	0.9	1.8	1.0	50.0	M	I
6	0.5	0.2	0.7	2.5	50.0	SM	II
7	0.6	0.6	1.2	1.0	50.0	SM	II
8	0.7	0.7	1.4	1.0	50.0	SM	II
9	0.6	0.6	1.2	1.0	50.0	SM	II
10	0.8	0.8	1.6	1.0	50.0	M	I
11	0.5	0.5	1.0	1.0	50.0	M	I
12	0.5	0.5	1.0	1.0	50.0	M	I
13	0.6	0.6	1.2	1.0	50.0	M	I
14	0.4	0.4	0.8	1.0	50.0	M	I

Table 4.3 Karyomorphology of *M. spinosa* (M-Metacentric; SM-Submetacentric)

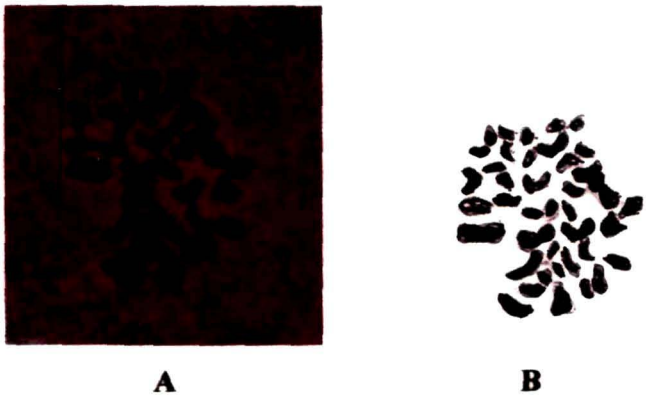
Chromosome pair	Long arm (μm)	Short arm (μm)	Total length (μm)	Arm ratio	Centromeric index	Centromeric position	Group
1	1.2	1.2	2.4	1.0	50.0	M	I
2	1.1	1.1	2.2	1.0	50.0	M	I
3	0.9	0.9	1.8	1.0	50.0	M	I
4	0.8	0.8	1.6	1.0	50.0	M	I
5	1.0	0.4	1.4	2.5	29.0	SM	II
6	0.8	0.4	1.2	2.0	35.0	SM	II
7	0.8	0.4	1.2	2.0	35.0	SM	II
8	0.9	0.9	1.8	1.0	50.0	M	I
9	0.8	0.8	1.6	1.0	50.0	M	I
10	0.8	0.4	1.2	2.0	35.0	SM	II
11	0.8	0.2	1.0	4.0	20.0	SM	II
12	0.7	0.7	1.4	1.0	50.0	M	I
13	0.8	0.4	1.2	2.0	35.0	SM	II
14	1.0	0.4	1.4	2.5	35.0	SM	II
15	0.8	0.4	1.2	2.0	35.0	SM	II
16	0.6	0.6	1.2	1.0	50.0	M	I
17	1.0	0.4	1.4	2.5	29.0	SM	II
18	1.0	0.4	1.4	2.5	29.0	SM	II
19	0.6	0.6	1.2	1.0	50.0	M	I
20	0.4	0.4	0.8	2.0	50.0	M	I
21	0.5	0.5	1.0	1.0	50.0	M	I
22	0.6	0.6	1.2	1,0	50.0	M	I

A B

Fig. 4.5 **A.** Mitotic chromosome of *Z. oxyphyllum* at metaphase stage; **B.** Camera lucida drawing (2n = 2*x* = 36)

A B

Fig. 4.6 **A.** Mitotic chromosome of *R. alceifolius* at mataphase stage; **B.** Camera lucida drawing (2n = 4*x* = 28)



A B

Fig. 4.7 **A.** Mitotic chromosome of *M. spinosa* at early metaphase stage; **B.** Camera lucida drawing (2n = 4*x* = 44).

4.4 Isolation of biochemical compounds from the plants

4.4.1 *Z. oxyphyllum*

The tender shoots of *Z. oxyphyylum* yielded the compound **ZO1**, which was obtained as yellowish oil. The IR spectrum presented in Fig. 4.8 of **ZO1** showed adsorption bands at 2958, 2926, 2854, 1729, 1600, 1580 cm^{-1} indicating the presence of an aromatic system C=N and ester group. Adsorption bands at 1462, 1380, 1274, 1123, 1072, 1039, 960 and 742 confirmed the long linear chain of the compound. In the ^{1}H NMR spectrum presented in Fig. 4.9, two doublets of doublets each integrating to two protons at δ 7.49 and δ 7.68 indicated the presence of the pyridyl system in the molecule. The multiplet signal at δ 4.21 integrating to one proton confirmed the presence of the ester group. The doublet at δ 0.93 and triplet at δ 0.88 each integrating to three protons indicated the presence of two methyl groups. The ^{13}C NMR spectrum as presented in Fig. 4.10 indicated the presence of four aromatic carbons, two methyl groups, five methylenes in addition to one each of CH, quaternary aromatic carbon and carbonyl carbon. The spectral data suggested that the compound **ZO1** was an alkaloid and the structure of the compound **ZO1** could be 2-methylheptyl isonicotinate (Fig. 4.11). The NMR spectral data was in good agreement with the earlier report (Bordoloi *et al.*, 2001).

4.4.1.1 Analytical data of ZO1

IR (KBr) cm^{-1} 2958, 2926 (CH_3), 2854 (CH_2), 1729 (C=O), 1600, 1580, 1462, 1380, 1274, 1123, 1072, 1039, 960, 742 (Fig. 4.8).

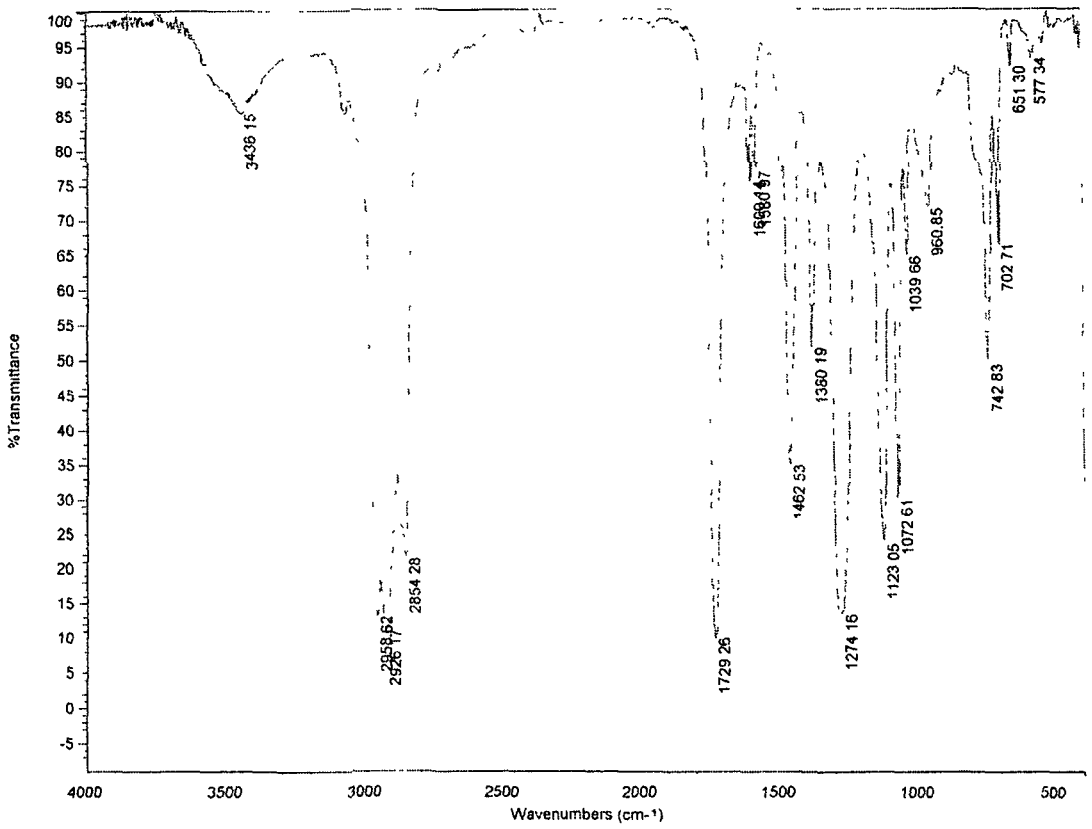


Fig. 4.8 IR spectrum of **ZO1**

^{1}H NMR ($CDCl_3$) δ 0.88 (3H, H-7), 0.93 (3H, H-8), 1.25-1.41 (6H, overlapping signals of H-4, H-5 and H-6), 1.55 (1H, H-2), 4.21 (1H, H-1), 7.49 (doublet of doublets, 2H) and 7.68 ((doublet of doublets, 2H) (Fig. 4.9).

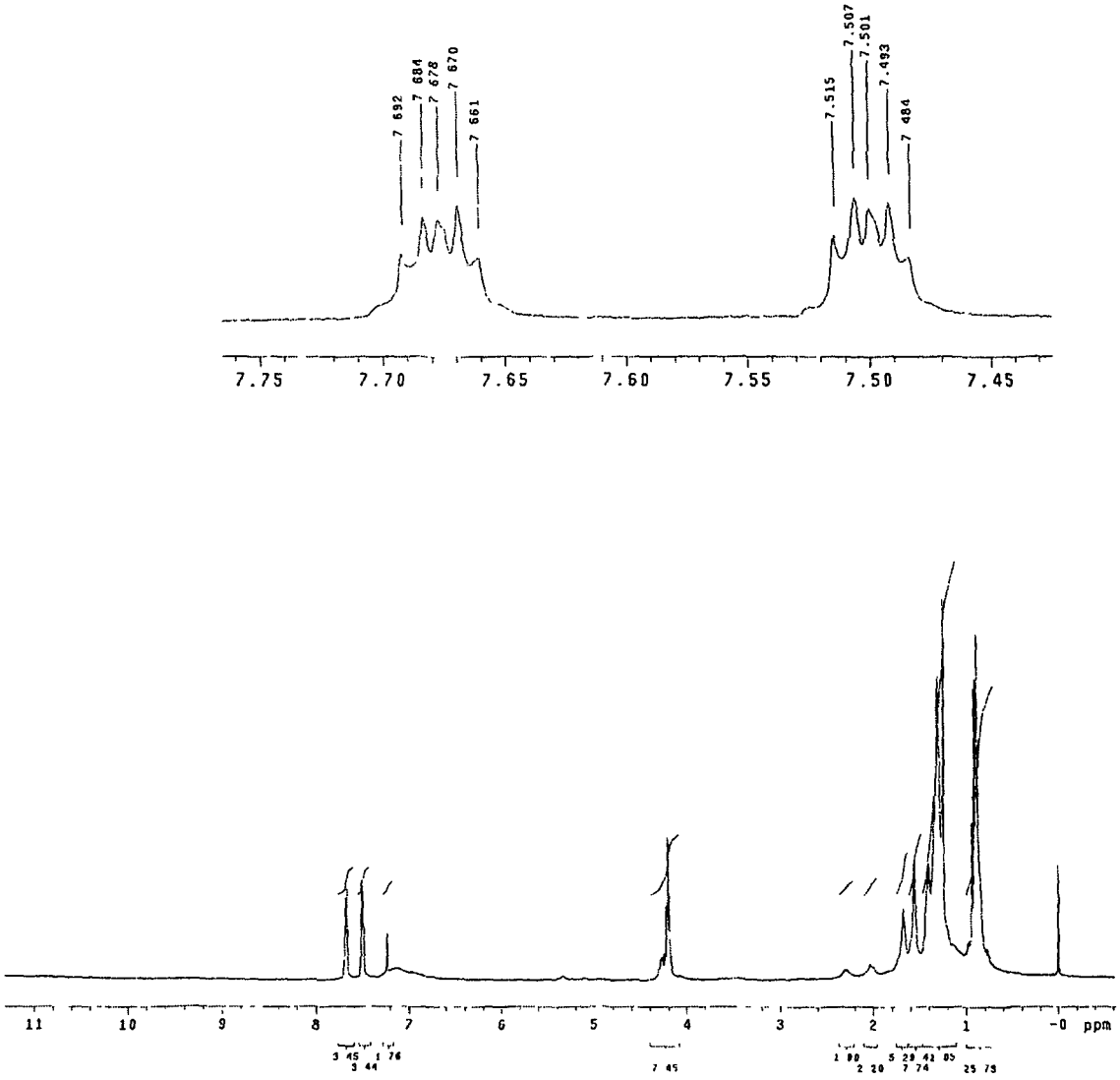


Fig. 4.9 ^{1}HNMR spectrum of **ZO1**

^{13}C NMR ($CDCl_3$) δ 12.0 (C-7), 14.23 (C-8), 23.92 (C-6), 24.75 (C-5), 29.90 (C-4), 30.53 (C-3), 39.69 (C-2), 68.21 (C-1), 129.52 (C-3' and C-5'), 131.55 (C-2' and C-6'), 132.37 (C-1') and 167.49 (COO) (Fig. 4.10).

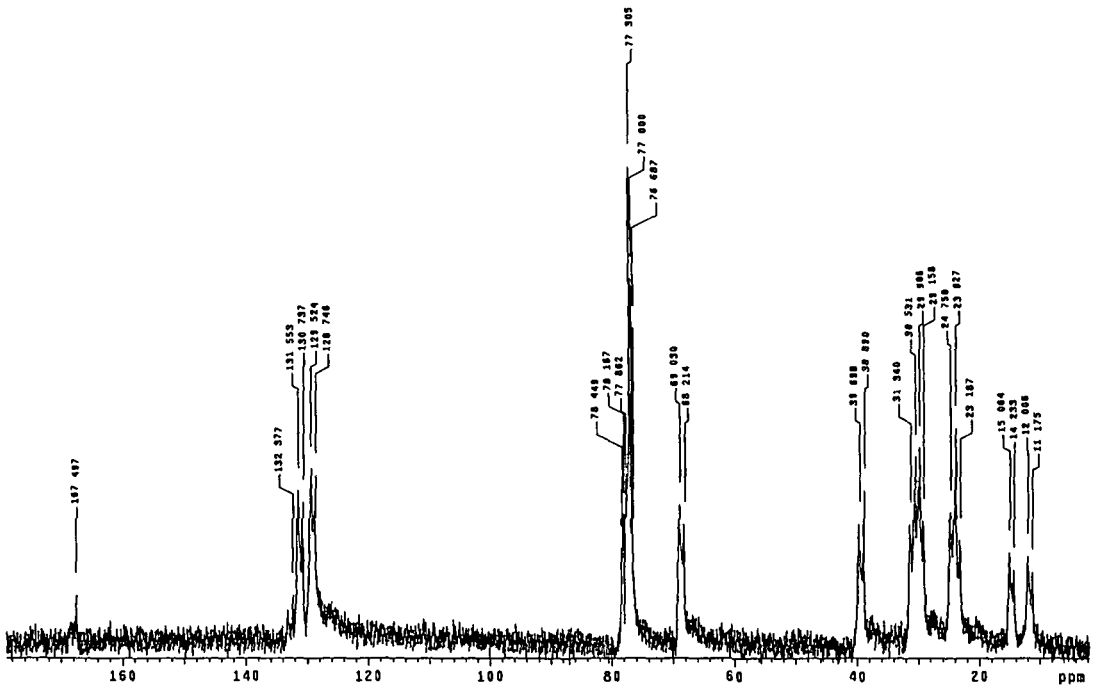


Fig. 4.10 ^{13}CNMR spectrum of **ZO1**

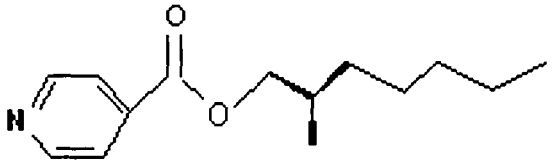


Fig. 4.11 Probable structure of **ZO1** (2-methylheptyl isonicotinate)

4.4.2 *R. alceifolius*

The tender leaves of *R. alceifolius* yielded the compound **RA1**. The compound was also obtained as yellowish oil. The IR (Fig.4.12) and ^{1}H NMR spectra (Fig. 4.13) were not good for identification and characterization of the compound. However, the signals of ^{13}C NMR spectrum in Fig. 4.14 at δ 11.1, 14.1, 23.4, 23.9, 29.4, 30.5, 38.8, 68.2, 128.6, 130.7, 132.3 and 167.5 suggested the structure of the compound could be 2-methylheptyl isonicotinate (Fig. 4.15).

4.4.2.1 Analytical data of RA1

IR (KBr) cm^{-1} 2920 (CH_3), 2851 (CH_2), 1709 (C=O), 1637, 1514, 1463,.1377, 1271, 1167, 723 (Fig. 4.12).

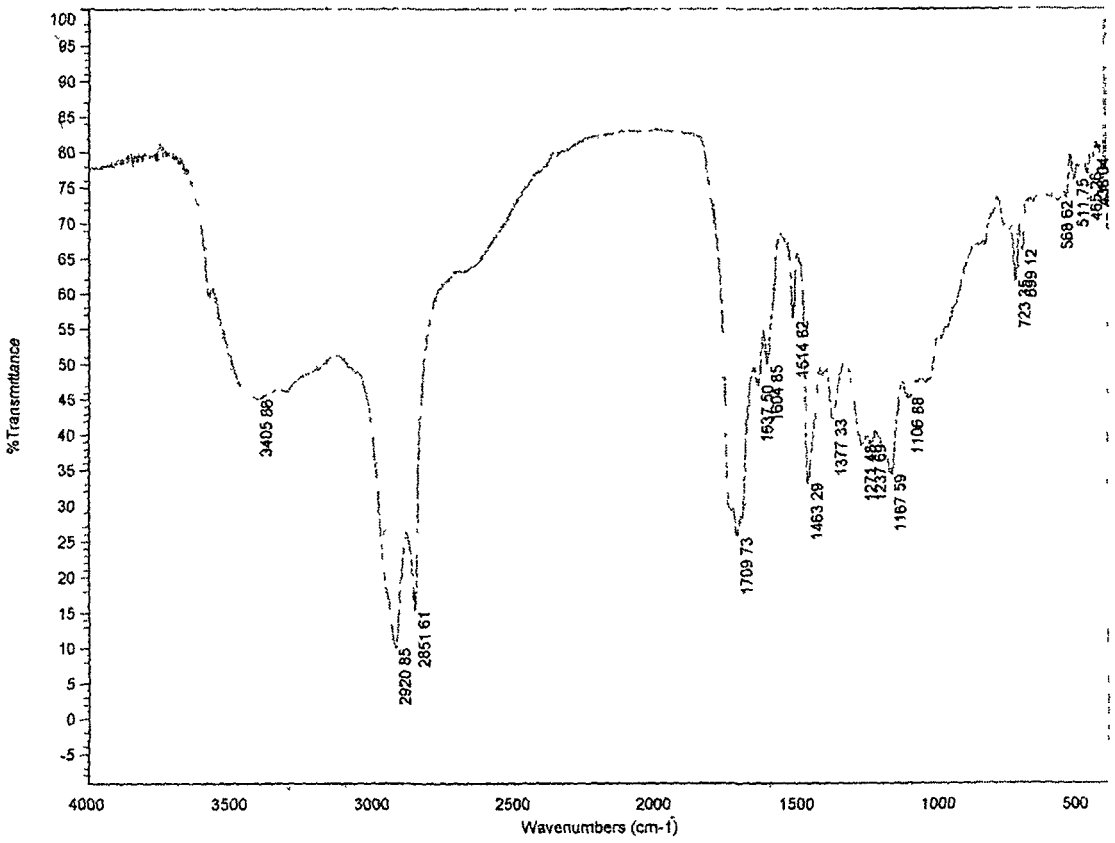


Fig. 4.12 IR spectrum of **RA1**

^{1}H NMR ($CDCl_3$) δ 0.87 (3H, H-7), 1.25 (overlapping signals of H-4, H-5 and H-6), 1.56 (1H, H-2), 7.23 (doublet of doublets, 2H) (Fig. 4.13).

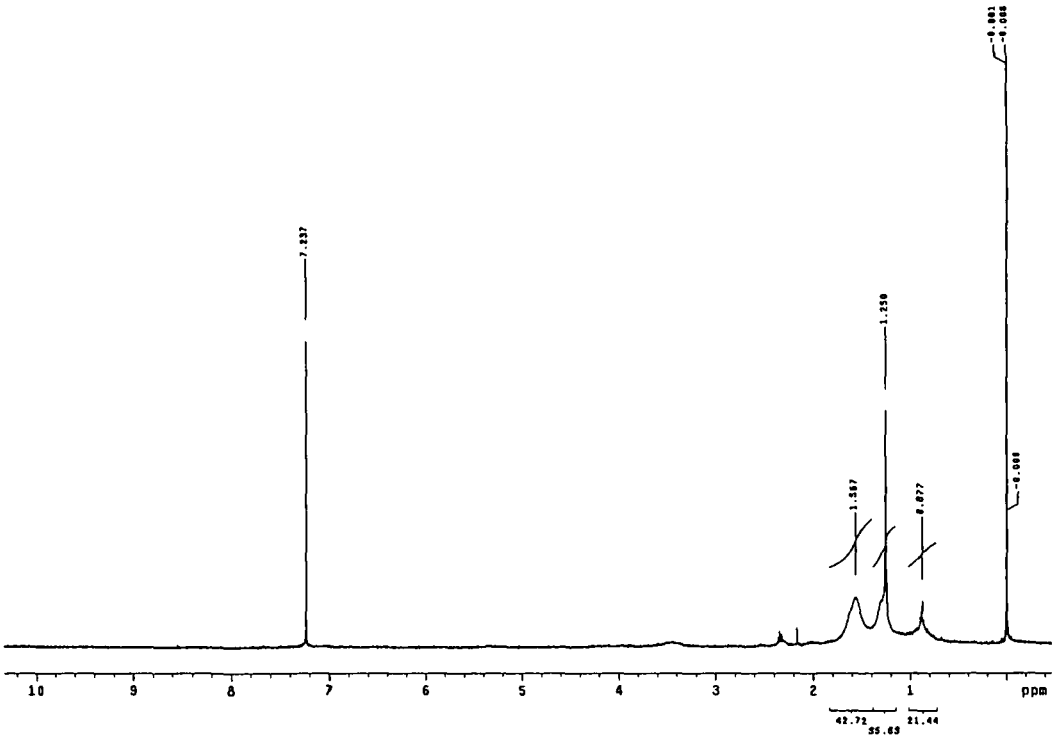


Fig. 4.13 ^{1}H NMR spectrum of **RA1**

^{13}C NMR ($CDCl_3$) δ 11.12 (C-7), 14.16(C-8), 23.41 (C-6), 23.93 (C-5), 29.46 (C-4), 30.52 (C-3), 38.89 (C-2), 68.22 (C-1), 128.67 (C-3' and C-5'), 130.71 (C-2' and C-6'), 132.31 (C-1'), 167.5 (COO) (Fig. 4.14).

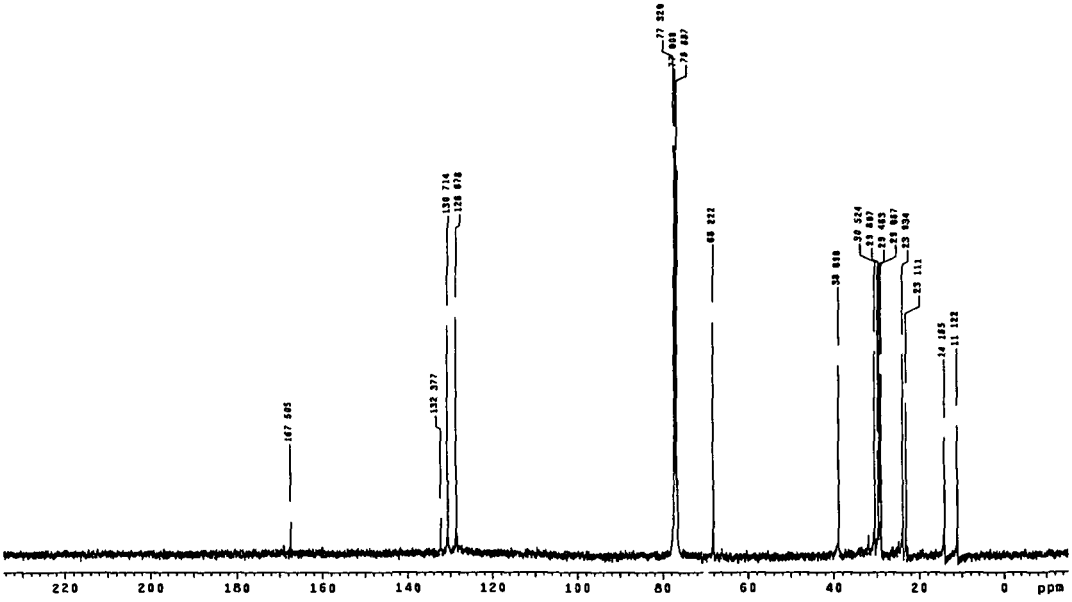


Fig. 4.14 ^{13}C NMR spectrum of **RA1**

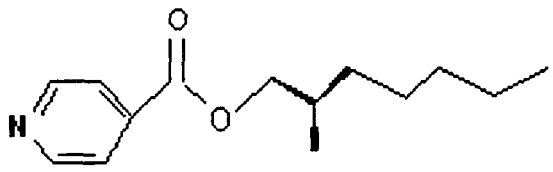


Fig. 4.15 Probable structure of **RA1** (2-methylheptyl isonicotinate)

4.4.3 *M. spinosa*

The mature fruits of *M. spinosa* yielded the compounds **MS1** and **MS2**. The compound **MS1** was yellowish gum. The IR spectrum (Fig. 4.16) of **MS1** showed adsorption band for OH group (3437 cm^{-1}), carbonyl group (1743 cm^{-1}) and a double bond (1689 cm^{-1}) in the molecule. In the ^{1}H NMR spectrum (Fig. 4.17), triplet at δ 5.35 due to H-12 (olefinic proton), double doublet at δ 3.65 (carbinolic region), seven tertiary methyl groups at δ 0.75, 0.78, 0.84, 0.87, 0.93, 0.96 and 1.24 confirmed the typical 3β-hydroxyolean-12-en-28-oic acid as reported in the previous literature (Mendez *et al.*, 1995; Chaiyadez *et al.*, 2004; Choi *et al.*, 2004 and Guvenalp *et al.*, 2006). In the ^{13}C NMR spectrum (Fig. 4.18), presence of the 28-carbonyl carbon at δ 177.7, and C-12 and C-13 at δ 125.7 and δ 137.7 (olefinic signals), respectively further supported the olean structure of the compound (Mendez *et al.*, 1995, Chaiyadez *et al.*, 2004 and Guvenalp *et al.*, 2006). These special features indicated that **MS1** belonged to an oleanane type triterpene having a carboxylic carbon. Based on the evidence, the compound **MS1** was identified to be oleanolic acid (Fig. 4.19).

MS2 was also obtained as a yellowish gum. The ^{1}H NMR spectrum (Fig 4.21) clearly supported the olean structure of the compound. Absence of the signal for 28-carbonyl carbon of carboxyl group in the ^{13}C NMR spectrum (Fig. 4.22), absence of a sharp peak for carbonyl group in IR spectrum (Fig. 4.20) and presence of a distinct peak at m/z 492 [M^{+} + CH_2OH + H_2O] in the MALDI TOF MS (Fig 4.23) suggested the structure of **MS2** to be oleanol (4.24).

4.4.3.1 Analytical data of MS1

IR (KBr) cm^{-1} 3437 (OH), 2927 (CH_3), 2854 (CH_2), 1743 (C=O), 1689 (C=O) (Fig. 4.16).

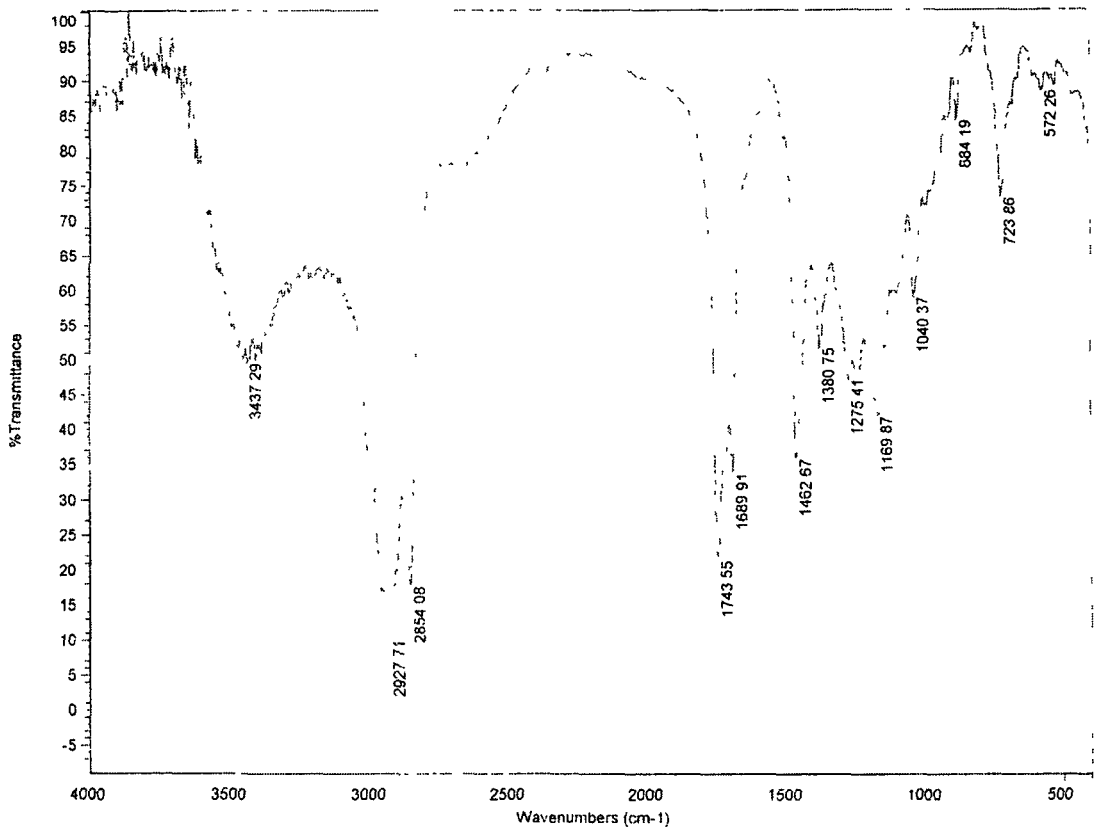


Fig. 4.16 IR spectrum of **MS1**

^{1}H NMR ($CDCl_3$) δ 5.35 (1H, H-12), 3.65 (1H, H-3), 2.75 (1H, H18), 1.24 (3H, CH_3-27), 0.96 (3H, CH_3-23), 0.93 (3H, CH_3-30), 0.87, (3H, CH_3-29), 0.84 (3H, CH_3-25), 0.78 (3H, CH_3-24), 0.75 (3H, CH_3-26) (Fig. 4.17).

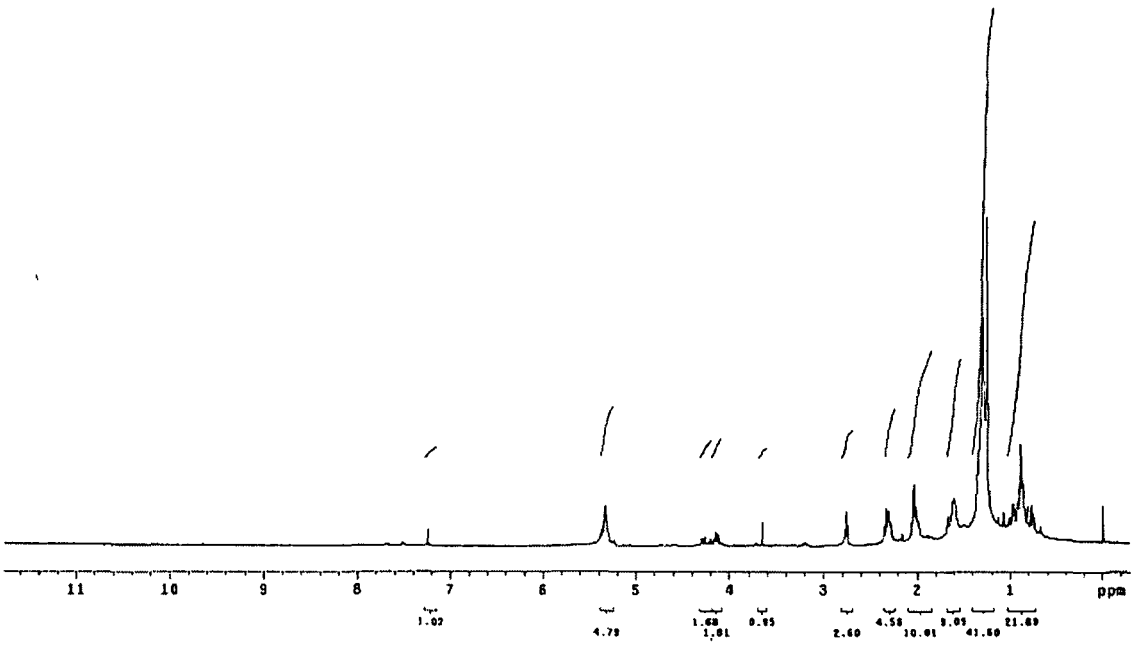


Fig. 4.17 ^{1}H NMR spectrum of **MS1**

^{13}C NMR ($CDCl_3$) δ 38.7 (C1), 27.3 (C2), 77.0 (C3), 38.7 (C4), 55.3 (C5), 18.5 (C6), 32.8 (C7), 38.7 (C8), 47.6 (C9), 36.7 (C10), 22.6 (C11), 125.7 (C12), 137.7 (C13), 39.6 (C14), 27.4 (C15), 23.0 (C16), 45.9 (C17), 39.6 (C18), 45.9 (C19), 30.5 (C20), 32.0 (C21), 33.0 (C22), 28.2 (C23), 15.7 (C24), 15.4 (C25), 17.1 (C26), 25.7 (C27), 177.7 (C28), 33.0 (C29), 23.7 (C30) (Fig. 4.18).

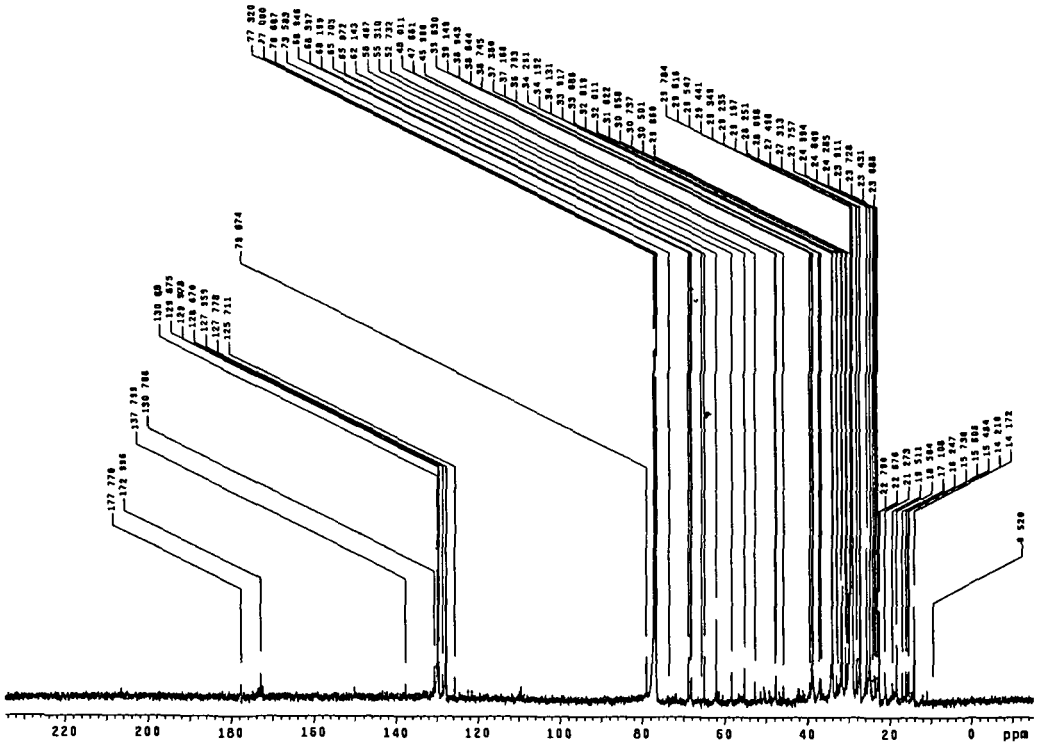


Fig. 4.18 ^{13}C NMR spectrum of **MS1**

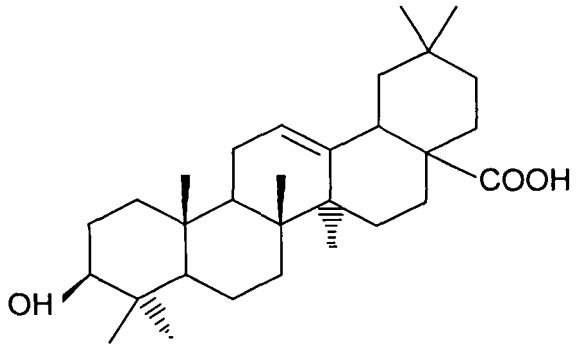


Fig. 4.19 Probable structure of **MS1** (Oleanolic acid)

4.4.3.2 Analytical data of MS2

IR (KBr) cm $^{-1}$ 3418 (OH), 2926 (CH_3), 2854 (CH_2), 1731, 1689 (4.21) (Fig. 4.20).

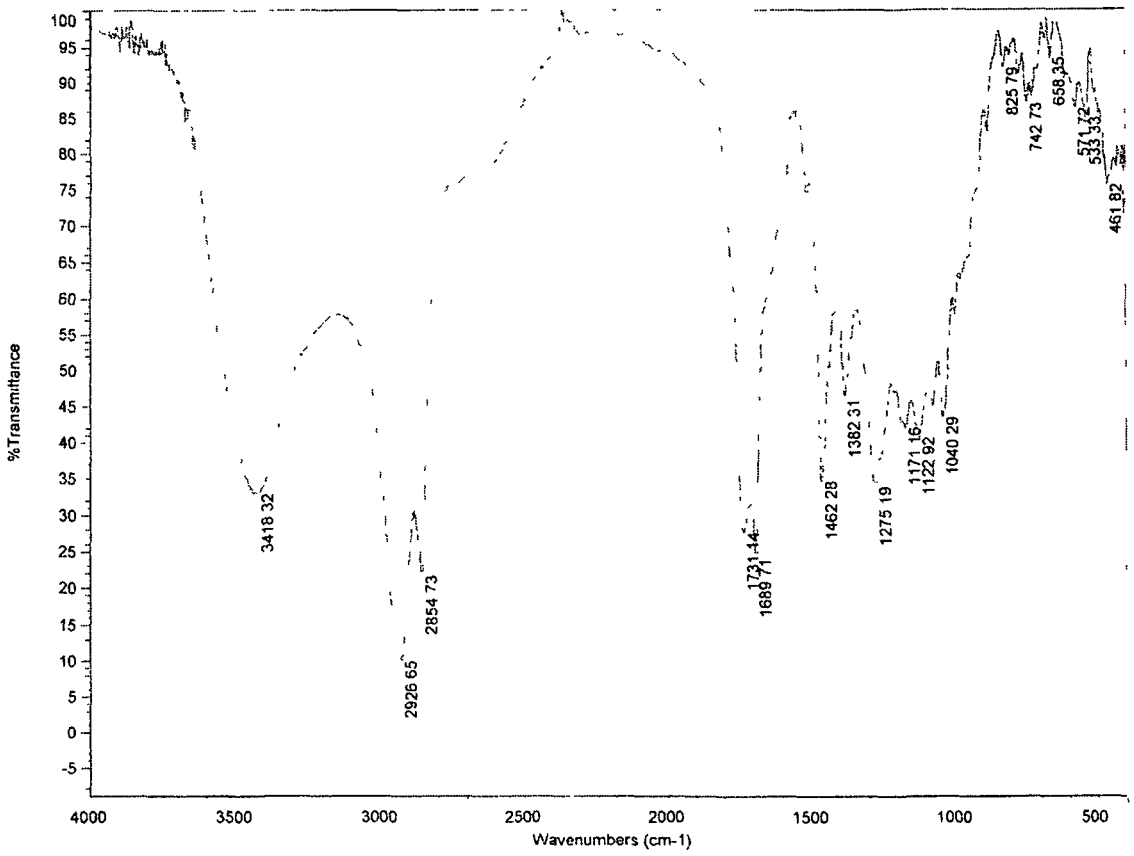


Fig. 4.20 IR spectrum of **MS2**

^{1}H NMR ($CDCl_3$) δ 5.34 (1H, H-12), 3.20 (1H, H-3), 2.76 (1H, H18), 1.25 (3H, CH_3-27), 0.98 (3H, CH_3-23), 0.93 (3H, CH_3-30), 0.87, (3H, CH_3-29), 0.86 (3H, CH_3-25), 0.79 (3H, CH_3-24), 0.77 (3H, CH_3-26) (4.22) (Fig. 4.21).

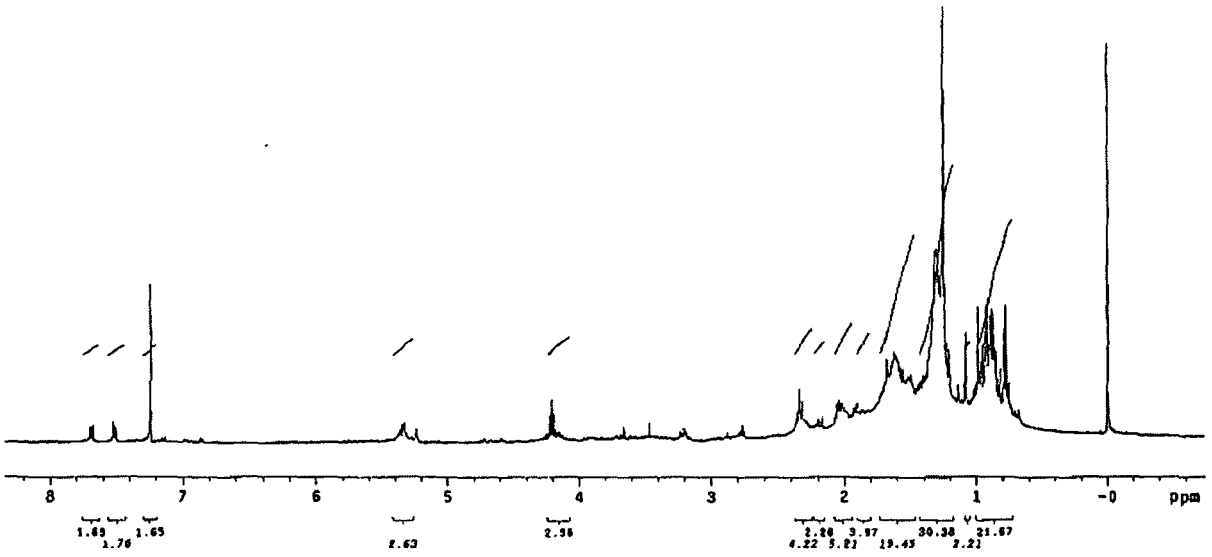


Fig. 4.21 ^{1}HNMR spectrum of **MS2**

^{13}C NMR ($CDCl_3$) δ 38.8 (C1), 28.1 (C2), 79.0 (C3), 38.9 (C4), 55.3 (C5), 18.4 (C6), 33.1 (C7), 39.1 (C8), 48.0 (C9), 37.1 (C10), 23.1 (C11), 125.7 (C12), 137.8 (C13), 39.1 (C14), 29.0 (C15), 23.7 (C16), 47.6 (C17), 39.1 (C18), 47.6 (C19), 30.5 (C20), 33.1 (C21), 33.8 (C22), 28.2 (C23), 15.7 (C24), 15.6 (C25), 17.1 (C26), 32.0 (C29), 23.9 (C30) (Fig. 4.22).

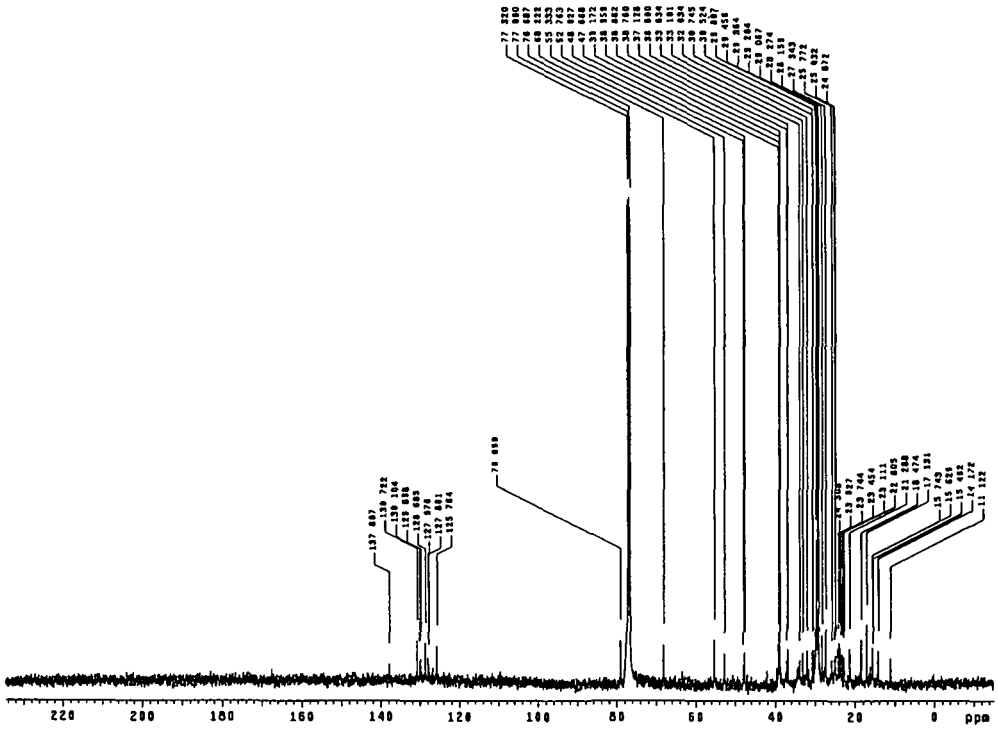


Fig. 4.22 ^{13}C NMR spectrum of **MS2**

MALDI TOF MS: m/z at 492 [M^+ + CH_2OH + H_2O] (Fig. 4.23).

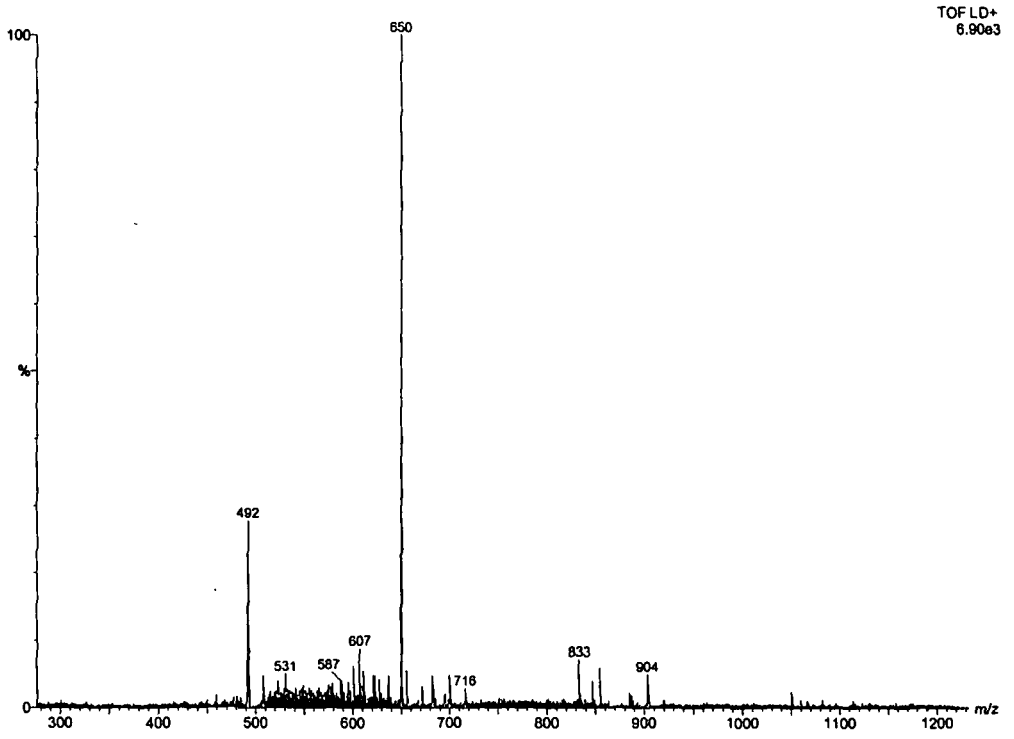


Fig. 4.23 MALDI TOF MS of **MS2**

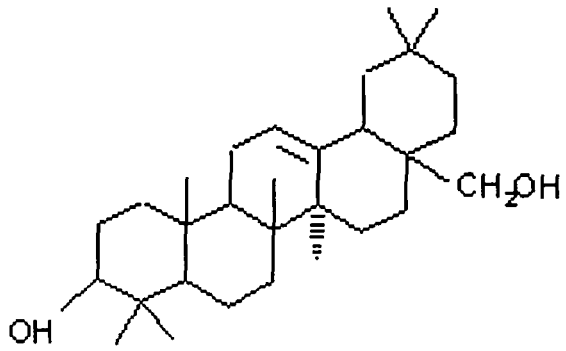


Fig. 4.24 Probable structure of **MS2** (Oleanol)

4.5 Antimicrobial assay

Antimicrobial activity of the isolated compounds (**ZO1, RA1, MS1** and **MS2**) was assessed against five microorganisms. Antimicrobial activity was observed in all the compounds against the tested microorganism strains. The diameter of inhibition zones of the compounds against the test microorganisms varied from 12.0 ± 0.50 mm to 24.0 ± 0.14 mm (Table 4.4). The compound **ZO1** showed the largest zone of inhibition (24.0 ± 0.14 mm) against *C. albicans* (Fig. 4.25 **E**) followed by compound **MS1** (23.0 ± 0.29 mm) against *S. aureus* (Fig. 4.25 **B**) revealing the presence of the highest antimicrobial activity. The compounds **ZO1** and **MS1** exhibited high antimicrobial activity against the test organisms and **MS1** showed the highest activity against *S. aureus* and *E. coli.*

Table 4.4 Antimicrobial activity of the compounds extracted from the plants

Sample	Diameter of inhibition zone (DIZ) in mm				
	B. subtilis	*S. aureus*	*E. coli*	*K. pneumoniae*	*C. albicans*
ZO1	20.0 ± 0.14	19.0 ± 0.38	18.0 ± 0.38	15.0 ± 0.14	24.0 ± 0.14
RA1	14.0 ± 0.14	16.0 ± 0.0	15.0 ± 0.50	10.0 ± 0.50	19.0 ± 0.50
MS1	18.0 ± 0.29	23.0 ± 0.29	22.0 ± 0.14	17.0 ± 0.50	19.0 ± 0.25
MS2	12.0 ± 0.14	14.0 ± 0.50	14.0 ± 0.29	12.0 ± 0.50	16.0 ± 0.29
Control	6.0	6.0	6.0	6.0	6.0

Results are the mean of three replicate ± standard error (SE).

* DIZ of >6 mm are considered indicative of activity.

4.6 Genomic DNA isolation and purification

Genomic DNA obtained through the modified procedure was of good quality and the yield of DNA more than double the amount as compared to the yield obtained through the original protocol (Table 4.7)

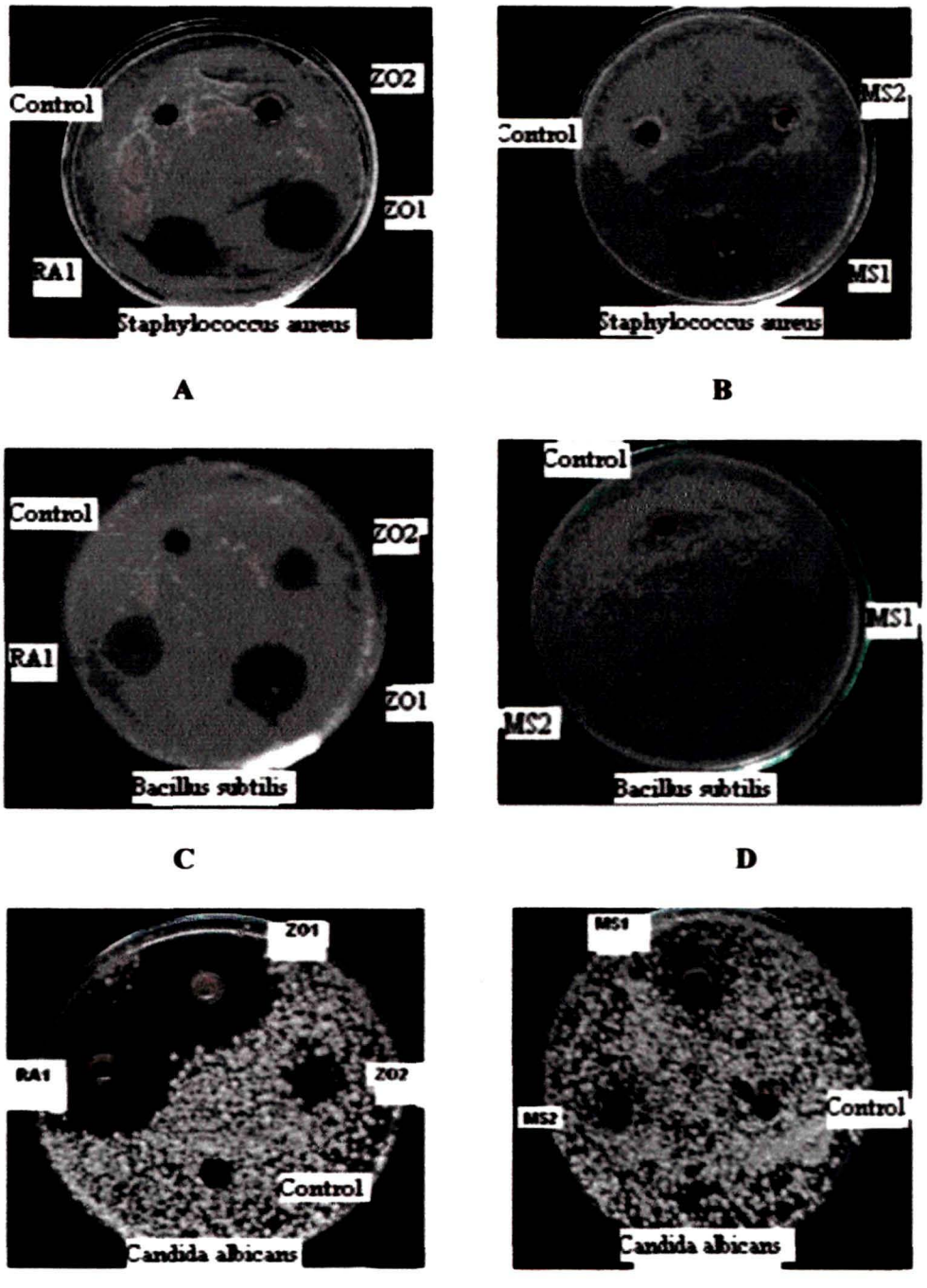


Fig. 4.25 Antimicrobial activity of the compounds isolated from the plants against *Staphylococus aureus* (A, B); *Bacillus subtilis* (C, D) and *Candida albicans* (E, F).

4.6.1 DNA quantification

Optical density of the isolated genomic DNA from *Z. oxyphyllum* at 260 nm = 0.051.

Therefore, the value corresponds the yield = 50 µg/ml x 0.051= 2.55 µg/ml

In 200 µl TE buffer the amount of DNA would be 2.55 x 200/5 µg = 102.0 µg.

3 g of plant tissue yielded 102.0 µg of genomic DNA.

The yield of genomic DNA per gram of leaf tissue = 102.0/3 = 34.0 µg.

Therefore, the yield of genomic DNA from *Z. oxyphyllum* per gram of fresh leaf tissue was 34.0 µg.

In the same way, the yield of genomic DNA from *R. alceifolius* and *M. spinosa* per gram of fresh leaf tissue was estimated to be 38.0 µg and 46.0 µg, respectively. Data obtained on yield of genomic DNA are presented in Table 4.5.

4.6.2 Purity of DNA

The purity of the isolated DNA from *Z. oxyphyllum* was checked by finding out the OD at A_{260} and A_{280} nm in an UV/VIS spectrophotometer.

Since, A_{260} = 0.051 and A_{280} = 0.029, therefore, A_{260}/A_{280} = 1.76

In the same way, the purity of genomic DNA (A_{260}/A_{280} ratio) from *R. alceifolius* and *M. spinosa* was determined and are presented in the Table 4.5.

Table 4.5 Yield and A_{260}/A_{280} ratio of the isolated DNA samples

Name of the species	A_{260}	A_{280}	A_{260}/A_{280} (A)	DNA yield (µg g^{-1} leaf tissue) (Y)
Z. oxyphyllum	0.051	0.029	1.76	34.0
R. alceifolius	0.0570	0.031	1.84	38.0
M. spinosa	0.0690	0.037	1.86	46.0

4.6.3 Different isopropanol precipitation temperature and duration

The yield and purity of isolated DNA using different isopropanol precipitation time and temperature was presented in Table 4.6, which showed that overnight isopropanol precipitation at room temperature (25-30°C) was the optimized step for the isolation of genomic DNA from the medicinal plants.

Table 4.6 Yield and purity of isolated DNA using different isopropanol precipitation time and temperature [I- Incubation time; P- Precipitation temperature; Y- DNA yield (μg g^{-1} leaf tissue); A- A_{260}/A_{280} ratio]

Z. oxyphyllum				*R. alceifolius*				*M. spinosa*			
I	P	Y	A	I	P	Y	A	I	P	Y	A
1 h	4°C	23	1.46	1 h	4°C	25	1.65	1 h	4°C	38	1.65
1 h	25-30°C	19	1.54	1 h	25-30°C	21	1.72	1 h	25-30°C	27	1.76
Over night	4°C	36	1.62	Over night	4°C	42	1.74	Over night	4°C	49	1.77
Over night	25-30°C	34	1.76	Over night	25-30°C	38	1.84	Over night	25-30°C	46	1.86

Table 4.7 Yield and purity of isolated DNA from Khanuja *et al.* (1999) and modified protocol (Y- DNA yield; A- A_{260}/A_{280} ratio)

Protocol	Plant species					
	Z. oxyphyllum		*R. alceifolius*		*M..spinosa*	
	Y	A	Y	A	Y	A
Khanuja *et al.* (1999)	14	1.41	18	1.49	21	1.61
Modified protocol	34	1.76	38	1.84	46	1.86

4.6.4 Restriction digestion

The quality of isolated genomic DNA was checked by restriction endonuclease digestion. Genomic DNA isolated from the plants was subjected to complete digestion with *Eco* RI, *Hin*d III and mixture of *Eco* RI and *Hin*d III after incubating at 37°C for 6 h.

4.6.5 Electrophoresis of DNA samples

The undigested DNA samples showed conspicuous bands of high molecular weight when electrphoresed in 0.8% agarose gel. The DNA and restriction endonuclease digestion profiles in agarose gel are presented in Fig. 4.26, 4.27 and 4.28.

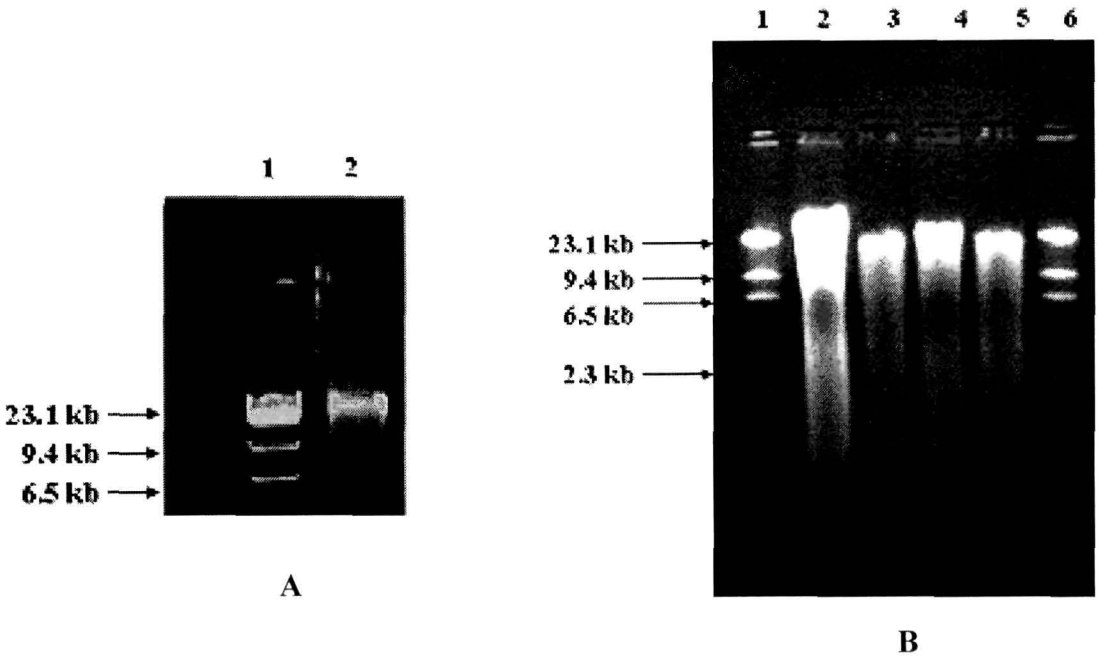


Fig. 4.26 Genomic DNA and restriction digestion profile from *Z. oxyphyllum*

(A) Lane 1: λ-DNA digested with *Hin*d III (molecular size marker); Lane 2: Genomic DNA sample **(B)** Lanes 1 and 6: λ-DNA digested with *Hin*d III (molecular size marker); Lane 2: undigested sample; Lane 3: DNA digested with *Eco* RI; Lane 4: DNA digested with *Hin*d III; Lane 5: DNA digested with *Eco* RI and *Hin*d III.

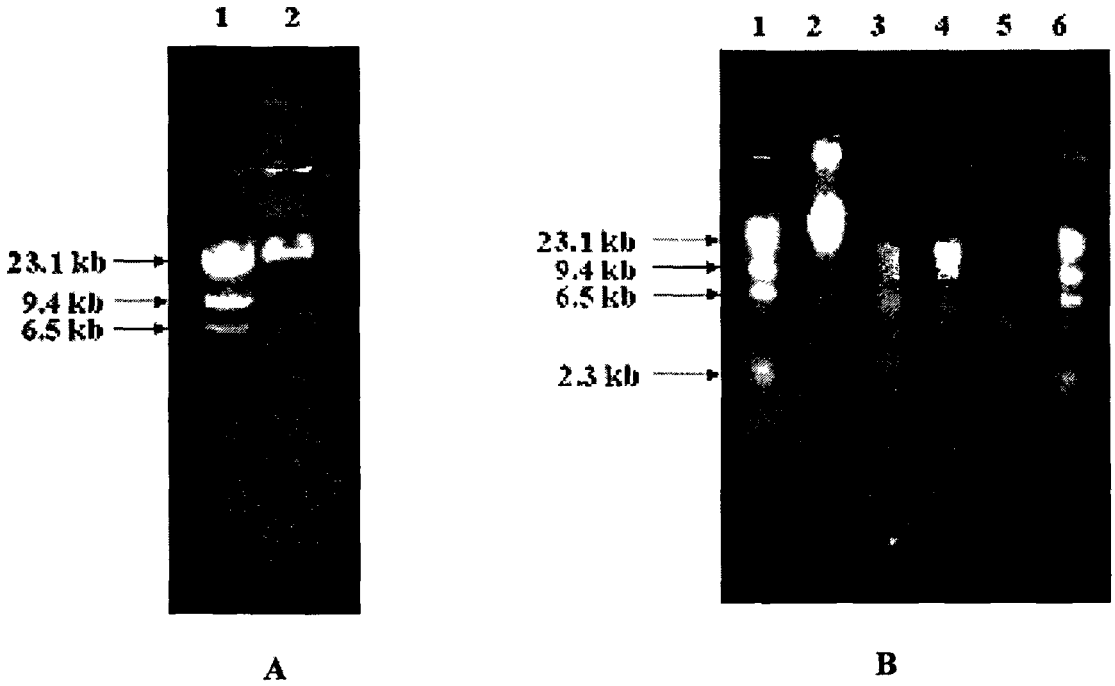


Fig. 4.27 Genomic DNA and restriction digestion profile from *R. alceifolius*

(A) Lane 1: λ-DNA digested with *Hind* III (molecular size marker); Lane 2: Genomic DNA sample **(B)** Lanes 1 and 6: λ-DNA digested with *Hind* III (molecular size marker); Lane 2: undigested sample; Lane 3: DNA digested with *Eco* RI; Lane 4: DNA digested with *Hind* III; Lane 5: DNA digested with *Eco* RI and *Hind* III.

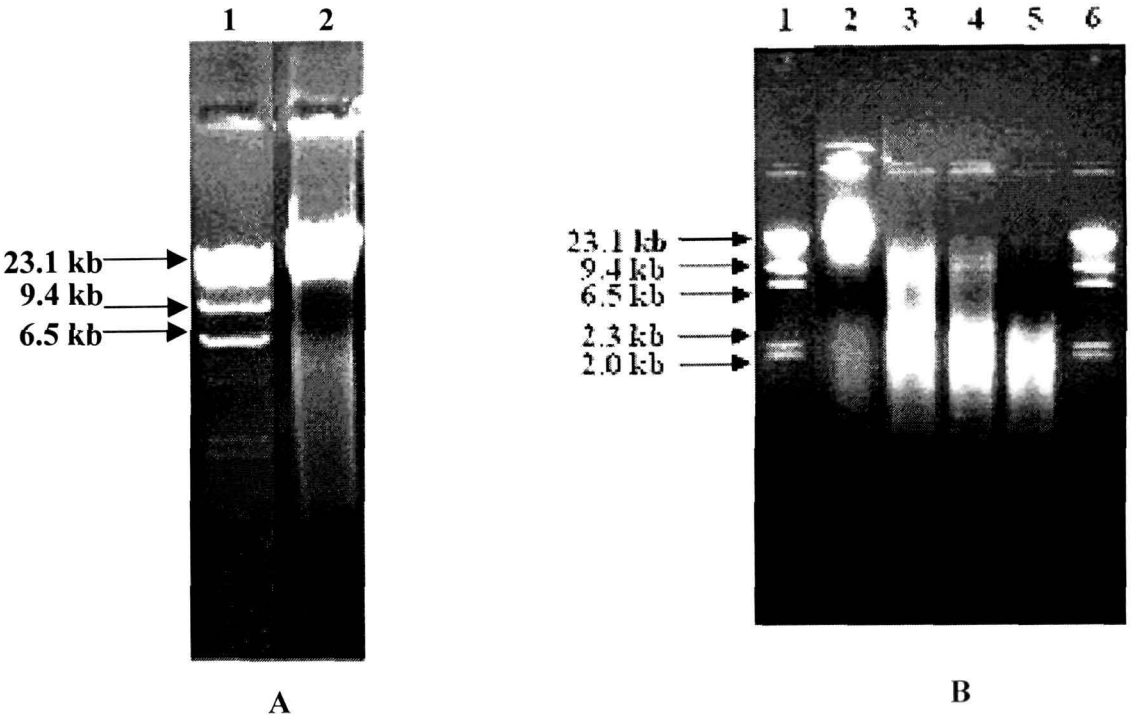


Fig. 4.28 Genomic DNA and restriction digestion profile from *M. spinosa*

(A) Lane 1: λ-DNA digested with *Hin*d III (molecular size marker); Lane 2: DNA sample **(B)** Lane 1 and 6: λ-DNA digested with *Hin*d III (molecular size marker); Lane 2: undigested sample; Lane 3: DNA digested with *Eco* RI; Lane 4: DNA digested with *Hin*d III; Lane 5: DNA digested with *Eco* RI and *Hin*d III.

4.7 Genome size determination

4.7.1 Flow cytometry

Cell nuclei at G_0/G_1 stage were obtained from sufficiently mature leaves of raised seedlings of the plants. Samples having clear high peaks were retained. Flow cytometric analysis of the isolated nuclei resulted in histograms of their DNA content as compared to the reference standard and represented one peak each of the G_0/G_1 nuclei of *Z. oxyphyllum, R. alceifolius* and *M. spinosa*. The 2C nuclear DNA content of the plants was presented in Table 4.8 while the histograms of the peak were presented in Fig. 4.29. The fluorescence peak of PI-stained nuclei from *P. sativum* was calibrated to the channel 200 (Fig. 4.29 A). The results from the PI-stained nuclei samples isolated from *Z. oxyphyllum*, *R. alceifolius* and *M. spinosa* showed G_0/G_1 peak in channel 167, 125 and 173 respectively (Fig. 4.29 B, C and D). The mean 2C nuclear DNA content of *Z. oxyphyllum* compared to that of *P. sativum* was equal to 0.835 (167/200) and hence the 2C DNA amount of *Z. oxyphyllum* was estimated to be 0.835 x 9.09 = 7.590 pg. The ratio of G_0/G_1 peak mean of *R. alceifolius* compared to that of *P. sativum* was found to be 0.625 (125/200) and the 2C DNA content estimated to be 0.625 x 9.09 = 5.681 pg. In the same way, the ratio of G_0/G_1 peak mean of *M. spinosa* as compared to *P. sativum* was found to be 0.865 (173/200) and the 2C DNA content estimated to be 0.865 x 9.09 = 7.862 pg. The DNA C-value or genome size for *Z. oxyphyllum*, *R. alceifolius* and *M. spinosa* were estimated to be 3.79 pg, 2.84 pg and 3.93 pg, respectively (Table 4.8). Using the conversion factor (1 pg = 0.978 x 10^9 bp) the genome size of *Z. oxyphyllum*, *R. alceifolius* and *M. spinosa* were found to be 3.70 x 10^9 bp, 2.77 x 10^9 and 3.84 x 10^9 bp, respectively (Table 4.8). The C-values ranged from 2.84 – 3.93 pg or 2.77 x 10^9 – 3.84 x 10^9 bp indicating the longer genome size in *M. spinosa* and small in the case of *R. alceifolius*.

Table 4.8 Nuclear DNA content of the plants determined by flow cytometry

(SE- standard error)

S N	Name of the species	Number of samples	2C DNA (pg)	SE	C DNA (pg)	C DNA (bp)
1	*Z. oxyphyllum*	3	7.590	0.14	3.79	3.70×10^9
2	*R. alcceifolius*	3	5.681	0.14	2.84	2.77×10^9
3	*M. spinosa*	3	7.862	0.29	3.93	3.84×10^9

4.7.2 Microscopy method

The genome size of medicinal plants determined by microscopy method (Konwar *et al.*, 2007) is presented in Table 4.9. The volume of tissue sections of all three species ranged from 1.6×10^{10} - 2.0×10^{10} μm^3. The volume of intercellular spaces in tissue sections varied between 3.2×10^9 - 4.0×10^9 μm^3. The average volume of single cell ranged from 76,180 - 90,250 μm^3 and weight of the tissue section was 0.03 g. By using the method, 2C content of *Z. oxyphyllum*, *R. alceifolius* and *M. spinosa* was estimated to be 7.19 pg, 5.42 pg and 7.49 pg, respectively. The DNA C-value or genome size for *Z. oxyphyllum*, *R. alceifolius* and *M. spinosa* was estimated to be 3.59 pg, 2.71 pg and 3.79 pg or 3.51×10^9 bp, 2.65×10^9 bp and 3.70×10^9 bp respectively.

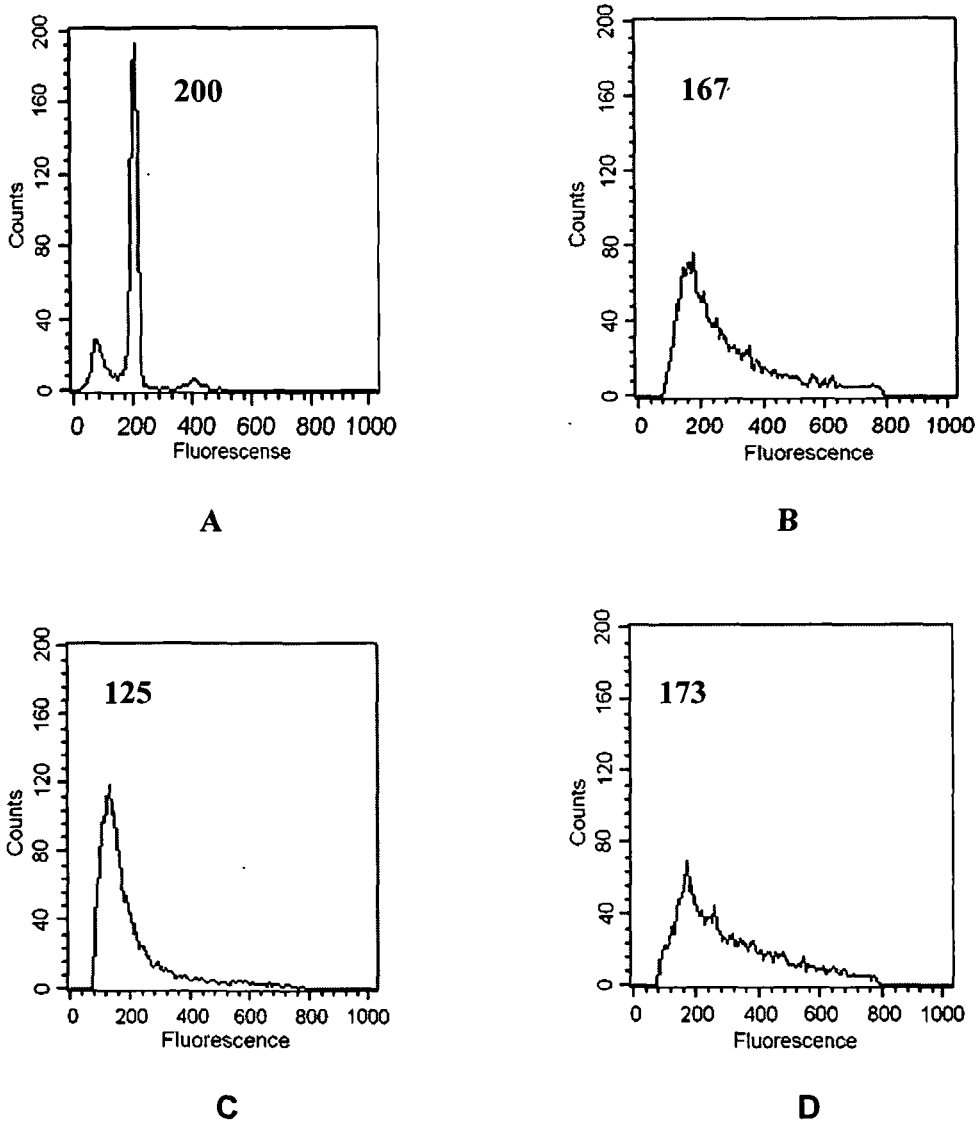


Fig. 4.29 Histograms of the nuclear DNA peak of the plants determined by flow cytometry

(**A**) The fluorescence of the nuclear DNA peak of PI-stained nuclei from *P. sativum* (2C = 9.09 pg DNA), which served as external reference standard. The gain of the instrument was adjusted so that G_0/G_1 peak was positioned at channel 200. The 2C DNA content of the plant species was determined after comparison with the fluorescence peak of the reference standard, whose 2C content was known

(**B**) The fluorescence peak of PI stained nuclei from *Z. oxyphyllum*

(**C**) The fluorescence peak of PI stained nuclei from *R. alceifolius*

(**D**) The fluorescence peak of PI stained nuclei from *M. spinosa*

Table 4.9 Nuclear DNA content of the plants determined by microscopy method

Plant species	Volume of the tissue section ($t\mu m^3$)	Volume of the intercellular space ($s\mu m^3$)	Average volume of single cell ($x\mu m^3$)	Weight of the tissue section (g)	2C-value (pg)	C-value (pg)	C-value (bp)
Z. oxyphyllum	1.6×10^{10}	3.2×10^{9}	90,250	0.030	7.19	3.59	3.51×10^{9}
R. alceifolius	2.0×10^{10}	4.0×10^{9}	76,180	0.030	5.42	2.71	2.65×10^{9}
M. spinosa	1.8×10^{10}	3.6×10^{9}	78,182	0.030	7.49	3.79	3.70×10^{9}

4.7.3 Comparison of two methods

The C-value or genome size of the medicinal plants obtained from flow cytometry and the new microscopy method was compared by plotting the values graphically to assess the extent of variation. The result is presented in Fig. 4.30.

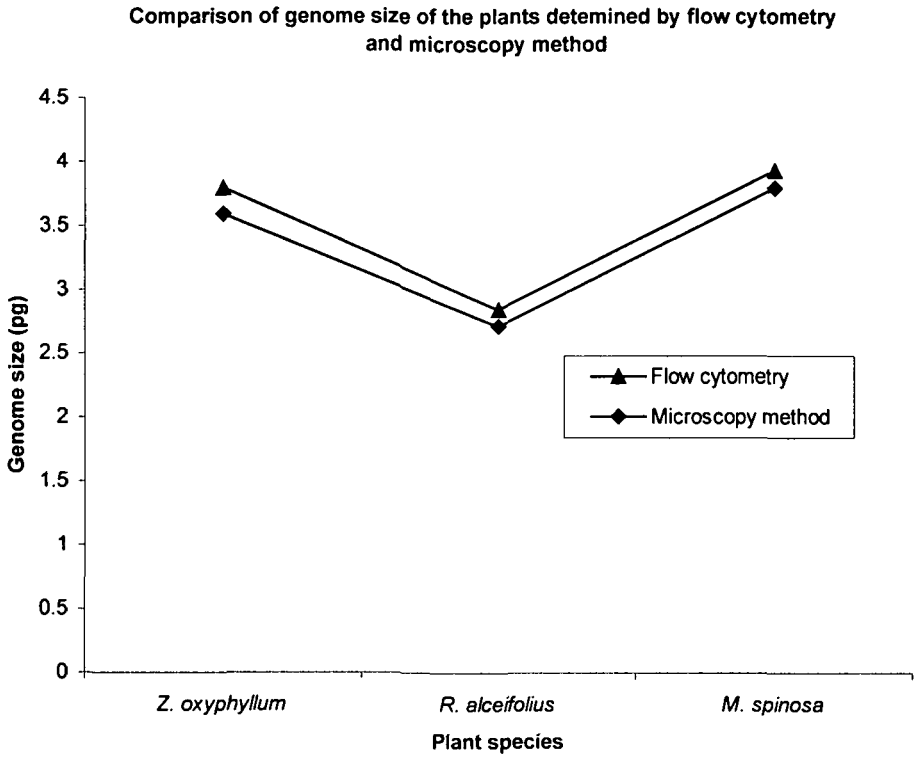


Fig. 4.30 Comparison of the genome size of the plants determined by both methods

Chapter 5
Discussion

Chapter 5 Discussion

5.1 Medicinal plant diversity

Among the documented 400 medicinal plants 148 were herbs, 87 trees, 70 shrubs, 45 climbers, 28 undershrubs, 8 grasses, 5 ferns, 4 palms, 3 bamboos and 2 sedges (Fig. 4.1). Herbs were the primary sources of medicines in terms of number of species followed by trees. This is perhaps because they are abundant and/or woody species that are frequently found in the forest and it is believed that the more abundant a plant is the more medicinal values it may possess (Coe and Anderson, 1996). The ease at which they can be collected, stored and transported and the ease at which bioactive compounds can be extracted are also factors that contribute to the preference of herbs (Shrestha and Dhillion, 2003). The most cited plant family was Fabaceae with 20 species, followed by Asteraceae, Euphorbiaceae, Cucurbitaceae, Rubiaceae and Zingiberaceae (Fig. 4.2). Leaf was the most widely used plant parts accounting for 148 plant species (Fig. 4.3) followed by fruit, root, bark and whole plant (Annexure II: photographs of some recorded medicinal plants).

5.1.1 Therapeutic uses

The recorded plant species were used in the treatment of a variety of human ailments (Fig. 4.4). Majority of the plant species described in the investigation are found to be used in the treatment of different forms of skin diseases such as skin cuts and wounds, scabies, eczema, ringworms, ulcers, allergy, carbuncles, abscesses etc. According to Spiewak (2000) skin diseases amount to as high as 34% of all occupational diseases. The tropical humid climate of Assam might be the reason that facilitates the development of many skin diseases. A study revealed that skin diseases constituted 6.3% of the total number of the patients who attended medical care and eczema was the largest group of skin diseases that occur in Assam (Das, 2003). Moreover, the majority of the people of Assam is involved in agriculture and related activities and is thereby frequently exposed to different sensitizers and dermatological infections. Next to skin diseases, the most frequently claimed uses

are stomach ailments such as dysentery and diarrhoea, dyspepsia, flatulence, expelling worms etc. Dermatological and gastrointestinal problems are the common ailments because of poor hygienic conditions, sanitation facility along with contaminated food and water. A number of plants were attributed to cure lung and respiratory diseases such as cough and cold, pneumonia, tonsillitis, sinusitis etc. Another frequently claimed use of the recorded medicinal plants are gynaecological problems such as relieving pain after child birth, menstrual trouble, dysmennorea, leucorrhoea, abortifacient, galactogue to nursing mothers etc.

Much of the information reported in this study concerning some therapeutic uses of *Amaranthus spinosus, Areca catachu, Flemingia strobilifera, Lasia spinosa, Litsea salicifolia, Meyna spinosa, Mikania micrantha, Phlogacanthus thyrsiformis, Pogostemon benghalense, Rhynchostylis retusa, Rubus alceifolius, Talauma hodgsonii, Wedelia chinensis, Zanthoxylum oxyphyllum* etc were found to be new in the literature of Indian medicinal plants and deserves further study.

The information provided in the investigation is limited and there is always a scope to initiate works in this direction. The recorded medicinal plants need to be investigated through phyto and pharmacological experiments to know the validity of their therapeutic uses. Scientific validation and clinical proof with these traditional herbal medicines might lead to potential drugs.

5.2 Morpho-phenological characters of the selected plants

The morphological characters of the selected plants were compared with the characters described by Kanjilal *et al.* (1940) in "Flora of Assam" and Bora and Kumar (2003) in "Floristic Diversity of Assam". The characters of all the species are in agreement with the characters as described by Kanjilal *et al.* (1940) and Bora and Kumar (2003). Description of *Z. oxyphyllum* was not found in "Floristic Diversity of Assam". In "Flora of Assam", the species *R. alceifolius* was mentioned as *R. moluccanus* and *M. spinosa* was named as *Vangueria spinosa* by Kanjilal *et al.* (1940). Bora and Kumar (2003) reported the species *R. moluccanus* as *R. alceifolius* (Syn. *R. moluccanus auct. non* Linn.) and *Vangueria spinosa* as *M. spinosa* after consulting with various taxonomic literature and herbarium species of Botanical

Survey of India. In the present investigations, new names of the species have been used. One interesting fact is that the height of the plants was not found in any of the literature mentioned. In the case of *M. spinosa* two plant types were observed: shrub and small tree and therefore the plant was mentioned as a large shrub or small tree. But in the case of *Z. oxyphyllum* the plant grows into a straggling tree unless pruned regularly. The phenological characters such as flowering and fruiting period along with the morphology of the plants described are expected to help the future workers in correct identification of the plants.

5.3 Chromosome number and karyotype of the plants

5.3.1 *Z. oxyphyllum*

Chromosome counts of *Z. oxyphyllum* were found to be 2n = 2*x* = 36. Chromosome arm ratio of the species varies from 1.0 – 4.0 μm while the centromeric index varies from 20.0 – 50.0. There are 12 chromosome pairs having median centromere and 6 chromosome pairs having submedian centromere. Stace *et al.* (1993) examined chromosomes for 9 tribes and 73 genera of Rutaceae for the probable chromosome base number and found that the basic chromosome number *x* = 18 was typical for the tribes *Zanthoxyleae* and *Toddalieae*. The present species belongs to the tribe *Zanthoxyleae* and therefore the basic chromosome number of *Z. oxyphyllum* is *x* =18 indicating the species to be a diploid.

5.3.2 *R. alceifolius*

The basic chromosome number of all species of *Rubus* contain *x* = 7 (Amsellem *et al.*, 2003; Naruhashi *et al.*, 2002). Naruhashi *et al.* (2002) made detailed study of the chromosome number of 37 species of *Rubus* in Taiwan and found that the basic chromosome number for all species was *x* = 7. Chromosome arm ratio of the present species was found to be 1.0 in all the chromosome pairs except one where the arm ratio was found to be 2.5. The centromeric index was found to be 50.0 in all the chromosome pairs. There are 10 chromosome pairs having median centromere and 4 chromosome pairs having submedian centromere. The chromosome counts of *R. alceifolius* revealed the number 2n = 4*x* = 28,

indicating the species to be a tetraploid. According to Amsellem *et al.* (2003) ploidy level is quite variable in the genus *Rubus*. This statement was found to be similar with the results of Naruhashi *et al.* (2002).

5.3.4 *M. spinosa*

The chromosome formula of the species was found to be $2n = 4x = 44$. Chromosome arm ratio of *M. spinosa* varies from 1.0 – 4.0. The centromeric index varies from 29.0 – 50.0. There are 12 chromosome pairs having median centromere and 10 chromosome pairs having submedian centromere. Kiehn (1995) made extensive chromosome survey of the family Rubiaceae with regard to detailed karyological characters and found that the length and structure of chromosome are stable characters for certain groups of Rubiaceae and in most cases the basic chromosome numbers are $x = 9$, 10 or 11. The chromosome count of *M. spinosa* revealed the total chromosome number to be 44, apparently a tetraploid one.

5.4 Isolation of biochemical compounds from the plants

5.4.1 *Z. oxyphyllum*

In the present investigation, 2-methylheptyl isonicotinate was isolated from the tender shoots of *Z. oxyphyllum*. In the previous studies, rhetsinine, sesamin, eudesmin, epieudesmin, syringaresinol, γ-fagarine, β-sitosterol, lupeol (Deshpande and Shastri, 1977) and zanthoxyline (Tiwari and Masood, 1979) were reported from the stem bark while zanthoxyphylline, corydine (Tiwari and Masood, 1978) and a lactone 3, 4-bis (3', 4'-dimethoxyphenylmethyl) dihydroxyfuran-2-one (Tiwari *et al.* 1980) were isolated from the roots. So far, no work has been reported from the tender shoots and probably this is the first report of the extraction of the compound 2-methylheptyl isonicotinate from *Z. oxyphyllum*. The compound was first isolated from the culture filtrate of *Streptomyces* sp. 201 (Bordoloi *et al.*, 2001). The compound was found to have high antibacterial and antifungal activity. It is a natural analogue of the established antituberculotic drug, isoniazid (Bordoloi *et al.*, 2001). Although, *Z. oxyphyllum* is being used to cure a number of ailments, so far, no report has been found about the use of the plant against tuberculosis. The plant

might be useful against tuberculosis. The isolation of 2-methylheptyl isonicotinate from the plant indicated that the higher plants also synthesize such potential compounds like that of bacteria, which could be antibacterial and antifungal. Though soil microorganisms or fungi produce most of the antibiotics, higher plants have also been a source of antibiotics (Trease and Evans, 1972).

5.4.2 *R. alceifolius*

The compound 2-methylheptyl isonicotinate was also isolated from the tender leaves of *R. alceifolius*. Probably this is also the first report of the extraction of the compound 2-methylheptyl isonicotinate from *R. alceifolius*. In the previous study, rubusic acid, tormentic acid and rubonic acid (Annonymous, 1990, 1991, 1993) were isolated from the whole plant.

Till now, the compound 2-methylheptyl isonicotinate has not been reported from other angiospermic plants.

5.4.3 *M. spinosa*

So far, very little work has been done on the phytochemistry of this plant. In the present investigation, oleanolic acid and oleanol were isolated from the mature fruits of *M. spinosa*. In the previous study, oleanolic acid was isolated from the leaf extract of this plant (Gogoi and Sarmah, 1995). This is the first report of the extraction of oleanolic acid and oleanol from the fruits of *M. spinosa*. Oleanolic acid is widely distributed in plant species all over the world. It has been marketed in China as an oral drug for human liver disorders (Liu, 1995). The oleanolic acid has anti-inflammatory (Ghosh *et al.*, 1983) and anti-HIV (Kashiwa *et al.*, 1998) activities. In recent years, it has been found to be a marked anti-tumor (Jie *et al.*, 2002) and wound healer (Gustavo *et al.*, 2006). The isolation of oleanolic acid and oleanol from *M. spinosa* provided scientific evidence to the ethnotherapeutic claims.

5.5 Antimicrobial assay

The isolated compounds (2-methylheptyl isonicotinate, oleanolic acid and oleanol) were found to be potential as antibacterial and antifungal in pharmaceutical

applications. In the previous investigation, 2-methylheptyl isonicotinate was found to have marked antibacterial activity against *Bacillus subtilis, Escherichia coli, Klebsiella* sp., *Shigella* sp. and *Proteus mirabilis* and antifungal activity against *Fusarium moniliforme, F. semitechtum, F. oxysporum, F. solani* and *Rhizoctonia solani* (Bordoloi *et al.*, 2001). In the present investigation also, the compound exhibited antimicrobial activity against *B. subtilis, E. coli, K. pneumoniae, S. aureus* and the yeast *C. albicans*. Oleanolic acid and oleanol also expressed different mode of antimicrobial activity against the test microorganisms (Table 4.4 and Fig. 4.25). Tang *et al.* (2000) earlier reported the antimicrobial activity of oleanolic acid. Further research is therefore necessary for *in vivo* evaluation of toxicity in animal and human studies. Medicinal plants and microorganims are the proper candidates for studying therapeutic activity and should receive continuous research attention (Bonjar and Nik, 2004). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments.

5.6 Genomic DNA isolation

Like other medicinal plants, the tissues of the plants studied contain secondary metabolites having medicinal properties, which interfere with the genomic DNA isolation process. A simple, efficient and reliable CTAB method therefore was standardized after modifying some of the key steps of the earlier CTAB protocol of Khanuja *et al.* (1999). This protocol was selected for the isolation of genomic DNA because of two reasons. Firstly, Khanuja *et al.* (1999) successfully isolated fairly high yield of quality DNA from fresh as well as dry tissues of many genera of medicinal and aromatic plants and secondly, the protocol eliminated the use of phenol, which made the protocol less hazardous. Spectrophotometric measurement of the isolated DNA samples (using the modified protocol) at 260 nm and 280 nm gave a desirable absorbance ratio A_{260}/A_{280} of 1.76 - 1.86 indicating the insignificant levels of contaminating proteins and polysaccharides. Thus, the protocol developed was effective enough in obtaining high yields of quality DNA (Table 4.7).

5.6.1 Use of PVP

PVP 1% in the extraction buffer resulted in poor yield and quality of genomic DNA isolated from these medicinal plants. This might be due to the interference of phenolic contents present in the plants. Therefore, PVP 4% was added into the extraction buffer to purge the phenolic contents, which facilitated better yield and quality of genomic DNA. Earlier report also suggested that addition of 4% PVP was helpful to remove polyphenols during DNA isolation (Kleb-Llanes *et al.*, 2002). Kim *et al.* (1997) and Warude *et al.* (2003) also reported that high concentration of PVP was helpful to remove polyphenols.

5.6.2 Optimization of temperature and duration for precipitation

To overcome the problem of contamination of isolated DNA by polysaccharides and polyphenols, the duration and precipitation temperature were optimized. In general, the quality and yield of isolated DNA depend on the precipitation temperature and the duration (Michiels *et al.*, 2003). Overnight isopropanol precipitation at room temperature (25-30°C) improved the yield and quality of the isolated DNA as compared to precipitation for 1 h (Table 4.6) as mentioned in the original protocol. Precipitation at low temperature also increased the yield of DNA but exhibited a clear reduction in purity (Table 4.6). The optimum temperature was determined to be 25-30°C. These modified steps were necessary to improve the yield and quality of the isolated genomic DNA of the medicinal plants.

5.6.3 High concentration of NaCl

The addition of high concentration of NaCl increased the solubility of polysaccharides, effectively decreasing co-precipitation of polysaccharides and DNA. Warude *et al.* (2003) reported that 1.5 M NaCl was effective in removing polysaccharides. Fang *et al.* (1992) found that 1 M NaCl facilitated the removal of polysaccharides by increasing their solubility in ethanol. Salts, such as NaCl, were added to modulate the concentration of cation in the extraction buffer (Kawata *et al.*, 2003).

5.6.4 Leaf tissue

The fully opened tender leaves have a high cell density and contain less polysaccharides or secondary metabolites (Towner, 1992). Therefore, fully opened tender leaves were used for genomic DNA isolation. This was consistent with the results as reported by Choudhury *et al.* (1999), Kim *et al.* (1997), Lodhi *et al.* (1994), Richards *et al.* (1994) and Rout *et al.* (2002).

5.6.5 CTAB

The CTAB method is widely used on plants due to its versatility (Richards *et al.*, 1994). It is used as a detergent in the extraction buffer to separate polysaccharides from that of DNA (Pirttila *et al.*, 2001). Richards *et al.* (1994) opined that polysaccharides interfere with biological enzymes such as polymerases, restriction endonucleases, and ligases. According to Kawata *et al.* (2003) CTAB is included in extraction buffers as a reagent for protein denaturation in DNA isolation process.

5.6.6 EDTA and β-mercaptoethanol

EDTA is often included in the extraction buffer to chelate magnesium ions, a necessary co-factor for nucleases (Kawata *et al.*, 2003; Puchooa, 2004). β-mercaptoethanol is a strong reducing reagent and used to prevent oxidation of polyphenols present in the crude plant extract (Kawata *et al.*, 2003; Puchooa, 2004; Pirttila *et al.*, 2001).

5.6.7 Restriction digestion

Restriction endonuclease digestion of the DNA samples with *Eco* RI and *Hind* III showed complete homogenous restriction when resolved in 0.8% agarose gel (Fig. 4.26, 4.27 and 4.28). The DNA samples were well digested with both the enzymes, indicating the absence of impurities and inhibitors. Genomic DNA isolated from *Z. oxyphyllum* was partially degraded; however it was found to be suitable for the restriction digestion. DNA was completely digested with the restriction enzymes as was observed in case of *R. alcceifolius* and *M. spinosa* (Fig.

4.27 and 4.28) and there was no evidence of the presence of nuclease. Digestion with restriction enzymes is often used to test the purity and suitability of the DNA for use in recombinant DNA technology (Henry, 1997). The quality of the isolated DNA samples was evident from the restriction digestion experiment and this indicated that the isolated DNA samples would be amenable to further processing in other DNA manipulation techniques such as southern blot and cloning.

The method described in the present investigation is simple, efficient and reliable, and resulted in purified high molecular weight genomic DNA. The method can be used in other plant species having high amounts of polysaccharides and polyphenols.

5.7 Genome size determination

Flow cytometry (Otto, 1990) and microscopy method (Konwar *et al.*, 2007) were followed to determine the genome size of the plants and both methods were found to be comparable from the accuracy point of view.

5.7.1 Flow cytometry

The flow cytometry is a popular method of genome size determination and in recent years, the method has become a preferred technique for estimating the nuclear DNA content because of its accuracy and ease (Rayburn *et al.*, 1989, Heslop-Harrison, 1995; Bennet and Leitich, 1995; Dolezel *et al.*, 1998). Flow cytometry is a means of rapidly measuring large number of nuclei from small amounts of tissue (Hoping, 1993). The flow cytometry analyses the relative fluorescence intensity, and hence relative DNA content. The genome size of an unknown sample can be determined only after comparison with the nuclei of a reference standard (Dolezol and Bartos, 2005).

5.7.1.1 Fluorochrome

Propidium iodide (PI) was used as the flurochrome of choice for the estimation of nuclear DNA content, because PI-based flow cytometry produced consistent results with those based on Feulgen microspectrophotometry (Johnson *et*

al., 1999). The second plant genome size workshop (2003), held at Royal Botanic Garden, Kew, London also recommended PI as the flurochrome of choice.

5.7.1.2 Nuclei isolation buffer

The composition of nuclei isolation buffer is critical to facilitate the release of nuclei free from the cytoplasm and in sufficient quantities, maintain the integrity of isolated nuclei and facilitate DNA staining. Otto buffers (Otto 1990) are phosphate/citric acid buffers having pH 7.3 and comprise separate nuclear isolation and staining steps. Therefore it was possible to keep isolated nuclei in Otto I buffer at room temperature for prolonged time periods without negative influence on staining of DNA (Dolezol and Bartos, 2005).

5.7.1.3 Reference standard

One key criterion in genome size determination is the choice and correct use of reference standard, which has been largely neglected (Dolezel *et al.*, 1998). *Pisum sativum* was selected as the reference standard for the flow cytometric analysis of the isolated cell nuclei because the nuclear genome of *P. sativum* is stable (Barnyl and Greilhuber, 1995; Baranyl *et al.*, 1996). The 2C value of the nuclear genome of this plant is 9.09 pg (Dolezel *et al.*, 1995), which is in the middle of the known range of genome sizes of the plants and would facilitate calibration of reference standards with higher or lower genome sizes (Dolezol and Bartos, 2005). Moreover, the plant is easy to grow and multiply, and high quality nuclei suspensions can be prepared from leaves, which appear to be free from compounds interfering with PI staining.

5.7.1.4 External reference standard

The use of internal standard gave poor reading of the results in peak qualities, probably resulting from interference between the staining solutions and the genomes of two species. To overcome this problem, external reference standard was used and the same was controlled after every three samples to check the calibration of the flow cytomter by adjusting the gain of pea to channel 200. Hendrix and Stewart

(2005) used both internal and external standards to determine genome size of *Gosypium* sp. and found that results obtained from both were statistically similar. Srisawat *et al.* (1995) used external reference standard for the determination of oil palm DNA content and found consistent results with the previous reports. According to Hendrix and Stewart (2005) external standard is preferable until the issues related to internal standardization of the species under study are fully understood.

This is the first attempt and report of the genome size and nuclear DNA content for *Z. oxyphyllum* and *M. spinosa*. The genome size of *R. alceifolius* was earlier determined to be 1.29 - 1.75 pg for triploid and tetraploid species respectively using flow cytometry by Amsellem *et al.* (2003). The difference in genome size of *R. alceifolius* observed in the present investigation might be due to intraspecific variations in the genome size because of differences of ecological conditions or evolution of the species. Another explanation of this observed variation could be due to the proportion of nuclei in the G_2 stage of the cell cycle, with double DNA content as compared to nuclei in the G_0 and G_1 stages.

5.7.2 Microscopy method

This method of genome size determination involved several simple steps including measurement of average volume of single cell, volume of intercellular space in the leaf tissue, cell number and DNA content per g of leaf tissue. These steps could be worked out in a general laboratory having the facility of isolating plant DNA. Although, flow cytometry is expected to yield accurate and reproducible determination of 2C DNA content of plant species, the problem starts with expeditions to more distant areas, when the transport and maintenance of the material become an issue. Moreover, the cost of establishing a flow cytometry laboratory may be prohibitive in certain areas (Dolezel and Bartos, 2005). The technique needs sophisticated instrument, which is not possible in all situations. Moreover, at times, this method gives contradictory results. The North Eastern region of India, regarded as biodiversity 'hot spot', is yet to establish a flow cytometry laboratory (Konwar *et al.*, 2007). The method provided almost identical

results for all these plant species having 1-3% variation. Thus, the method is expected to help in characterizing the vast unexplored plant resources of the region.

5.7.3 Comparison of both methods

From Tables 4.9 and 4.10, and Fig. 4.30, very little difference of C-values of the plants was observed, and the difference being 0.22 pg in the case of *M. spinosa*. Therefore, the new method of genome size determination was found to be comparable to the popular technique of flow cytometry. The accuracy of results derived would depend on the correctness of the steps involved in both methods. Two critical points were taken into account in the microscopy method. Firstly, the method for the isolation of genomic DNA was such that it could isolate almost all the DNA from the nuclei, and secondly, the accurate determination of the intercellular space in the concerned plant species (3.2×10^9 - 6.1×10^9 μm). The technique of estimating nuclear DNA content of a nucleus using this method is expected to be innovative, unique and first of its kind (Konwar *et al.*, 2007).

Plant genome size is an important biological characteristic with the relationship to systematics, ecology and distribution. Knowledge of genome size of a species is essential for assessing the coverage of genomic library, estimating the copy number of a gene in genome and developing strategies for gene cloning based on genome mapping (Arumugunathan *et al.*, 1999). An accurate determination of genome size provides basic information for breeders and molecular geneticists (Lee *et al.*, 2005). Researchers continue to report new relationships between genome size and characters of interest for the plant breeders. A comparison on the amount of nuclear DNA is helpful in cytotaxonomic and evolutionary studies. Knowing the genome size and DNA content of these three plants species will help in improving our knowledge base and elucidate the systematic relationship between closely related species.

Chapter 6
Conclusion

Chapter 6 Conclusion and future works

6.1 Conclusion

From the present investigation following conclusions were drawn:

1. The chromosome numbersof *Z. oxyphyllum*, *R. alceifolius* and *M. spinosa* were determined to be 2n = 2*x* = 36, 2n = 4*x* = 28 and 2n = 4*x* = 44, respectively. From the chromosome formula it is evident that the first species is a diploid one while the last two species are tetraploid.
2. From the tender shoots and leaves of *Z. oxyphyllum* and *R. alceifolius* antibacterial and antifungal compound 2-methylheptyl isonicotinate was isolated while oleanolic acid and oleanol were isolated from the mature fruits of *M. spinosa*. The isolated compounds were found to be potential as antibacterial and antifungal agents against *B. subtilis, E. coli, K. pneumoniae, S. aureus* and yeast *C. albicans*.
3. A simple, efficient and reliable CTAB-based method was standardized for the isolation of genomic DNA of medicinal plants. The method yielded a high amount (38 - 46 μg g^{-1} fresh leaf tissue) of quality DNA free from contaminants. DNA from all the samples was effectively subjected to complete digestions with *Eco* RI, *Hind* III and mixtures of *Eco* RI and *Hind* III restriction endonuclease enzymes.
4. The flow cytometric determination of genome size of the medicinal plants ranged from 2.84 - 3.93 pg or 2.0 x 10^9 - 3.93 x 10^9 bp indicating larger genome in the case of *M. spinosa* and smaller in *R. alceifolius*. By using the new microscopy method, the genome size of the plants were estimated to be 2.71 - 3.79 pg or 3.51 x 10^9 - 3.70 x 10^9 bp. Data indicated that the new microscopy method of genome size determination was comparable with the popular technique of flow cytomtry. The microscopy method was found to be innovative and less expensive.
5. The study highlighted the vast diversity of medicinal plants in the region, which could be commercially exploited. There is an urgent need to explore

and document the plants of North East India used by different communities for medicinal purpose.

6.2 Future works

In nature, plants undergo rapid polyploidization and therefore, the selected plant species need to be investigated in the context of different localities of the region to find out the variability at ploidy level. The study of karyotype of the plants would help to understand the evolution pattern, reproduction behaviour and also to identify the species from other related or allied species.

The investigation of active principles from the plants might lead to the discovery of new therapeutic drugs. The antimicrobial assay of the isolated compounds needs further investigation such as use of positive control and determination of MIC value. Determining the bioactive potentiality of phytochemicals against bacteria and fungi would provide vital information in combating the situation arising out of development of resistant bacteria and fungi against common antibiotic and antifungal drugs.

The optimized protocol of DNA isolation could be applied to isolate genomic DNA from the plants rich in polyphenols and other secondary metabolites. Flow cytometric determination of genome size of plants needs further characterization such as taking samples from different localities and geographical regions, use of different nuclei isolation buffer, use of *Hordeum vulgare* and *Allium cepa* as external and internal reference standards. Molecular study including isolation of pure genomic DNA, restriction digestion and genome size determination would provide the basis at molecular level for future improvement in desired direction by utilizing genetic engineering tools.

The recorded medicinal plants would provide a database for future phytochemical, pharmacological, cytological and molecular investigations. Research efforts should therefore be directed for the major diseases for which suitable drugs are not available in the modern system of medicine and where herbal medicines have possibility of offering new drugs.

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List of Publications

In Journal

1. Buragohain, J. and Konwar, B.K. (2007). Ethnomedicinal plants used in skin diseases by some Indo-Mongoloid communities of Assam. *Asian J. Exp. Sci.*, **21** (2): 281-288.

2. Buragohain, J. and Konwar, B.K. Genome size determination of *Zanthoxylum oxyphyllum* and *Meyna spinosa* by flow cytometry: a preliminary study. *Journal of Cell and Tissue Research* (In Press).

3. Buragohain, J. and Konwar, B.K. An efficient and reliable method of DNA extraction from *Meyna spinosa*: a traditional medicinal plant from North East India. *Journal of Plant Biochemistry and Biotechnology* (In Press).

Paper presented in National Seminar

1 Buragohain, J. and Konwar, B.K. (2006). Antimicrobial activity of the fruits of *Meyna spinosa* Roxb ex Link: a potential medicinal plant of North East India. Paper presented in the National Seminar on "*Value Addition to Bioresources of NE India, Post Harvest Technology and Cold Chain*" held at Gauhati University, Guwahati on 19-21 May, 2006.

2. Buragohain, J. and Konwar, B.K. (2006). Isolation of genomic DNA from *Zanthoxylum oxyphyllum* for assessment of genetic diversity. Paper presented in the UGC sponsored National Seminar on "*Biodiversity and Indigenous Knowledge System*" held at Rajiv Gandhi University, Arunachal Pradesh on 25-26 October, 2006.

Annexture I

List of recorded medicinal plants and their uses

S N	Name of the Species	Family	Local name	English name	Flowering & fruiting	Morphological Characteristics	Part used	Medicinal Use
1	*Abelmoschus esculentus* (L) Moench. MBBT/0261	Malvaceae	Bhendi	Lady's finger	May-Sep	Hairy shrub; leaves cordate, 3-5 lobed, coarsely toothed, scabrous; flowers yellow with crimson center; capsules pyramidal, oblong with longitudinal ridges.	Fruit	Expectorant
2	*Abroma augusta* L. MBBT/0029	Sterculiaceae	Ulot kambal, gorokhia korai	Devil's cotton, Cotton abroma	Apr-Aug	Small tree; leaves alternate, 3-5 lobed with very palmate veins; stems and leaves are covered with soft bristly hairs; flowers dark maroon, in terminal panicles; fruits capsule.	Root	Uterine tonic
3	*Abrus precatorius* L.	Fabaceae	Latumoni	Crab's eye vine	Jul-Nov	Climbing shrub; leaves paripinnate, leaflets 10-18 pairs, opposite oblong; flowers white, tinged with pink, in axillary pedunculate racemes; seeds scarlet red with a black spot	Root	Uterine tonic, leucoderma
4	*Abutilon indicum* (L.) Sweet	Malvaceae	Jopa, Junuka goch	Indian mallow	Apr-Oct	Pubescent undershrub; leaves cordate or ovate-orbicular, acute, long petiolate; flowers yellow, axillary, solitary, on long peduncles; capsules subgloboose, many seeded.	Leaf	Toothache, fever
5	*Acacia fernesiana* (L.) Willd. MBT/0158	Mimosaceae	Tarua-kadam	—	Sep-Mar	Thorny shrub; branches zigzag, leaves bipinnate, pinnae 10-14, stipules spiny;	Stem bark,	Malaria

						flowers in axillary head, bright yellow, fragrant.		
6	*Acalypha indica* L. MBBT/0225	Euphorbiaceae	Bishohori, Muktajuri	Indian acalypha	Apr-Sep	Erect annual herb; leaves with long petioles, ovate or rhomboid-ovate; flowers unisexual, in axillary spikes; male flowers minute, followed by a tuft of sterile flowers; female flowers scattered; capsules small, seed pale brown.	Whole plant	Skin diseases, ulcers
7	*Achras zapota* L. MBBT/0197	Sapotaceae	Sopeta	Sapota, Tree potato, Gum chicle	Apr-Aug	Tree with laticiferous barks; leaves leathery; flowers small, solitary; fruits berry.	Fruit	Diarrhoea
8	*Achyranthes aspera* L. MBBT/0047	Amaranthaceae	Bioni-hakuta, Ubhuta bonsoth	Prickly chaff flower	Oct-Apr	Erect herb, leaves opposite, ovate-elliptic rounded or narrowed down at the base; flowers small, greenish white, pink in long spikes	Leaf, root	Swellings and wounds of nipples, cough, sore throat, abscesses
9	*Acorus calamus* L.	Araceae	Bosh	Sweet flag	Nov-Jan	Aromatic herb with creeping rhizomatous rootstock; leaves linear; spadix long peduncled; capsules oblong.	Rhizome	Dyspepsia, flatulence, whooping cough
10	*Adenanthera pavoniana* L.	Mimosaceae	Ronga-chandan	Coralwood tree, Redwood tree	Apr-Sep	Medium sized tree; leaves bipinnate, pinnae 3-6 pairs, opposite, leaflets many, alternate, ovate-oblong; flowers pale yellow, in short peduncled racemes; fruits falcately curved pods, the valves spirally twisted after dehiscence; seeds lenticular-globose, brilliant scarlet	Leaf, bark	Useful in ulcers
11	*Aegle marmelos* (L.)	Rutaceae	Bel	Bael fruit	Apr-Aug	Middle sized spinous tree;	Leaf,	Peptic ulcer,

	Corr. MBBT/0229			tree, Bengal quince		leaves alternate, trifoliate; flowers greenish white, long, fragrant, borne on axillary cymose panicles; fruits globose, oblong or pyriform; pulp orange, sweet	fruit	chronic dysentery, constipation
12	*Aerva sanguinolenta* (L.) Bl.	Amaranthaceae	Soru-arokson	—	Apr-Sep	Undershrub; leaves alternate and opposite, variable, ovate-lanceolate; flowers white, in axillary and terminal compact spikes; fruits capsule	Tender shoot	Galactogue to nursing mothers
13	*Agerartum conyzoides* L. MBBT/0017	Asteraceae	Gendhela bon	Goat weed	Jan-Dec	Aromatic, erect, hispid herb; leaves opposite, ovate; heads blue or white in terminal corymb; Fruits achene.	Leaf	Cuts and wounds
14	*Alangium chinese* (Lour.) Harms. Syn. *A. begonifolia* Roxb. MBBT/0175	Alangiaceae	Sika morolia, maroli goch	—	May-Sep	Small tree with straggling branches; leaves simple, alternate, ovate-orbicular, shortly lobed; flowers white, in axillary dichotomous peduculate cymes	Leaf, stem bark	Malaria
15	*Albizia lebbeck* (L.) Willd. MBBT/0200	Mimosaceae	Siris-goch	Siris tree	Mar-Aug	Deciduous tree; leaves abruptly bipinnate, main rachis with a large gland above the base and one below the uppermost pair of pinnae, pinnae 2-4 pairs, leaflets 5-9 pairs with glands between the bases; flowers white, fragrant, in globose umbrella-heads; fruits charcteristic pods, pale yellow; seeds 4-12, pale brown, oblong.	Bark, flower, seed	Expectorant, skin diseases, chronic cough, bronchitis
16	*Albizia lucidior* (Steud.) Niels. Syn.	Mimosaceae	Moj		Apr-Jan	Evergreen tree; leaf rachis with a large cup-shaped gland;	Bark	Cuts, burns

	A. lucida (Roxb.) Benth.					leaflets 2-3 pairs, oblong-lanceolate; flowers creamy white, in corymbose heads on terminal panicle; pods thin, brown, shining.		
17	*Albizia odoratissima* (L. f.) Benth.	Mimosaceae	Kola-sirish	Black siris	Apr-Aug	Tree with irregularly cracked bark; leave abrupty pinnate, alternate, leaflets unequal sided, obtuse or rounded at the apex, dark green; flowers white, sessile, numerous, in small globose 5-10 flowered heads; fruits shortly stalked pods, brown; seeds flat, yeelow.	Bark	Ulcers and skin diseases
18	*Allium cepa* L. MBBT/0141	Liliaceae	Piyaj	Onion	Dec-Feb	Herb with aromatic fleshy underground bulb; leaves linear, hollow, cylindric and fleshy; flowers many, white in globular umbels.	Bulb	Digestive, carminative, blood purifier, skin diseases
19	*Allium sativum* L.	Liliaceae	Nohoru	Garlic	Dec-Feb	Foetid perennial herb; bulbs compound, underground, covered over by outer white thin scales; leaves simple, long flat, linear; flowers small, white, in rounded umbels mixed with small bulbils.	Bulb	Digestive, carminative, blood purifier, ringworm, scabies
20	*Alocasia indica* (Roxb.) Schott.	Araceae	Man kachu	—	Jan-Jun	Stout coarse herb; leaves green, triangular, saggitate; petiole stout, long; flowers monoecious; spathe yellowish green with foetid smell.	Rhizome	Abdominal pain
21	*Alocasia macrorriza* (L.) G.Don	Araceae	Bor kochu	—	Jan-Mar	Large rhizomatous herb; leaves broad, saggitate, basal lobes much shorter, petioles	Rhizome, tender leaf	Abscesses, tonsillitis

						dark brown; spathes green with yellowish glaze.		
22	*Aloe barbadensis* Mill. Syn. *A. vera* Tourn. ex L.	Liliaceae	Chalkonwa ri	Indian aloe	Sep-Jan	Coarse looking herb; leaves succulent, green, large, densely crowded; flowers bright yellow.	Leaf	Burn injuries, wounds, abscesses, fever
23	*Alpinia galanga* (L.) Willd.	Zingiberaceae	Kulanjan	Greater galanga	Apr-Jun	Aromatic herb; tuber deep orange brown, pungent; leaves glossy on both sides; flowers greenish white, in densely flowered, branched panicles; fruits orange red.	Rhizome	Rheumatism, bronchitis
24	*Alpinia nigra* (Gaertn.) Burtt. Syn. *A. allughas* (Retz.) Rosc.	Zingiberaceae	Tora	—	May-Aug	Tall herb; rootstock tuberous; leaves oblong-lanceolate, acuminate; flowers pinkish white, in terminal panicles; capsule globose.	Rhizome	Bronchitis, scabies and other skin diseases
25	*Alstonia scholaris* (L.) R. Br. MBBT/0120	Apocynaceae	Chatiana	—	Oct-Mar	Evergreen tree; leaves oblong-lanceolate, narrowed at base, coriaceous; flowers white, in terminal umbeliform cymes; follicles drooping.	Latex, Stem bark	Scabies, chronic diarrhoea, dysentery, malaria
26.	*Alternanthera sessilis* (L.) R.Br. ex DC. MBBT/0007	Amaranthaceae	Mati - kanduri	—	Jan-Dec	Prostrate herb; rooting at nodes; leaves opposite, linear-lanceolate; flowers white, in axillary, sessile heads.	Tender shoot	Dysentery
27	*Amaranthus spinosus* L. MBBT/0054	Amaranthaceae	Hati-khutura	Spiny amaranth	Jan-Dec	Erect or diffuse spinous herb; spines axillary, paired; leaves ovate-elliptic, petiolate; flowers greenish white, sessile, in axillary clusters or terminal spikes	Root, tender shoot	Diarrhoea, galactogue to nursing mothers*, eczema
28	*Amaranthus tricolor* L. var. *tristis*	Amaranthaceae	Bishalya karani	—	Jun-Nov	Erect or procumbent herb; leaves broadly ovate, base	Leaf	Cuts and wounds

						tapering into petiole; flowers green, in dense axillary clusters.		
29	*Amaranthus viridis* L. MBBT/0090	Amaranthaceae	Khutura	—	Jan-Dec	Erect herb; leaves ovate or rhomboid oblong; flowers greenish white, in axillary or terminal panicled spikes.	Tender shoot	Improves eyesight
30	*Amomum subulatum* Roxb.	Zingiberaceae	Bor-elachi	Greater cardamon, Nepal cardamon	Jan-Dec	Erect herb; leaves spiral, oblong-lanceolate; flowers yellowish white, in dense, short-peduncled, globose spikes; bracts red; capsules globose, red or brown.	Fruit	Aphrodisiac
31	*Amorphophalus paeoniifolius* (Dennst.) Nicolson Syn *A. campanulatus* (Roxb.) Bl. ex Decne	Araceae	Ol-kochu	—	Jul-Oct	Annual herb; leaves palmately lobed, crowded on the main stem; stem hairy; flowers cream coloured, unisexual, in a spadix enclosed by a spathe.	Tender shoot, corm	Sinusitis, dysentery, piles, rheumatism
32	*Ananus comosus* (L.) Merr.	Bromelliaceae	Anaras, mati-kothal	Pineapple	Apr-Aug	Perennial herb with a short stout stem; leaves spirally and compactly arranged, linear-lanceolate, margins spiny, toothed; heads terminal, ovoid; bracteoles reddish, triangular-ovate to oblong-ovate; fruits composite, succulent, bearing a crown of leaves.	Fruit	Abortifacient, constipation
33	*Andographis paniculata* (Burm.f.) Wall. ex Nees. MBBT/0176	Acanthaceae	Chirota, Kalmegh		Nov-May	Erect much branched herb with quadrangular stem; leaves opposite, linear or elliptic-lanceolate; flowers white, in axillary or terminal	Root, leaf	Malaria, diarrhoea, abscesses

						paniculate racemes; capsules linear-oblong.		
34	*Anona squamosa* L.	Annonaceae	Atlas-kothal		Jun-Sep	Small tree with spreading branches; leaves oblong-lnceolate; flowers yellow, axillary, solitary or few flowered together; fruits tubercled, pulp sweet.	Leaf	To kill lice in the hair
35	*Anthocephalus chinensis* (Lamk.) Rich.ex Walp. Syn. *A. cadamba* (Roxb.) Mig. MBBT/0190	Rubiaceae	Kadam goch		Dec-May	Deciduous tree, branches horizontal; leaves ovate elliptic or ovate-lanceolate; flowers orange yellow, in terminal peduncled, solitary globose heads; corolla funnel shaped; pseudocarps fleshy.	Leaf	Abscesses
36	*Aquillaria malaccensis* Lamk. Syn. *A. agalocha* Roxb. MBBT/0191	Thymeliaceae	Sachi-goch, Agaru	Agarwood, Aloe wood, Eaglewood	May-Aug	Tree; leaves oblong-lanceolate or elliptic; flowers white, in terminal umbellate cymes; fruits obovoid.	Root	Abdominal pain
37	*Areca catachu* L.	Arecaceae	Tamul		Apr-Jun	Tall unbranched palm; stem ending in a crown of leaves; leaves long, pinnatisect; flower white, monoecious, in branched spadix; nuts ovoid, smooth	Fruit (nut)	Septic ulcer*
38	*Argemone mexicana* L.	Papavaraceae	Shialkata	Mexican poppy	Jan-Jul	Erect prickly herb with yellow juice; leaves sessile, elliptic-obovate, pinnatified; flowers yellow, solitary, sessile; sepals concave, prickly outside; fruits oblong prickly, capsule.	Latex	Eye disease
39	*Argyreia nervosa* (Burm. f.) Boj. Syn. *A. speciosa* Sweet. MBBT/0097	Convolvulaceae	Tokoria alu	Elephant creeper	Aug-Nov	Perennial woody climber; leaves ovate-orbicular, cordate at the base; flowers funnel shaped, light purple; fruits	Leaf	Emolient, wounds, skin diseases

						globose, orange when ripe.		
40	*Aristologia tagala* Cham.	Aristologiaceae	Nilakantha		Apr-Dec	Perenial climber; stem softly woody; leaves alternate, petiolate, simple, exstipulate; fruit capsule.	Leaf	Cough
41	*Artemisia caruifolia* Roxb.	Asteraceae		Worm wood	Jul-Sept	Annual herb with stout stems; leaves sessile, deep green, multifid, segments very narrow and weak; heads green, many flowered.	Leaf	Fever, chronic diarrhoea, intestinal troubles
42	*Artemisia nilagricia* (Clarke) Pamp. Syn. *A. vulgaris* L. MBBT/0044	Asteraceae	Nagdona	Indian wormwood	Jul-Nov	Tall perennial herb; leaves pinnatisect, lobes deeply incised; flowers whitish yellow, in greenish panicled racemes.	Leaf, whole plant	Burn injuries, measles
43	*Artocarpus heterophyllus* Lamk. MBBT/0106	Moraceae	Kothal	Jackfruit	Mar-Aug	Evergreen tree; leaves elliptic or obovate, dark green above; flowers in cylindrical, axillary and terminal heads, embraced by leathery sheath; fruits oblong, tubercled.	Fruit	Swellings of feet and arms
44	*Artocarpus lacucha* Hom. Syn. *A. lakoocha* Roxb.	Moraceae	Bohot	—	Apr-Aug	Tree with dark brown bark; leaves elliptic-ovate or oblong; flowers monoecious, orange yellow, in axillary shortly stalked heads; fruits subglobose.	Fruit	Dysentery
45	*Arundo donax* L.	Poaceae	Nol	—	Aug-Dec	Perennial strong grass; culms high, jointed, hollow; Leaves linear to lanceolate; inflorescene a large panicle; spikelets long, awned, caryopsis oblong.	Rhizome	Lactifuge
46	*Asparagus rcemosus*	Liliaceae	Shotmul	Asparagus	Nov-Jan	Sraggling scandent spinous	Root	Tonic

	Willd. MBBT/0071					undershrub; root tuberous; cladodes in tufts; flowers white, in short solitary or fascicled racemes; berries globose.		
47	*Averrhoea carambola* L.	Averrhoaceae	Kordoi	Carmbola, Gooseberry	Jun-Nov	Evergreen tree; leaves compound, leaflets 5-11; flowers variegated with white and purple, in axillary racemes; fruits ovoid, 5-angled and 5-furrowed.	Fruit	Jaundice
48	*Azadirachta indica* A.Juss. MBBT/0124	Meliaceae	Neem	Margosa tree	Mar-Jun	Deciduous tree; leaves imparipinnate, crowded at the end of the branches, leaflets oblong, ovate-lanceolate or lanceolate; flowers pale white, in axillary racemes; capsules ovoid or obovoid, 3-celled.	Leaf	Anthelmintic, skin diseases, small pox, chicken pox and measles
49	*Bacopa monnieri* (L.) Penn. Syn. *Herpestis monnieri* (L.) Kunth. MBBT/0019	Scrophulariaceae	Brahmi	Thyme leaved gratiola	Jun-Aug	Prostrate or creeping herb; creeping at nodes; leaves obovate-oblong or spathulate; flowers pale blue or whitish, axillary, solitary; capsules 2-valved, 2-celled.	Whole plant	Cough, blood purifier, nervous disorders, brain tonic
50	*Baccaurea ramiflora* Lour.	Euphorbiaceae	Leteku	—	Apr-Aug	Small tree; leaves alternate, elliptic-oblong, narrowed to the base; flowers yellow, dioecious, in densely fascicled racemes; fruit ovoid-globose, fleshy.	Stem bark, fruit	Infected umbilicus of newly born baby*, constipation, prickly heat*
51	*Bambusa arundinacea* (Retz.) Willd.	Poaceae	Bah	Thorny bamboo	Jan-Mar	Tufted bamboo; culms spiny, leaf blade long, linear-lanceolate, shortly petioled; culm sheath hairy outside, blades triangular, hairy inside;	Sprout	Dyspepsia, anthelmintic

						spikelets 4-6 flowered; caryopsis oblong.		
52	*Bambusa balcooa* Roxb.	Poaceae	Bhaluka-bah	Plain bamboo	Feb-May	Stout tall bamboo; culms thick, leaf blades long, lanceolate, petioled; spikelets 6-8 flowered, in dense heads	Culm	Quick healing of wounds
53	*Bambusa tulda* Roxb.	Poaceae	Bijuli bah	—	Jan-Jun	Tufted bamboo; leaves long, linear-lanceolate, rough on margin; spikelets 7-12 flowered, in loose head; caryopsis hairy at top.	Shoot	Appetizer, respiratory complaints
54	*Barringtonia acutangula* (L.) Gaertn.	Barringtoniaceae	Hijal	Indian oak	Jun-Oct	Small tree; leaves crowded near the tip of the branchs, narrowed to base; flowers reddish, in long terminal pendulous racmes; fruit ovoid, blunty, quadrangular.	Twig	Toothache
55	*Basella alba* L. var. *rubra* (L.) Stew. MBBT/0079	Basellaceae	Puroi	Indian spinach	Oct-Dec	Fleshy, twining herb; stem greenish pink; leaves ovate-elliptic to oblanceolate; flowers pinkish green, in axillary pedunculate spikes; urticles ovoid, globose.	Leaf	Allergy
56	*Bauhinia variegata* L.	Caesalpiniaceae	Kural	—	Dec-Aug	Middle sized tree; leaves as long as broad; flowers large, variegated, white, purple veined, in lateral corymb; pods slightly curved.	Root	Inflammation of throat, abscesses
57	*Begonia roxburghii* DC	Begoniaceae	Noga tengesi	—	Jun-sep	Succulent herb; leaves broadly ovate, obliquely cordate; flowers pinkish red, in axillary dichotomous branched cymes; capsules 4-lobed.	Root	Scabies
58	*Belamcanda*	Iridaceae	Surjyakanti	Blackberry	Aug-Dec	Perennial herb; root sock	Root,	Abscesses, burn

	chinensis DC		phool	lily		creeping; leaves isobilateral, equitant; flowers stalked, bracteate, in spathaceous cymes; capsules obovoid.	leaf	injury
59	*Benincasa hispida* (Thunb.) Cogn.	Cucurbitaceae	Kumura	Ash gourd	Jul-Oct	Tendril climber; leaves slightly pentangular, petioles long, tendrils bifid, extra-axillary; flowers yellow, axillary, pedunculate, monoecious; pepo large, cylidric.	Fruit	Jaundice
60	*Bischofia javanica* Bl.	Euphorbiaceae	Uriam	Bishop wood	Apr-Dec	Deciduous tree; leaves alternate, trifoliate; leaflets elliptic-ovate; flowers greenish, in panicled racemes; fruits globose.	Fruit	Dysentery
61	*Bixa orellana* L.	Bixaceae	Jorot goch	Arnetto dye tree	Mar-Aug	Shrub; leaves ovate; flowers pinkish white, in panicles; capsules brown, spiny; seeds with bright red waxy coating.	Stem bark	Dysentery, kidney trouble
62	*Blumea lacera* (Burm.f.) DC.	Asteraceae	Kachidoria, kukursuta	—	Jan-May	Erect herb; leaves simple, lower petioled and upper subsessile, elliptic-oblong or obovate; flowers yellow, in terminal and axillary head.	Leaf, whole plant	Cuts and wounds, piles
63	*Boerhaevia diffusa* L.	Nyctaginaceae	Ponounowa	Spreading hog-weed	Jun-Nov	Diffuse much branched herb; branches swollen at nodes, pinkish; leaves opposite ovate-oblong, unequal; flowers pink, in terminal and axillary panicled umbels; fruits 5-ribbed.	Whole plant	Abscesses, dyspepsia
64	*Bombax ceiba* L.	Bombacaceae	Simolu	Silk cotton tree, Red cotton tree	Jan-May	Large tree; leaves digitately 5-7 foliate; leaflets elliptic lanceolate; flowers bright red	Root, spine	Diabetes, abscesses, pimples

						and orange yellow, large, solitary or clustred at the end of the branches; fruits oblong-ovoid capsule, 6-valved; seeds many, white silky hairy.		
65	*Borreria articularis* (L.f.) Will	Rubiaceae	Gahori bon	—	Jul-Nov	Much branched herb with quadrangular stems; leaves hispid, opposite, elliptic-ovate; flowers small, white, axillary in clusters; capsules globose, hairy.	Whole plant	Abdominal pain
66	*Brassica juncea* (L.) Czera. et Coss. MBBT/0087	Brassicaceae	Jati-lai	Indian mustard	Nov-Jan	Annual herb; leaves broadly ovate, coarsely dentate, middle leaves oblong, upper leaves linear; flowers yellow, in racemes; fruits siliqua.	Leaf, seed	Fever, skin diseases, cold infection
67	*Brassica napus* L. Syn. *B. campestris* L. sub.sp. *napus* (L.) Hook.f.et Thoms.	Brassicaceae	Sariah	Rape	Nov-Jan	Erect herb with fusiform roots; basal laves lyrate, upper ones entire or dentate; flowers golden yellow; seeds pale yellow.	Seed	Pimples
68	*Butea monosperma* (Lamk.) Taub.	Fabaceae	Polash	Flame of the forest	Apr-Sep	Tree; leaves trifoliate, leaflets brodly ovate or rhomboid; flowers flame coloured, in racemes; pods oblong, leathery.	Flower, Seed, latex	Urinary trouble, sore eyes, anthelmintic, ringworm, pimples
69	*Caesalpinia bonduc* (L.) Roxb.	Caesalpiniaceae	Letaguti	—	Aug-Dec	Scandent prickly shrub; Leaflets opposite, oblong or elliptic; flowers yellow, in dense peducled racemes; pods oblong, elliptic, prickly.	Seed	Pneumonia
70	*Caesalpinia cuculata* Roxb. Syn. *Mezoneurum cucullatum* (Roxb.)	Caesalpiniaceae	Bagh-achura	—	Nov-Jun	Straggling prickly shrub with dark brown branches; leaflets ovate-elliptic; flowers yellow, in terminal or axillary	Root	Swelling of joints

	Wt. Et Arn.					racemes; pods oblong, flat, 1-seeded.		
71	*Caesalpinia pulcherima* Swart.	Caesalpiniaceae	Radhachura	Dwarf goldmohar	Aug-Dec	Large shrub; leaves compound with 20-24 stalkless leaflets; flowers yellowish red or yellow, arranged in a pyramidal fashion, in peduncled cymes; pods flat with 6-8 seeds	Leaf, flower	Watery evacuation of the bowels, fever
72	*Cajanas cajan* (L.) Millsp. Syn. *C. indicus* Spr. MBBT/0072	Fabaceae	Rohor mah	Pigeon pea	Oct-Jan	Erect shrub with silky hair; leaves compound, leaflets oblong lanceolate, densely silky beneath; flowers yellow, in terminal panicles or corymbose racemes; fruits pod, seeds vary in colour.	Leaf	Jaundice
73	*Calamus rotang* L.	Arecaeae	Raidang bet	Rattan	Mar-Jan	Scandent slender palm; Stem yellowish, cylindrical, armed with short flat spines on the leaf sheaths; leaflets trigonous, rachis flat near the base, 1-ribbed; flowers unisexual, in long spadix; spathes elongate; fruits subglobose, pale yellow.	Shoot	Prevention of measles, skin diseases
74	*Calamus tenuis* Roxb.	Arecaeae	Jati-bet	—	May-Nov	Scandent armed palm; leaves pinnate, leaflets many, linear-lanceolate, rachis armed with recurved prickles; spadix long, spathe tubular; flowers unisexual, small in spikes; fruits subglobose.	Tender shoot	Blood purifier
75	*Calotropis procera* (Ait.) R. Br.	Asclepiadaceae	Akon	—	Apr-Sep	Tomentose shrub; leaves elliptic, ovate or obovate; flowers purplish white, in umbellate lateral cymes;	Latex, Leaf	Carbuncles, rheumatic pain, chest pain

						follicles long, recurved.		
76	*Camellia chinensis* (L.) O.Ktze. MBBT/0084	Theaceae	Chah-goch	Tea plant	Jan-Dec	Evergreen shrub; leaves alternate, elliptic-ovate or lanceolate; flowers white, fragrant, solitary or 2-4 together; capsules 3-seeded.	Leaf	Water sores
77	*Canarium bengalense* Roxb.	Burseraceae	Dhuna-goch	—		Tree with clear, amber coloured resin; leaves imparipinnate, leaflets 13-15, lanceolate; flowers in panicles; drupes ellipsoid, dark purple, aromatic.	Bark	Dysentery
78	*Cannabis sativa* L.	Canabinnaceae	Bhang	—	Apr-Dec	Aromatic herb; leaves alternate, upper ones often 1-3 foliate while lower ones 5-11 foliate; leaflets sessile, lanceolate, flowers white, small; male flowers in axillary cymes; female flowers *axillary, solitary; fruits* compressed nuts.	Leaf	Piles, skin cuts
79	*Capsicum frutescens* L. MBBT/0189	Solanaceae	Jolokia	Green pepper chilli	Feb-May	Branched undershrub; leaves elliptic-ovate; flowers white, axillary; fruits many-seeded berry.	Leaf, fruit	Dysentery
80	*Capsula bursa-pastoris* (L.) Medic.	Brassicaceae	Gonga moola	Shepherd's purse	Dec-Apr	Annual herb; leaves radical, basal leaves form a rosette, blade unequally deply divided; floweres white, in racemes; fruit triangular, flattened, heart shaped.	Leaf	Blood pressure, diarrhoea
81	*Carallia lucida* Roxb. Syn. *C. integerima* DC.	Rhizophoraceae	Mahi thekera, kon thekera	—	Dec-Mar	Evergreen small tree; leaves ovate, coriaceous with close parallel veins; flowers sessile; berry 1-locular, 1-seeded.	Fruit	Contagious ulcer

82	*Cardiospermum halicacabum* L. MBBT/0012	Vitaceae	Lota-kopal-phoota	Balon vine	Apr-Oct	Climbing herb, tendrils axillary; leaves bipinnate; flowers white, on long slender peduncled cymes with a pair of opposite tendrils below; fruits capsule.	Leaf	Swellings of muscles
83	*Carica papaya* L. MBT/0193	Caricaceae	Amita	Papaya	Apr-Oct	Shrub; stem fistular; leaves large, deeply lobed, forming a crown at the top; flowers white, axillary; fruits spherical or cylidrical; seeds black.	Fruit	Burn injury, snakebite, ringworm, pimples, sore eyes, jaundice, antifertility, liver disorder, and constipation
84	*Carissa carandus* L.	Apocynacceae	Korja tenga	Christ's thorn	Apr-Aug	Shrub armed with spreading spines in pairs; leaves elliptic oblong,; flowers white, in corymbose cymes; berries globose, 4-seeded.	Stem bark, fruit	Chest pain, stomach problems
85	*Caryota urens* L.	Arecaceae	Sewa	Toddy palm wine palm, jaggery palm	Feb-Jul	Tall stout palm; leave large, bipinnate; spadix large, interfoliar; flowers monoecious, a female between two males; fruits globose, dark purple.	Root	Galactogue to nursing mothers
86	*Cascabela thevetia* (L.) Lipp. Syn. *Thevetia perviana* (Pers.) K. Schum.	Apocynaceae	Korobiphul	Trumpet flower	Apr-Nov	Shrub; leaves alternate, linear or linear-lanceolate; flowers fragrant, yellow, in subterminal cymes; drupes 4-angled, subglobose.	Stem bark, seed	Boils, antifertility, malaria.
87	*Cassia alata* L. MBBT/0026	Caesalpiniaceae	Khorpat	Ringworm bush	Apr-Oct	Perennial shrub; leaves pinnate, leaflets 10-12 pairs, obovate-oblong, base unequal; flowers bright yellow, in long racemes; pod flat, winged.	Leaf	Scabies, ringworm

88	*Cassia fistula* L. MBBT/0182	Caesalpiniaceae	Sonaru	Golden flower, Indian laburnum	Apr-Sep	Small tree; leaflets 4-8 pairs, opposite, ovate-lanceolate; flowers yellow, in axillary drooping racemes; pods indehiscent, darkbrown when ripe.	Root, leaf, fruit	Common cold, constipation, septic ulcer
89	*Cassia occidentalis* L. MBBT/0032	Caesalpiniaceae	Medelua	Cofea senna	Apr-Sep	Erect undershrub; leaflets 3-5 pairs, ovate or elliptic-lanceolate; flowers orange yellow, in short axillary corymbose racemes; pods slightly recurved.	Leaf	Ringworm, itches
90	*Cassia sophera* L. MBBT/0075	Caesalpiniaceae	Medelua	—	Jun-Nov	Undershrub; leaflets 4-8 pairs, opposite, lanceolate; flowers yellow, in terminal or axillary corymbose racemes; pods slightly curved.	Leaf	Ringworm, scabies, insect bite
91	*Cassia tora* L. MBBT/0181	Casalpiniaceae	Dadigdiga, Bilokhoni	—	Jul-Nov	Undershrub; leaflets 3 pairs, opposite, obovate-oblong; flowers yellow, in subsessile pairs, in leaf axils; pods obliquely septate.	Leaf	Ringworm, asthma
92	*Catambium malaccense* (Burm.f.) Syn. *Alpinia malaccensis* (Burm.f.) Rosc.	Zingiberaceae	Bor tora	—	Jun-Sep	Shrub; leaves villous beneath; racemes erect; flowers white; lip ovate, yellow with variegated center of deep yellow or red; fruits globose, yellow to orange colour when mature.	Rhizome	Remedy of sores
93	*Catheranthus roseus* G. Don. Syn. *Vinca rosea* L. MBBT/0033	Apocynaceae	Nayantora	Periwinkle	Jan-Dec	Undershrub; Leaves glossy, oblong; flowers white or purplish pink; fruits cylindrical.	Leaf	Diarrhoea, diabetes, anticarcinogenic
94	*Catunaregam spinosa* (Thunb.)	Rubiaceae	Bihmona	—	May-Oct	Spiny shrub; spines axillary; leaves obovate, narrowed	Fruit	Asthma, bronchitis,

	Tirven. Syn. *Randia dumetorum* (Retz.) Lam.					down into a petiole; flowers creamy white, solitary or few flowers together; berries globose, fleshy.		pneumonia, fever
95	*Celastrus paniculatus* Willd.	Celastraceae	Jutimali	Black oil plant	May-Oct	Woody climber; leaves alternate, ovate or obovate; flowers unisexual, greenish white, in terminal compound panicled cymes; capsules globose, 3-valved, yellow when ripe.	Leaf, bark	Menstrual trouble, abortion
96	*Celosia argentia* L. MBBT/0064	Amaranthaceae	Sweta-murga	—	Aug-Nov	Erect herb with angular stem; leaves alternate, linear to ovate-lanceolate, narrowed down into a short petiole; flowers pinkish white, in terminal spikes; capsules ellipsoid-ovoid.	Whole plant	Wounds, sores, skin eruptions
97	*Celtis tetrandra* Roxb.	Ulmaceae	Sukuta	—	Jan-Aug	Tree with grey white wood; leaves ovate, oblique at the base; flowers greenish, in tomentose cymes; drupes globose.	Tender leaf	Relieves pain after childbirth
98	*Centella asiatica* (L.) Urban. Syn. *Hydrocotyl asiatica* L. MBBT/0010	Apiaceae	Bor-manimuni	Asiatic pennywort, Indian pennywort	Apr-Sep	Prostrate creeping herb; rooting at nodes; leaves reniform, long petiolate; flowers red, in axillary solitary umbels; fruits laterally compressed.	Whole plant	Cuts and wounds, headache
99	*Chenopodium album* L. MBBT/0092	Chenopodiaceae	Jilmil sak	Lamb's quarters	Nov-Mar	Erect herb; stem ribbed; leaves oblong-lanceolate, toothed or lobed; flowers white, in terminal, paniculate clusters; utricles depressed.	Tender shoot	Constipation, cough
100	*Chromolina odorata*	Asteraceae	Baghdhoka	—	Dec-Mar	Erect aromatic shrub; leaves	Leaf	Antiseptic to cuts

.	(L.) King et Robin. Syn. *Eupatorium odoratum* L. MBBT/0068		, Jarmani bon.			opposite, ovate; flowers white, in terminal and axillary corymbose heads; achenes ribbed.		and wounds
101	*Chrysophyllum lanceolatum* (Bl.) DC. Syn. *C. roxburghii*	Sapotaceae	Bon pitha	—	—	Evergreen tree with milky juice; leaves elliptic-oblong; flowers in dense axillary clusters; berry rusty tomentose, yellow when ripe, globose.	Fruit	Vermifuge
102	*Cicer arietinum* L.	Fabaceae	But-mah	Bengal gram	Apr-Jul	Erect herb; leaves imparipinnate, leaflets small, oval; flowers pink, blue or white; pods pubescent, seeds reddish brown.	Leaf	Abortifacient
103	*Cinnamomum tamala* (Hamilt.) Nees et Brem. MBBT/0135	Lauraceae	Tezpat	—	Apr-Jun	Tree with rough bark; leaves elliptic-oblong; flowers in panicles; drupes black when ripe.	Leaf	Cold, cough, pharyngitis
104	*Cissamperos pareira* L. MBBT/0232	Menispermaceae	Tubuki lota	—	Dec-May	Climber; leaves ovate, orbicular, palmately nerved; flowers greenish yellow, in pendulous cymes; fruits drupe.	Root, leaf	Dropsy, fever
105	*Cissus quadriangularis* Wall.ex.Wt & Arn	Vitaceae	Harjura lota	—	Apr-Aug	Climber or trailer; stem fleshy, quadriangular, 4-wiged, contracted at the nodes; leaves simple, cordate; flowers white, in axillary cymes; fruits globose, red when ripe.	Stem	Wounds, bone fracture
106	*Cissus repens* Lamk.	Vitaceae	Nol-tenga	—	Apr-Jun	Trailing herb; stem fleshy; leaves cordate-ovate, membranous, dentate; flowers white, in umbels; fruits globose.	Tender leaf	Stomach ailments

107	*Citrus aurantifolia* (Christm.) Swingle	Rutaceae	Gol-nemu	Common lime	Jul-Dec	Shrub; leaves elliptic-ovate with narrowly winged petiole; flowers white in racemes; fruits greenish yellow, skin thin.	Fruit	Dysentery, scabies
108	*Citrus grandis* (L.) Osb. Syn. *C. maxima* (Burm.f.) Merr. MBBT/0134	Rutaceae	Robab-tenga, Bor tenga	Shadock	Jul-Dec	Shrub; leaves alternate, ovate-oblong, petiole winged; flowers white, solitary or in axillary clusters; berries globose, spongy.	Fruit	Expels intestinal worms, blood purifier
109	*Citrus limon* (L.) Burm. MBBT/0138	Rutaceae	Kaji nemu	Lemon	Mar-Jul	Prickly shrub; leaves ovate-oblong; flowers purplish white; fruits oblong or round, bright yellow when ripe.	Fruit	Flatulence, dysentery, diarrhoea, prickly heat
110	*Cleome gynandra* L. Syn. *Gynandropsis gynandra* (L.) Briq. MBBT/0080	Capparaceae		—	Jan-Dec	Erect glandular herb; leaves trifoliate; leaflets long, obovate, sessile; flowers purplish white, in corymbose racemes; fruits capsule, seeds black.	Whole plant	Earache
111	*Clerodendron colebrookianum* Walp. MBBT/0004	Verbenaceae	Nephaphu	—	Aug-Nov	Evergreen shrub; leaves with basilaminar nectaries in clusters and laminar nectaries scattered on abaxial surface; flowers white, in terminal corymbose cymes; fruit globose, dark green.	Tender leaf	Hypertension
112	*Clerodendron indicum* (L.) O. Kuntze. Syn. *C. siphonanthus* R. Br. MBBT/0205	Verbenaceae	Akal-bih	—	Sep-Dec	Tall herb with hollow, ridged stem; leaves whorled, linear-lanceolate; flowers creamy white, in axillary and terminal paniculate cymes; drupes 4-lobed.	Root, leaf	Carbuncles
113	*Clerodendron serrartum* (L.)	Verbanaceae	Nangal-bhanga	—	Aug-Oct	Shrub; leaves ovate-elliptic, sharply serrate; coriaceous, in	Root, leaf	Dysentery, cuts and wounds

	Moon					whorls of three; flowers bluish purple; drupes 4-lobed, purple		
114	*Clerodendron viscosum* Vent. Syn. *C. infortunatum* Gaertn.f. MBBT/0114	Verbenaceae	Dhopat tita	—	Mar-Jul	Undershrub; leaves opposite, ovate-cordate; flowers white, tinged with pink in terminal panicles; drupes globose, black when mature.	Leaf	Malaria
115	*Clitoria ternatea* L. MBBT/0040	Fabaceae	Aparajita	Butterfly pea	Sep-Mar	Climbing vine; stems slender; leaflets 5-7, elliptic-oblong; flowers blue or white, papilionaceous, solitary; pods many seeded.	Leaf	Earache, fever, ulcer
116	*Coccinia grandis* (L.) Voigt. Syn. *Cephalendra indica* Naud. MBBT/0063	Cucurbitaceae	Kunduli	—	May-Aug	Herbaceous climbers; leaves ovate-cordate, palmatilobed; flowers dioecious, white, solitary, bell shaped; berries oblong-ovoid, red when ripe	Root	Diabetes
117	*Coix lachrya-jobi* L. MBBT/0074	Poacceae	Kawri-moni	Job's tear	Jul-Sep	Stout herb; culms branching at lower nodes; leaves linear-lanceolate, cordate at the base; spikes suberect; male spikelet terminal, female spikelets surrounded by bracts.	Root	Menstrual trouble
118	*Colocacia esculenta* (L.) Schott.	Araceae	Kochu	Taro, Cocoyam	Jun-Sep	Rhizomatous herb; leaves peltate, triangular, ovate, petioles long, green; spathes yellow; spadix shorter than spathe; flowers unisexual; fruits many-seeded berry.	Petiole	Cough.
119	*Colocacia fontanesii* Schott.	Araceae	Kola kochu	—	May-Sep	Rhizomatous herb; leaves peltate, triangular, ovate, petioles long, blackish purple; spathes yellow; spadix shorter than spathe; flowers unisexual; fruits many-seeded berry.	Petiole	Skin cuts

120	*Commelina benghalensis* L. MBBT/0122	Commelinaceae	Kona-simolu	—	Jul-Nov	Decumbent ascending herb; leaves ovate-elliptic or oblong-lanceolate, oblique at the base, spathes pubescent, with oblique mouth; flowers white.	Stem	Sore eyes
121	*Coptis teeta* Wall	Ranunculaceae	Mishimi tita	Gold thread, coptis	—	Rhizomatous perennial herb; rhizome golden yellow, woody, bearing many fibrous roots; leaves ternatisect, leaflets ovate-lanceolate, pinnatifid; flowers small, white, on slender leafless scape; fruis many seeded follicles.	Root	Malaria, stomach pain
122	*Corchorus capsularis* L.	Tiliaceae	Titamora	White jute	May-Aug	Herb with cylindrical straight stem; leaves thin, oval, narrow, pointed; flowers yellow, solitary; capsules short, globose, ribbed, 5-valved.	Tender leaf and shoot	Blood purifier, fever, prevents pox and measles
123	*Corchorus olitorius* L.	Tiliaceae	Mithamora	Jute	May-Aug	Herb with cylindrical straight stem; leaves thin, oval, narrow pointed; flowers yellow, solitary; capsules short, globose, ribbed, 5-valved.	Tender leaf and shoot	Same as above
124	*Cordia dichotoma* Forst.f. MBBT/0111	Cordiaceae	Bowal	—	Aug-Dec	Small tree; leaves alternate, elliptic or ovate; flowers white, in terminal and axillary peduculate cymes; drupes globose, yellowish purple when ripe.	Stem bark, Leaf, Seed	Inflammation of the body, skin eruptions
125	*Coriandrum sativum* L. MBBT/0089	Apiaceae	Dhania	Coriander	Dec-Feb	Annual bright green aromatic herb; leaves finely dissected, flowers white in compound	Whole plant, seed	Appetizer, strengthens stomach,

						umbels.		flatulence, menstrual trouble
126	*Costus speciosus* (Koen.ex.Retz.) J.E. Smith	Costaceae	Jam lakhuti	—	Jun-Sep	Tall herb; stem spirally twisted; rootstock creeping, tuberous; leaves spirally arranged, elliptic or obovate, subsessile; flowers white, large, in terminal globose or ovoid spikes, bracts red;	Rhizome	Jaundice
127	*Crassacocephalum crepidioides* (Benth.) Moore. Syn. *Gynura crepidioides* Benth.	Asteraceae	Kapah-phalia bon	—	Jul-Sep	Succulent herb; leaves alternate, obovate-oblong or lanceolate; heads reddish yellow, solitary or corymbose; achenes narrow, ribbed; pappus white.	Leaf	Sprain
128	*Crataeva nuravia* Buch-Ham. Syn. *C. religiosa* Auct.non Forst.f. MBBT/0123	Capparaceae	Borun goch	3-leaved caper	Apr-Jul	Middle sized deciduous tree; leaves palmately trifoliate; leaflets ovate-lanceolate; flowers creamy, in terminal corymbose racemes; berries globose having foetid smell.	Bark	Urinary trouble during fever
129	*Crinum asiaticum* L. MBBT/0132	Amaryllidaceae	Bon nohoru	—	Aug-Nov	Bulbous herb; leaves linear-lanceolate, long, flat, narrow with a sheathing base; flowers white, fragrant, in umbels; capsules subglobose.	Bulb	Emetic in food poisoning
130	*Crotalaria pallida* Ait. Syn. *C. mucronata* Desv. ; *C. striata* DC. MBBT/0042	Papilionaceae	Ghonta-karna	—	Jun-Dec	Undershrub; leaves trifoliate, leaflets obovate; flowers yellow, in long terminal racemes; pods long, oblong-cyllindric.	Leaf	Scabies, ringworm
131	*Croton caudatus* Geisel.	Euphorbiaceae	Lota mahudui	—	Apr-Oct	Scandent shrub; young parts stellate, hairy; leaves ovate or ovate-orbicular, serrate;	Leaf	Kidney trouble

						flowers pale yellow, in long terminal racemes; capsules globose.		
132	*Croton joufra* Roxb.	Euphorbiaceae	Goch-mahudi	—	Feb-Jun	Shrub with greyish-white barks; leaves aromatic, oblong-lanceolate, coriaceous; flowers light yellow, in terminal or axillary racemes; capsules ovoid, 3-lobed.	Leaf	Dysmenorrhea
133	*Croton tiglium* L. MBBT/0148	Euphorbiaceae	Konibih	Purging cotton, croton oil tree	Jun-Dec	Shrub; leaves oblong, young leaves brownish to slightly reddish; flowers pale yellow, in terminal racemes; fruits oblong, obtusely 3-lobed, glabrous, 5-seeded.	Tender shoot	Constipation, carbuncles
134	*Cucumis sativus* L. MBBT/0186	Cucurbitaceae	Tioh	Cucumber	Apr-Jul	Climbers with extra axillary tendrils; flowers yellow, solitary, axillary; fruits pepo.	Whole plant, fruit	Insect repellent, diuretic
135	*Cucurbita maxima* Duch. ex Lamic	Cucurbitaceae	Ronga-lao	Red gourd squash	Nov-Mar	Hispid, trailing herb; leaves reniorm, sub-orbicular, shallowly 5-lobed, hispid; calyx lobes linear; fruits vary in shape, size and colour.	Seed	Sexual vigour
136	*Curanga amada* Juss.	Scrophulariaceae	Bhui-tita	—	May-Aug	Diffuse herb; leaves opposite, ovate, crenate; flowers in short terminal or pseudo axillary racemes; capsules orbicular, include in much enlarged calyx.	Leaf	Appetizer, remedy of fever
137	*Curculigo orchioides* Gaertn.	Hypoxidaceae	Nagini	—	July-Oct	Small, stemless herb with a tuberous rootstock; leaves long petioled, lanceolate; flowers distichous, lower bisexual, rest all male.	Tuber	Cuts and wounds, impotency

138	*Curcuma amada* Roxb.	Zingiberaceae	Amada	Mango ginger	May-Jun	Herb; rhizome aromatic with mango like smell; leaves oblong acuminate; flowers pale yellow, in peduncullate spikes.	Rhizome	Diarrhoea
139	*Curcuma aromatica* Salisb.	Zingiberaceae	Bon-halodhi, keturi, ketkuri	Wild turmeric, yellow zedoary	April-June	Herb; rhizome aromatic; leaves oblong, narrowed down into long petioles; flowers pale yellow, in long pedunculate spikes.	Rhizome	Sprains, ringworm, scabies
140	*Curcuma caesia* Roxb.	Zingiberaceae	Kola-halodhi	Black zedoary	May-Jun	Herb with a fleshy rhizome; leaves and rhizome aromatic; leaves simple, sheathing, entire, linear-lanceolate, black stripe on midrib; flowers pinkish bisexual, in spikes.	Rhizome	Sprain
141	*Curcuma domestica* Valet. Syn. *C. longa* L.	Zingiberaceae	Halodhi	Turmeric	May-Jun	Herb with aromatic rhizome; leaves sheathing, linear-lanceolate; flowers slightly pink tinged, in spikes.	Rhizome	Removes weakness after childbirth, removes wart, blood purifier
142	*Cuscuta reflexa* Roxb. MBBT/0069	Cuscutaceae	Akashi-lota	Dodder	Nov-Mar	Parasitic twiner; stem slender, yellowish-green; leaf absent; flowers white, tubular, solitary or in clusters.	Stem	Wounds, jaundice
143	*Cyclosorus extensus* (Moore) Ching. Syn. *Amphineuron opulentum* (Kaulf.) Holtt.	Thelipteridaceae	Bihlongoni dhekia	—	Fertile: Jul-Feb	Terrestrial fern; rhizome, short, creeping; stipes short, hairy in grooves; scales linear-lanceolate, brown; lamina large pinnate, pinnae 20-27 pairs, sessile; sori mostly confirmed to lobes of pinnae.	Leaf	Insect biting
144	*Cynodon dactylon* (L.) Pers. MBBT/0184	Poaceae	Dubori-bon	Dhub grass, Bermuda	Jan-Dec	Perennial prostrate grass; rooting at nodes; leaves linear-lanceolate; inflorescencce 4-5	Whole plant	Cuts and wounds, piles, leucorrhoea

				grass		digitate spike; spikelets compressed, sessile, 1-flowered		
145	*Cynoglossum glochidiatum* Wall. ex Benth. Syn. *C. wallichi* G. Don	Boraginaceae	Bor-bioni hakuta	—	Jul-Dec	Erect hispid herb; leaves ovate-lanceolate, hairy; flowers white, in capitate cymes; nutlets glochidiate.	Root	Vomiting
146	*Cyperus rotundus* L.	Cyperaceae	Keya-bon	Nut grass	Jul-Dec	Perennial erect sedge with stoloniferous rhizome; leaves linear, acuminate; spike in simple or compound umbels; nuts oblong, trigonous.	Tuber	Stomach discomfort
147	*Dactyloctenium aegypticum* (L.) P. Beauv.	Poaceae	Bobosa-bon	—	Jun-Nov	Annual grass; rooting at nodes; leaves linear-lanceolate, distichous; inflorescence a 2-6 digitate spike; spikelets long, compressed, 3-5 flowered.	Culm	Asthma.
148	*Dalbargia sisso* Roxb. ex DC. MBBT/0151	Fabaceae	Sisu-goch	Sisso	Mar-May	Deciduous tree; leaflets alternate, ovate; flowers yellowih white, in axillary panicles; pods linear-lanceolate, 1-2 seeded	Leaf, bark	Gonorrhoea, dysentery
149	*Datura metel* L. Syn. *D. fastusa* L. MBBT/0021	Solanaceae	Boga dhatura	—	Jan-Apr	Undershrub; leaves ovate-lanceolate; flowers white, axillary, solitary; capsules globose, spinous.	Leaf, flower	Asthma, cough
150	*Datura stramonium* L. MBBT/0076	Solanaceae	Dhatura	—	Jan-Mar	Undershrub; leaves ovate-oblong; flowers white, axillary, solitary; capsules ovoid, prickly.	Leaf	Eczema
151	*Daucus carota* L. MBBT/0235	Apiaceae	Gajor	Carrot	Jan-Mar	Small herb with conical roots; leaves cauline, ramal,	Root	Scabies

						petiolate, compound; flowers in compound umbel; fruit cremocarp, splitting into 2 mericarps.		
152	*Desmodium caudatum* (Thunb.) DC.	Fabaceae	Bor bioni-hakuta	—	Jun-Nov	Shrub with trifoloiate leaves; leaflets ovate-oblong; flowers white, in axillary racemes; pods pendulous, jointed beset with minute hooks.	Root	Haemospermia
153	*Desmodium triflorum* (L.) DC.	Fabaceae	Tripadi	—	Aug-Nov	Prostrate herb; rooting at nodes; leaves trifoliate, leaflets obovate-lanceolate; flowers purplish, 1-3 together, axillary; pods curved.	Whole plant	Dysentery
154	*Derris eliptica* (Roxb.) Benth	Fabaceae	Etam-chali	—		Woody climber; leaves imparipinnate; flowers pinkish white, in lax racemes.	Fruit, Bark	Fish poison
155	*Dillenia indica* L.	Dilleniaceae	Ou-tenga	Elephant apple	Jul-Dec	Tree with spreading branches; leaves elliptic to oblanceolate; flowers creamy white, solitary, terminal; pseudocarps globose or subglobose.	Fruit	Dysentery, flatulence, constipation
156	*Dioscorea bulbifera* L.	Dioscoreaceae	Gothia alu	Air yam, potato yam	Jun-Oct	Large twining herb; bulbils axillary tubercled; leaves simple, alternate, broadly ovate-cordate; flowers in axillary panicled spikes; capsules oblong.	Tuber	Piles
157	*Dioscorea esculenta* (Lour.) Burk	Dioscoreaceae	Mua alu	Lesser yam	Jul-Oct	Twining herb; tubers cylindrical, lobed, flesh white; stem prickly at base; leaves ovate-cordate.	Tuber	Swellings of muscles
158	*Diospyros malaberica* (Desv.)	Ebenaceae	Kendu	—	May-Sep	Tree with blackish bark; leaves oblong-lanceolate;	Seed	Dysentery

	Kost.					flowers pale yellow, in axillary corymbose clusters; female flowers solitary; berries subglobose.		
159	*Diplazinum esculentum* (Retz.) Sw.	Athyriaceae	Khuwa dhekia	—	Fertile: Jul-Feb	Terrestrial fern; rhizome erect, dark brown; stipes erect, tufted; fronds large, lamina 2-pinnate; sori linear, continuous along almost whole length on both sides of the veins, brown; sporangia shortly stalked.	Tender shoot	Blood purifier
160	*Diplocyclos palmatus* (L.) Jeffery. Syn. *Bryonia laciniosa* L.	Cucurbitaceae	Kau-kerela	—	Apr-Aug	Climbing herb with simple tendril; leaves angled or lobed; flowers white, solitary; fruits elliptic or ovoid, reddish when ripe.	Fruit	Diabetes
161	*Dracena angustifolia* Roxb.	Agavaceae	Jam lakhuti, Hati kuhiar	—	Sep-Nov	Tall shrub; leaves linear, sessile, drooping, crowded at the top; flowers pinkish white, in terminal panicles; berries globose.	Root,	Jaundice
162	*Drymaria cordata* (L.) Willd. ex Roem. et Schult. Syn. *D. driandra* Bl. MBBT/0037	Caryophyllaceae	Lai-jabori	—	Mar-Sep	Diffuse or suberect herb; rooting at nodes; leaves ovate-cordate; flowers white, in axillary or terminal peduncled cymes. Capsules ovoid.	Whole plant	Sinusitis
163.	*Duchesnea indica* (Andr.) Focke. Syn. *Fragaria indica* Andr. MBBT/0102	Rosaceae	Goru-khis	Indian strawberry	Aug-Dec	Prostrate herb; leaflets obovate; flowers yellow, solitary on long axillary peduncles; berries globose, bright red, fleshy.	Fruit	Dysentery
164	*Dysoxylum binectariferum* (Roxb.) Hook. f. ex Bedd.	Meliaceae	Bandar-dema	—	Apr-Dec	Medium sized tree; leaves paripinnate, leaflets 6-8, ovate-oblong; flowers white, in large axillary panicles;	Seed	Ulcers, leprosy

						capsules obovoid, 4-celled.		
165	*Ecbolium viride* (Forsk.) Alston. Syn. *E. linnaeanum* Kurz.	Acanthaceae	Nilakantha	—	Sep-Nov	Undershrub; leaves oblanceolate, sinuate; flowers blue, in dense terminal spikes; capsules broadly ovate.	Root	Abdominal pain
166	*Eclipta prostrata* L. Syn. *E. alba* (L.) Hassk. MBBT/0035	Asteraceae	Keheraj	—	Jul-Sep Mar-May	Erect or prostrate herb; leaves opposite, linear-oblong, lanceolate; heads white, in trminal, axillary, solitary peduncles; achnes winged on the margins, pappus absent.	Whole plant	Promotes growth of hair, skin diseases
167	*Eichornia crassipes* (Mart.) Solms.	Pontideriaceae	Meteka	—	Sep-Nov	Aquatic herb; stems erect or oblique; leaves saggitate, petiole ssheathing; flowers bluish, long pedicelled, in many flowered racemes; capsules subglobose or oblong.	Flower	Sore eyes
168	*Elaeagnus conferata* Roxb. Syn *E. latifolia* L.	Elaeagnaceae	Mirika-tenga	—	Nov-Mar	Scandent spiny shrub; leaves elliptic-oblong, silvery white, shining beneath; flowers yellow, in 1-5 flowered clusters; drupes elliptic oblong.	Leaf	Alcoholic intoxication
169	*Elaeocarpus floribundus* Bl. MBBT/0008	Elaeocarpaceae	Jolphai, Belphoi	Olive	Jul-Nov	Tree; leaves alternate, ovate-elliptic, flowers white, in axillary racemes; drupes oblong smooth, green.	Fruit	Cough, bronchitis
170	*Elaeocarpus sphaericus* (Gaertn. f.) Schum. Syn. *E. ganitrus* Roxb.	Elaeocarpaceae	Rudraksha	Utrasum-bead tree	—	Evergreen tree; leaves oblong-lanceolate; flowers white, axillary, in dense racemes; drupes ovoid or globose, longitudinally grooved.	Fruit	Brain disorders
171	*Elephantopus*	Asteraceae	Bon-lai	Prickly	Sep-Jan	Erect herb; leaves radical,	Whole	Diarrhoea,

	scaber L. MBBT/0038			leaved elephant's foot		ovate-oblong; heads pinkish, achenes ribbed, pappus 4-5 bristles, white.	plant	bronchitis
172	*Elsholtzia blanda* (Benth.) Benth.	Lamiaceae	Bon-tulosi	—	Nov-Jan	Perennial shrub; leaves swet scented, lanceolate, serrate; flowers white, in long spikes; nutlets ellipsoid.	Root	Dysentery
173	*Eleusine indica* (L.) Gaertn. MBBT/0188	Poaceae	Bobosa bon	—	Jun-Dec	Annual tufted grass; leaves distichous, linear, flat; inflorescence a terminal umbel of 2-4 digitate spikes; spikelets 2-seriate, 3-6 flowered; caryopsis oblong.	Whole plant	Post partum aid to mothers
174	*Emila sonchifolia* (L.) DC. MBBT/0199	Asteraceae	Bon-kopohua	—	Jan-Dec	Variable annual herb; leaves radical and cauline, lyrate-pinnatifid, basal leaf petioled; flowers purplish, in corymbose heads; achenes oblong, 5-ribbed.	Whole plant	Antipyretic
175	*Entada scandens* Benth	Fabaceae	Ghila	Nicker bean, Mackay bean, sea bean	Apr-Aug	Climber with angular stem; leaves bipinnate, ending in a bifid tendril; flowers pale yellow, in spikes; pods jointed, woody; seeds flat, orbicular.	Seed	Hair shampoo
176	*Enhydra fluctuans* Lour.	Asteraceae	Helonchi sak	—	Nov-Jan	Herb; stem prostrate, rooting at nodes; leaves opposite, sessile, dentate, glandular; heads axillary or terminal, sub-sessile, heterogamous	Tender shoot	Laxative, prickly heat
177	*Erecthites valerianaefolia* (Wolf.) DC	Asteraceae	Bon kopah	Fireweed. Pile weed	Jan-Dec	Succulent herb; stem grooved; leves variable, upper ones lobed, ovate-cordate; flowers white, in reddish heads; fruits achene.	Leaf	Cuts and wounds

178	*Eryngium foetidum* L. MBBT/0002	Apiaceae	Man dhania	—	May-Oct	Aromatic herb; leaves spathulate, spinous toothed; flowers white, in umbelate, peduculate, heads; fruits ellipsoid.	Leaf	Flatulence, stomach trouble
179	*Erythrina stricta* Roxb.	Fabaceae	Ronga modar	—	Mar-Jun	Medium sized prickly tree; leaflets broad ovoid; flowers deep red, in terminal racemes; pods curved.	Leaf	Abscesses
180	*Erythrina variegata* L. Syn. *E. indica* Lamk.	Fabaceae	Boga-modar	—	Apr-Jun	Deciduous tree; leaves trifoliate, leaflets round; flowers white, in terminal racemes.	Bark	Jaundice
181	*Euphorbia antiquorum* L.	Euphorbiaceae	Siju	—	Nov-Jan	Flesy shrub; stem cylindrical, 3-5 angled with upwardly curving, jointed, thorny branches; involucres ternate, forming short peduncled cymes.	Latex	Burn injuries, boils, warts
182	*Euphorbia hirta* L.MBT/0009	Euphorbiaceae	Gakhiroti bon	—	Jan-Dec	Procumbent hairy herb; leaves opposite, elliptic or ovate-oblong, hairy; cyathea axillary or terminal; capsules globose, 3-lobed.	Tender shoot	Lactation in nursing mothers
183	*Euphorbia ligularia* Roxb. Syn. *E. nerifolia* L.	Euphorbiaceae	Siju	—	Dec-May	Succulent shrub; branches 5-angled, spines arising from thick tubercles; leaves alternate, obovate-oblong, fleshy; involucres yellowish, forming 3-flowered cymes; capsules 3-lobed.	Latex	Burn injuries, boils, warts
184	*Ficus auriculata* Lour. Syn. *F. roxburghii* Wall ex	Moraceae	Atha-dimaru	—	Apr-Oct	Tree; leaves ovate-orbicular, sub-criaceous; receptacles in fascicles on the trunk or	Bark	Itches and other skin diseases

	Mig.					leafless branches; purplish when ripe.		
185	*Ficus. benghalensis* L.	Moraceae	Bor-goch, bot goch	Bannyan tree	Apr-Nov	Evergreen tree; aerial roots forming prop roots; leaves alternate, elliptic to ovate; receptacles globose, axillary, sessile, red when ripe.	Latex	Abscesses, septic ulcer, cracked heels
186	*Ficus racemosa* L.	Moraceae	Mau-dimoru	—	Feb-Sep	Deciduous tree; leaves alternate, ovate-oblong, glabrous; receptacles subglobose, stalked.	Fruit	Diabetes
187	*Ficus. religiosa* L. MBBT/0172	Moraceae	Anahat goch	—	Apr-Sep	Tree with spreading branches; leaves broadly ovat, apex acuminate to a long tail; receptacles subglobose, axillary, dark purple when red.	Bark	Itches and other skin diseases
188	*Flacourtia jangomas* (Lour.) Raeusch.	Flacourtiaceae	Poniol	Indian plum	Mar-Aug	Medium sized tree with spines; leaves ovate or ovate-lanceolate; flowers unisexual yellow, in axilary raccemes; fruits globose, dark purple.	Stem, bark, leaf, latex	Diarrhoea, cracked heels
189	*Flemingia strobilifera* (L.) R.Br. MBBT/0001	Fabaceae	Makhioti	—	Mar-July	Erect shrub; leaves unifoliate, ovate-lanceolate; flowers in short cymes on terminal and hairy racemes; bracts rounded, folded; corolla white; pods oblong, 2-seeded, enclosed by bracts.	Root	Menstrual irregularities*, ringworm
190	*Floscopa scandens* Lour. MBBT/0055	Commelinaceae	Soru-konasimolu	—	Aug-Dec	Subscandent herb; leaves lanceolate, narrowed down to base; flowers white, in dense terminal panicles; capsules subglobose, compressed.	Leaf	Sore eyes
191	*Garcinia cowa*	Clusiaceae	Kuji-	—	Mar-Sept	Medium sized tree with	Fruit	Diarrhoea,

	Roxb. ex DC.		thekera			greyish brown bark; leaves elliptic-lanceolate; flowers pinkish yellow, in terminal or axillary cymes; berries ovoid, globose, acidic orange inside when ripe.		dysentery, flatulence
192	*Garcinia lancifolia* (G.Don.) Roxb. MBBT/0051	Clusiaceae	Rupahi-thekera	—	Apr-Oct	Shrub or tree; leaves lanceolate, narrowed down to the base; flowers creamy pinkish red, in few flowered cymes; berries obovoid, orange yellow.	Fruit	Same as above
193	*Garcinia pedunculata* Roxb.	Clusiaceae	Bor-thekera	—	Feb-Jun	Tree; leaves ovate-oblong; flowers terminal, pedunculate; berry large, smooth, rounded, acidic; seeds reniform.	Fruit	Same as above
194	*Garcinia xanthochymus* Hook. f. ex. Anderson	Clusiaceae	Tepor-tenga	—	Mar-Sep	Tree with drooping branches; latex yellow; leaves linear-oblong; flowers whitish, in axillary fascicles; fruits globose with persistent calyx.	Bark, fruit	Alcohol intoxication, dysentery
195	*Gloriosa superba* L	Liliaceae	Ulot-chandal, Ulu-chandan	Glory lily	Apr-Jun	Herbaceous annual climber; rootstock solid, fleshy- yellow, cylidric; Leaves stalkless, opposite, tips of the leaves acts as tendrils; flowers multicoloured, uniquely structured, large axillary, solitary; flower colour progresses from tips to the bases, from green to yellow passing through orange and scarlet to crimson.	Tuber	Expulsion of worms, abortion, abdominal pain itching
196	*Gmelina arborea* Roxb. MBBT/0127	Verbenaceae	Gomari	—	Mar-Jun	Tree; leaves ovate, long petiolate; flowers yellow, in	Leaf	Indigestion, flatulence

						axillary or terminal panicles; drupes pyriform.		
197	*Gomphrena celosioides* Mart.	Amaranthaceae	Leheti	—	Sep-Nov	Annual herb; leaves opposite; flowers white, in heads; bracts leafy, bracteoles purplish; perianth 5, membranous.	Whole plant	Blood purifier
198	*Gossypium herbaceum* L. MBBT/0063	Malvaceae	Kopah	Cotton	Nov-Jan	Shrub; leaves ovate or orbicular, 3-7 lobed, cordate at base; floweres solitary, axillary; capsules subglobose, seeds with long wooly hairs.	Leaf, flower	Dysentery, ear troubles, burn injury, scabies, inflammations
199	*Hedyotis corymbosa* (L.) Lamk. Syn. *Oldenlandia corymbosa* L. MBBT/0116	Rubiaceae	Bon-jaluk	—	Jun-Dec	Decumbent herb; stem quadrangular; leaves linear-lanceolate; flowers white, in1-3 flowered pedunculate axillary cymes; capsules ovoid.	Tender shoot	Body ache, peptic ulcer
200	*Hedyotis diffusa* Syn. *Oldenlandia diffusa* Roxb. MBBT/0078	Rubiaceae	Bon-jaluk	—	Apr-Dec	Diffusely prostrate herb; leaves linear or linear-lanceolate; flowers white, sessile or shortly pedicelled, solitary, axillary; capsules ovoid.	Tender shoot	Same as above
201	*Hedyotis vertecillata* (L.) Lamk. MBBT/0126	Rubiaceae	Bon-jaluk	—	Mar-Dec	Prostrate diffuse hispd herb; leaves linear-lanceolat; flowers white, in axillary clusters; capsules ovoid, hispid.	Root	Pneumonia
202	*Heliotropium indicum* L. MBBT/0168	Boraginaceae	Hatisuriya bon	Indian geleotrope	May-Sep	Annual herb; Leaves alternate, broadly ovat; flowers violet or bluish violet, in terminal and axillary coiled spikes; nutlets globose.	Leaf	Insect bite, pimples, wounds
203	*Hibiscus cannabinus*	Malvaceae	Bon bhendi	Deccan	May-Sep	Undershrub with prickly	Leaf	Dysentery

	L. MBBT/0020			hemp		straight stems; lower leaves cordate, upper leaves deeply palmate with 3-7 lobes; flowers yellow, axillary, solitary; capsules oval.		
204	*Hibiscus mutabilis* L. MBBT/0027	Malvaceae	Sthala padma	—	Aug-Oct	Shrub; leaves angled, palmately lobed; flowers white or pink in morning, deep red subsequently, peduncles long, axillary and solitary.	Flower bud	Menorrhagia
205	*Hibiscus rosa-sinensis* L. MBBT/0159	Malvaceae	Jobaphul	China rose	Jan-Dec	Shrub; leaves ovate, stipulate, epicalyx 6-8; flowers red, solitary axillary; fruits capsule, scizocarpic	Leaf	Antidandruff shampoo
206	*Hibiscus sabdarifolia* L. MBBT/0059	Malvaceae	Tengamora	—	Nov-Dec	Erect shrub; Stem and leaves often tinged with red; leaves palmatifid, 3-5lobed, glandular beneath; flowers yellow or pink, solitary; calyx fleshy; capsules ovoid;	Leaf, fruit	Stomach ailments.
207	*Hiptage benghalensis* (L.) Kurz. Syn. *H. madablota* Gaertn.	Malpighiaceae	Madhoi-maloti	—	Jan-June	Climbing shrub; leaves elliptic or ovate-lanceolate; flowers white, fragrant, long, in axillary or terminal racemes; fruits oblanceolate, winged.	Root	Pneumonia
208	*Holarrhena pubescens* (Buch-Ham.) Wall ex DC. Syn. *H. antydysenterica* Wall. MBBT/0174	Apocynaceae	Dudhkuri, Kutoj	—	May-Oct	Large shrub; leaves opposite, ovate to elliptic-oblong; flowers creamy white, fragrant, in terminal cymes; follicles slender, long, curved.	Bark	Dysentery, jaundice, leprosy
209	*Homalomena aromatica* (Roxb.) Schott.	Araceae	Gondh-kochu	—	Jun-Oct	Robust herb with creeping aromatic rhizome; leaves cordate, accuminate; spathes obscure, greenish yellow;	Rhizome	Stomach ailments

						fruits berry.		
210	*Houttuynia cordata* Thunb. MBBT/0155	Saururaceae	Mosondori, Mosundori	—	Jun-Aug	Prostrate aromatic herb; leaves ovate, cordate at the base, gland dotted; flowers in dense axillary spikes subtended by an involucre of bracts; bracts white; fruits subglobose casule.	Leaf	Flatulence, diarrhoea, dysentery
211	*Hydrocotyl javonica* Thunb. Syn. *H. nepalensis* HK	Apiaceae	Manimuni	—	Jul-Nov	Annual prostrat herb; rooting at nodes; leaves orbicular or roundwith with long petioles; floweres pink-purple, in many flowered, axillary umbels; fruits flat and round.	Leaf	Dysentery
212	*Hydrocotyl sibthorpioides* Lamk. Syn. *H. rotundifolia* Roxb. ex DC. MBBT/0015	Apiaceae	Soru-manimuni	—	Apr-Aug	Prostrate diffuse herb; leaves orbicular, cordate; flowers sessile, in umbels; fruits orbicular, reddish brown.	Whole plant	Dysentery, skin diseases
213	*Ichnocarpus frutescens* R.Br.	Apocynaceae	Dudhkuri lota	Black creeper	Sep-Dec	Twining shrub; leaves opposite, ovate or elliptic or eliptic-lanceolate; flowers white, in axillary and terminal cymes; follicles linear	Root	Fever, diabetes
214	*Impatiens balsamina* L. MBBT/0018	Balsaminaceae	Dam-deuka	Garden balsam	Feb-Aug	Erect soft herb; swollen at nodes; leaves elliptic-lanceolate; flowers pinkish, axillary, solitary or fascicled; capsules 5-valved and splits longitudinally with a jerk scattering the seeds.	Stem, leaf	Jaundice, corns, urinary trouble
215	*Imperata cylindrica* L.	Poaceae	Ulu	Thatch grass	Mar-Jun	Annual grass; leaves linear, edges sharp; inflorescence a panicle, silky with long hairs;	Root	Vermifuge

						spiklets with purple stigma and yellow anthers.		
216	*Ipomoea aquatica* Forsk. Syn. *I. reptans* Poir. MBBT/0045	Convolvulaceae	Pani-kolmow	Swamp cabage	Aug-Feb	Aquatic trailing herb; rooting at nodes; stem hollow; leaves ovate-oblong; flowers purple, solitary or in few flowered peduncled cymes; capsules subglobose.	Tender shoot	Diabetes, galactagogue to nursing mother, prickly heat
217	*Jasminum sambac* (L.) Alt.	Oleaceae	Duamalii	Jasmine		Spreading shrub; leaves in pairs, broad at the base; flowers white, very fragrant.	Flower	Inflammation of the mammary glands of nursing mother
218	*Jatropha curcas* L. MBBT/0228	Euphorbiaceae	Bhotera, Ronga bongali-era	—	May-Nov	Shrub with watery latex; leaves ovate, slightly 3-5 lobed, cordate at base, long petioled; flowers yellow, in panicled cymes; capsules subglobose, lobed.	Stem	Swollen gums
219	*Jatropha gossipifolia* L.	Euphorbiaceae	Bhotera, Bongali-era	—	Aug-Dec	Small shrub, young parts reddish purple; leaves alternate, palmately lobed, cordate or subcordate at base; flowers purplish red, in subcorymbose cymes; capsules oblong, 3-lobed.	Bark, leaf	Gastro-entertitis, sprain
220	*Justicia adhatoda* L. Syn. *Adhatoda vasica* Nees; *A. zeylanica* Medic. MBBT/0098	Acanthaceae	Bahak tita	—	Nov-Apr	Evergreen shrub; leaves opposite, ovate or elliptic-lanceolate; flowers white with purple stripes, in dense axillary leafy spikes; capsules 4-seeded.	Leaf	Cough
221	*Justicia gendarussa* Burm. Syn. *Gendarussa vulgaris* Nees.	Acanthaceae	Jatrasiddhi, Titakhari	—	Mar-Sep	Undershrub; leaves opposite, linear-lanceolate, shortly petioled; flowers white, in teminal paniculate or axillary	Leaf	Skin diseases

	MBBT/0118					spikes; capsules 4-seeded.			
222	*Kaempferia galanga* L.	Zingiberaceae	Gathion	—	Mar-May	Herb with aromatic rhizome and tuberous roots; leaves broadly elliptic; inflorescence terminal, on a leafy shoot; flowers white.	Rhizome	Emollient, cough	
223	*Kalonchoe pinnata* (Lamk.) Pers. Syn. *Bryophyllum pinnatum* (Lamk.) Kurz.	Crassulaceae	Dupor tenga, pate gaja	—	Nov-Mar	Suculent herb; leaves ovate or elliptic; flowers purplish, in long panicles; follicles 4, membranous.	Leaf	Diuretic, gall blader stones, wounds, sprain	
224	*Kayea assamica* King et Prain	Clusiaceae	Sia-nahor	—	Apr-Jul	Tall evergreen tree with reddish wood; bark brownish-grey; leaves elliptic, lanceolate; flowers white in terminal or axillary fascicled panicles; fruits depressed, globoose.	Fruit	Fish poison	
225	*Lagenaria siceraria* (Molina) Standl. Syn. *L. vulgaris* Ser. MBBT/0185	Cucurbitaceae	Jati-lao	Bottle guard	Feb-May	Pubescent, tendril climber; stem 5-angular; leaves long petioled, 5-lobed, flowers white, unisexual, solitary; fruits bottle or ' dumbell shaped, almost woody when mature, seeds oblong with marginal groove.	Flower, fruit	Burn injury, pox	
226	*Lagerstroemia speciosa* Pers. Syn. *L. reginae* Roxb.; *L. flos-reginae* Retz. MBBT/0195	Lythraceae	Ajar	—	Apr-Jun	Tree; leaves opposite, oblong elliptic or oblong-lanceolate; flowers purple, in terminal panicles; capsules woody, ellipsoid or subglobose.	Leaf	Promotes flow of urine	
227	*Lantana camara* L. MBBT/0082	Verbenaceae	Gu-phool	—	Jan-Dec	Spiny, aromati shrub; leaves ovate, acuminate, toothed; flowers white, pink or yellow, in many flowered, peduncled	Leaf	Healing of wounds	

						head; fruits ovoid.		
228	*Laportia crenulata* Wedd. Syn. *Dendrenide sinuata* (Bl.) Chew	Urticaceae	Surat	—	Aug-Jan	Hairy shrub; leaves alternate, broad, elliptic ovate, cordate at base; flowers white, in short dichotomous cymes; achenes globose.	Root	Septic ulcer
229	*Lasia spinosa* (L.) Thw.	Araceae	Chengmora	—	Dec-Feb	Stout prickly herb with thick creeping rhizome; leaves long petioled, hastate, sagittate or palmatifid; spadix short, cylindrical green.	Rhizome	Irregular menstruation* leucorrhoea*
230	*Lawsonia inermis* L. Syn. *L. alba* Lamk.	Lythraceae	Jetuka	Henna plant	Jul-Nov	Large shrub; leaves ovate-lanceolate, pale green; floweres white, in terminal panicles.	Bark, leaf, flower	Skin diseases, prickly heat, burn injury, headache
231	*Leea indica* (Burm.) Merr. MBBT/0131	Leeaceae	Kukura-thengia	—	Apr-Nov	Tall shrub; leaves 2-3 pinnate, leaflets ovate-lanceolate; flowers white, in axillary, corymbose cymes; beries subglobose, 3-6 obed.	Leaf, root	Diarrhoea
232	*Leonurus japonicus* Houtt. Syn. *L. sibiricus* L. MBBT/0066	Lamiaceae	Rong-doron	Siberian mother wort	Feb-May	Erect herb; stem ribbed; leaves opposite, aromatic, deeply divided into lobes; flowers pink, in axillary vertecelate cyme; fruit a nutlet.	Leaf	Sore eyes
233	*Lepidium sativum* L.	Brassicaceae	Halim-sak	Garden cress	Apr-Aug	Glabrous, annual herb; radical leaves pinnate or pinnatisect, long petioled; cauline leaves lobed or pinnatifid, petiole gradually diminishing; flowers white, in terminal racemes.	Tender shoot	Useful for liver
234	*Leucas plukeneti* (Roth.) Spr. Syn. *L. aspera* (Wild.) Spr.	Lamiaceae	Doron bon, drun bon	—	Jan-Dec	Erect herb; leaves linear or narrowly oblong-lanceolate; flowers white, in terminal or	Leaf	Sinusitis.

	MBBT/0065					axillary whorls; calyx curved, 10-toothed; nutlets brown.		
235	*Limnophila indica* (L.) Druce	Scrophulariaceae	Amsoi	—	Nov-Jan	Erect hispid herb, rooting at nodes; stem hollow; leaves opposite and whorled, linear-oblong; flowers white, pedicillate, axillary; capsules ellipsoid.	Tender shoot	Digestive
236	*Lindernia pusilla* (Willd.) Bold. Syn. *Vandellia scabra* Benth	Scrophulariaceae	Gakhiroti-bon	—	Sept-Jan	Straggling annual herb; rooting at nodes; leaf opposite, smooth, slightly cordate; flower violet, axillary, and solitary; fruits capsule.	Whole plant	Galagtogue to nursing mother
237	*Lindernia ruellioides* (Colsm.) Pennel. Syn. *Bonnaya reptans* (Roxb.) Spr. MBBT/0023	Scrophulariaceae	Kasidoria bon	—	Oct-Feb	Prostrate herb; leaves opposite, obovate-oblong, serrate; flowers white, in racemes; capsules slender.	Whole plant	Tonsillitis
238	*Litsea glutinosa* (Lour.) Robinson; Syn. *L. sebifera* Pers.	Lauraceae	Baghnola	—	May-Nov	Tree, leaves ovate-lanceolate or elliptic-oblancolate; flowers greenish yellow, in compound umbels; fruits subglobose.	Leaf	Dysentery*
239	*Litsea cubeba* (Lour.) Pers. Syn. *L. Citrata* Bl.	Lauraceae	Mezankari	—	April-Nov	Deciduous tree with aromatic smell; young shoots silky; leaves ovate-lanceolate, membranous; flowers greenish white, in capitate umbels, solitary or in corymbose; fruits ovoid.	Fruit	Stomachic
240	*Litsea salicifolia* (Roxb. ex Nees.) Hook.f. MBBT/0249	Lauraceae	Dighloti	—	Feb-June	Shrub with silky branches; leaves alternate, elliptic or narrows lanceolate; flowers greenish yellow, in axillary, clustered umbels; fruits black.	Leaf	Dysentery*

241	*Ludwigia octovalvis* (Jacq.) Raven. Syn. *Jussiaea suffruticosa* L.	Onagraceae	Pani jolokia	—	Jan-Dec	Erect herb; leaves sessile, linear-lanceolate; flowers yellow, axillary, solitary; capsules cylidric, 8-ribbed.	Whole plant	Fungal infections of toes
242	*Luffa acutangula* (L.) Roxb.	Cucurbitaceae	Jika	Ribbed gourd	Mar-Sep	Tendril climber; leaves cordate, suborbicular, 5-7 lobed; flowers yellow, unisexual, in axillary racemes; fruits 10-ribbed, oblong, narrow at the base; seeds black, compressed.	Flower	Night blindness
243	*Luffa aeygiptica* Mill. Syn. *L. cylindrica* (L.) Roem.	Cucurbitaceae	Bhul	Sponge gourd	Mar-Sep	Tendril climber; Stem 5-angular; leaves cordate, suborbicular, 5-lobed; flowers yellow, unisexual, in axillary racemes; fruits smooth, cylindrical; ripe fruit contains sponge like fibres; seeds black, narrowly winged.	Fruit	Antidandruff shampoo
244	*Lygodioum flexuosum* (L.) Sw.	Lygodiaceae	Kopow-dhekia	—	Fertile: Feb-Dec	Twining fern with creeping rhizome; leaflets pinnate, simple or terminal leaflets forked; fertile leaflets narrower; sori protruding from the margin.	Whole plant	Ulcer
245	*Machilus bombyciana* King ex Hook.f.	Lauraceae	Som	—	Mar-Jun	Tree; bark grey and warty; leaves oblong-lanceolate, coriaceous; flowers pale white, in panicles; fruits globose.	Fruit	Anthelmentic
246	*Maesa indica* (Roxb.) DC	Myrsinaceae	Awa-pat, Maiki-biring	—	Feb-Nov	Shrub; leaves elliptic-oblong, or ovate lanceolate; flowers white, long pedicelled, in simple or compound axillaryracemes; berries	Shoot	Strengthens the gums, headache

						globose, brownish.		
247	*Mallotus phillipinensis* (Lamk.) Muell & Arg.	Euphorbiaceae	Senduri gooti	—	Oct-Apr	Small tree; leaves broadly ovate orbicular, rounded at base; flowers yellow, in terminal panicled racemes; capsules 3-lobed, spiny.	Bark	Stomach pain
248	*Mangifera indica* L. MBBT/0120	Anacardiaceae	Aam	Mango	Mar-Jun	Evergreen tree; leaves oblong-lanceolate; flowers creamy, in terminal panicles; drupes ovoid.	Tender leaf, flower, fruit, latex	Digestive, diuretic, chronic dysentery, sore eyes
249	*Malva verticillata* L. Syn. *M. parviflora* L. MBBT/0088	Malvaceae	Lofa	—	Dec-Mar	Undershrub; stem with hairs; leaves palmately lobed; flowers yellow; fruits ovoid.	Leaf	Stomach ailments
250	*Maranta arundinacea* L.	Marantaceae	Pati-alu	Arrowroot	June-Oct	Erect, slender herb; rhizome, fleshy, obovoid, cylindrical, and covered with pale scales; leaves ovate-oblong to ovate-lanceolate; flowers white, in clusters on diverging inflorescence branches.	Rhizome	Vermifuge
251	*Marsilea quadrifolia* L.	Marsileaceae	Pani-tengesi	—	Fertile: Aug-Nov	Amphibious fern with long, creeping rhizomes; stipes long with 4 leaflets; sporocarps oval to bean shaped, borne on short, lateral branches of the petioles.	Whole plant	Diuretic, emollient
252	*Melastoma malabathricum* L.	Melastomaceae	Phutuka	Indian rhodo-dendron	Jan-Dec	Perennial shrub; young parts, leaf veins and leaf stalks dark purple, hairy; leaves opposite, oblong-lanceolate; flowers purple; fruits pulpy and fleshy.	Leaf	Cuts and wounds
253	*Melia azedarach* L. MBBT/0113	Meliaceae	Ghora neem	—	Mar-Jul	Tree; leaves imparipinnate, crowded at the end of the	Bark, leaf	Skin diseases

						branches, leaflets lanceolate; flowers white, in axillary panicles; drupes oblong, 1-seeded.		
254	*Meliosma pinnata* Roxb.	Sabiaceae	Hengunia-sak	—	Apr-Aug	Tree; leaves imparipinnate, leaflets opposite, oblong-lanceolate, subcoriaceous; flowers white, in terminal panicles; drupes globose.	Bark	Dysentery
255	*Meliotus alba* Lamk. Syn. *M. indica* L. MBBT/0110	Fabaceae	Bon-methi	—	Mar-Apr	Annual herb with much branched tap root; leaves petiolate, trifoliate, obovate; flowers yellow, in dense flowered, axillary, typical racemes; fruits pod,	Leaf	Bowel complaint
256	*Melochia corchorifolia* L. MBBT/0030	Sterculiaceae	Bon-mora	Wild jute	Jul-Sep	Erect, hairy herb; leaves oblong-ovate; flowers pinkish white, in dense terminal or axillary clusters; capsules small, globose.	Root bark	Sore lip
257	*Mentha arvensis* L.	Lamiaceae	Podina, Poduna	Mint	Nov-Jan	Erect aromatic herb with suckers; leaves opposite, shortly petioled, oblong, ovate or lanceolate; flowers lilac, in axillary distant whorls; nutlets smooth.	Leaf	Stomachache, dyspepsia, appetizer, nasal bleeding
258	*Mesua ferrea* L. MBBT/0136	Clusiaceae	Nahor	Iron-wood tree	Mar-May	Evergreen tree; bark reddish brown; leaves thick, lanceolate, coriaceous, red when young; flowers white, very fragrant, axillary or terminal, solitary or in pairs; fruits ovoid with a conical points, surrounded by the enlarged sepals; seeds 1-4	Leaf, flower	Dysentery, pimples, snake repellent

						angular, dark brown, smooth.		
259	*Merremia umbellata* (L.) Hall. Syn. *Ipomea cymosa* Roem & Schutt. MBBT/0121	Convolvulaceae	Kolia-lota	—	Apr-Sep	Climber; leaves oblong lanceolate, cordate at the base; flowers light yellow, in umbelliform cymes, capsules ovoid.	Leaf	Inflammation
260	*Meyna spinosa* Link. Syn. *Vangueria spinosa* Roxb. MBBT/0250	Rubiaceae	Kutkura	—	Mar-Nov	Small tree; spines axillary, straight; leaves ovate-elliptic; flowers greenish white, in axillary cymes; fruits globose or obovoid drupes.	Leaf, fruit, seed	Hair shampoo*, cracked heels*, piles, abortion*, pimples*
261	*Microsorium punctatum* (L.) Copel. Syn. *Pleopeltis punctata* (L.) Bedd.	Polypodiaceae	Mirioni-murha	—	Fertile: May-Feb	Epiphytic or lithophytic fern; frond linear-lanceolate, shortly stiped; sori small, round, irregularly scattered on the apical part of the lamina.	Leaf	Cuts and wounds
262	*Mikania micrantha* Kunth. Syn. *M. scandens* Willd. MBBT/0067	Asteraceae	Prem-lota, Japani-lota	—	Sep-Jan	Twining herb; leaves opposite, ovate or ovate-cordate; flowers white, in corymbose heads; achenes 5-angled.	Leaf	Stomach pain* dysentery*, cuts and wounds
263	*Mimosa pudica* L. MBBT/0153	Mimosaceae	Lajuki lota	—	Jan-Dec	Prickly herb; pinnae 4, digitately arranged, sensitive; pinnules 12-20 pairs, linear-lanceolate; flowers pinkish purple, in axillary peduncled, globular heads; pods prickly, 3-4 jointed.	Root, leaf	Jaundice, sexual vigour, septic ulcer
264	*Mimusops elengi* L.	Sapotaceae	Bokul	—	May-Sep	Evergreen tree; leaves ellipting oblong or lancolate; flowers creamy white, fragrant, axillary, solitary or in fascicles of few; berries ovate or ellipsoid, 1-seeded.	Bark, seed	Pyorrhea, toothache
265	*Mirabilis jalapa* L.	Nyctaginaceae	Godhuli	Four o'	Jan-Dec	Undershrub; leaves narrow,	Leaf	Boils, sores

	MBBT/0073		gopal	clock plant		pointed, ovate; flowers variously coloured from white, pink to red and sometimes striped; Seeds black, twice the size of a pepper.		
266	*Mollugo pentaphylla* L. MBBT/0179	Aizoaceaea	Setkopora	Indian chickweed	Aug-Oct	Erect herb; leaves whorled or opposite, linear-lanceolate; flowers white, in terminal cymes; capsules globose, 3-lobed.	Leaf	Urinary trouble
267	*Momordica charantia* L. MBBT/0230	Cucurbitaceae	Tita-kerela	Bitter gourd	May-Aug	Annual climbers with angled and grooved stems; tendrils simple and elongate; leaves orbicular, cordate, deply divided into 5-7 lobes; flowers unisexual, yellow on long peduncles; fruits 3-valved, ribbed.	Root, fruit	Diabetes, piles
268	*Momordica cochinchinensis* Spreng. MBBT/0170	Cucurbitaceae	Bhat-kerela	—	May-Sep	Climber with rootstock; leaves sub-orbicular, 5-lobed; flowers white, solitary; fruits ovoid or ellipsoid.	Fruit, seed	Cough, chest complaint
269	*Monochoria hastata* (L.) Solms. Syn. *M. hastaefolia* Prel. MBBT/0166	Pontederiaceae	Bhat meteka	—	Aug-Nov	Aquatic herb; leaves sagittate, petioles sheathing; flowers bluish, long pedicelled, in racemes; capsules subglobose.	Leaf	Digestive
270	*Morinda angustifolia* Roxb.	Rubiaceae	Achu-goch	—	Feb-Oct	Shrub; leaves elliptic oblong narrowed down into petiole; flowers white, in axillary cymes or globose heads; fruits fleshy, 1-seeded.	Leaf	Sore feet
271	*Moringa oleifera* Lamk. Syn. *M. pterigosperma* Gaertn. f.	Moringaceae	Sajina	—	Dec-Jan	Deciduous tree; leaves 3-pinnate on long, greenish rachis; flowers greenish white, in panicles; fruits 9-ribbed,	Leaf, flower, fruit	Cuts and wounds, pox, measles, skin diseases

	MBBT/0070					seeds winged.		
272	*Morus australis* Poir. Syn. *M. indica* Thunb. MBBT/0144	Moraceae	Nuni	Common mulberry	Mar-Jun	Shrub; leaves lobed, serrate; flowers white, in catkin; fruits ovoid, shining, crimson, black when ripe.	Flower	Constipation
273	*Mucuna pruriens* (L.) DC.	Fabaceae	Bandar-kekua	Cowhage	Jun-Jan	Annual climber; leaves trifoliate, leaflets broadly ovate, elliptic or rhomboid-ovate; flowers purple, in axillary, peduncled 6-30 flowered racemes; pods turgid, longitudinally ribbed, densely clothed with persistent pale brown or grey irritant bristles.	Root	Diuretic
274	*Mukia maderaspatana* (L.) Roem	Cucurbitaceae	Trikosaki	—	Apr-Sep	Scandent or prostrate climber; leaves ovate or subdeltoid, cordate at the base; flowers yellow, unisexual; fruits globose, brownish yellow, finally turning red.	Whole plant	Burning sensation, flatulence, colic
275	*Murdannia malabarica* (L.) Bruch. Syn. *Aneilima nudiflorum* R. Br. Mbbt/0058	Commelinaceae	Kureli	—	Jul-Nov	Diffuse herb, rooting at nodes; leaves linear-lanceolate; flowers bluish-purple, in terminal panicle; capsules subglobose.	Root	Jaundice
276	*Murraya koenigii* (L.) Spreng. MBBT/0128	Rutaceae	Narasingha	Curry-leaf plant	Apr-Jun	Strongly scented shrub; leaves imparipinnate, leaflets 9-25, alternate, ovate-lanceolate. Flowers white, in terminal corymbose panicles; berries ovoid.	Leaf	Dyspepsia, dysentery
277	*Musa bulbiciana* Colla. Syn. *M. troglodytarum* L.	Musaceae	Athia-kol	—	Jul-Sep	Stoloniferos shrub; leaves oblong, truncate at the apex; inflorescencce pendulous;	Fruit	Dysentery, diarrhoea, anthelmintic

						bracts pink; fruits pale yellow, angular at maturity; pulp white; seeds globose, black, warty		
278	*Musa sapientum* L. Syn. *M. paradisiaca* L. var. *sapientum* Kuntze	Musaceae	Pura-kol, Kach-kol	Plantain	Sep-Jan	Shrub with rhizomatous stem and pseudostem; leaves oblong-lanceolate with imbricating sheath and stout midrib; inflorescence spadix; fruits with a few seeds or absent.	Fruit	Diarrhoea
279	*Mussaenda frondosa* L. MBBT/0192	Rubiaceae	Chobai-atha	—	Apr-Nov	Shrub; leaves ovate to oblong-lanceolate; flowers orange yellow, in terminal dense cymes.	Whole plant	Jaundice, fever
280	*Myrica nagi* Thu. MBBT/0085	Myricaceae	Noga-tenga	—	Jan-Apr	Tree; leaves imparipinnate, leaflets 4-6 pairs, opposite; flowers white, in terminal panicles; fruits subglobose, pink when mature.	Stem bark	Asthma, diarrhoea, cough
281	*Naravella zeylanica* (L.) DC.	Ranunculaceae	Gop-choi	—		Scandent climber; roots tuberous, stem wiry with strong tendrils; leaves 3-foliate, leaflets modified into a 3-branched tendril; leaflets ovate-lanceolate; flowers yellow, fragrant, in axillary and terminal panicles; fruits aggregate of achenes.	Stem	Toothache
282	*Natsiatum herpaticum* Buch-Ham ex. Arn.	Icacinaceae	Hukati-lota	—	Nov-Feb	Climbing shrub; leaves ovate or suborbicular; flowers pale yellow, in axillary racemes; drupes ovate, compressed.	Leaf	Cuts and wounds
283	*Nelumbo nucifera*	Nelumbonaceae	Padum	Sacred	Jul-Nov	Handsome aquatic herb;	Root,	Ringworm, high

	Gaertn. Syn. *N. speciosum* Willd.			lotus		rhizomes elongate, creeping; leaves peltate, petioles very long; flowers solitary, large, fragrant, white or rosy with a centrally located yellow, spongy torus in which carpels are sunken; fruits ovoid, nut like achenes.	leaf	fever, skin irritation, headache
284	*Nerium indicum* Mill. Syn. *N. oleander* L.	Apocynaceae	Korobi phool	Indian oleander	Sep-Dec	Evergreen shrub with milky latex; leaves 3 in a whorl, linear, dark green; flowers red, rose colured or white, fragrant; fruits follicles.	Flower	Kills the lice in hair
285	*Nicotiana tabacum* L.	Solanaceae	Dhopat	Tobbaco	—	Glandular erect herb; leaves ovate, oblong-lanceolate or elliptic; flowers light red, white or pink, in many flowered panicled racemes; capsules ovoid.	Leaf	Sores, wounds, scabies
286	*Nigella sativa* L.	Ranunculaceae	Kola-jira	Black cumin	Apr-Jun	Small herb; leaves 2-3 pinnatisect, cut into linear or linear-lanceolate segments; flowers pale blue, solitary.	Seed	Jaundice, fever
287	*Nyctanthes arbor-tristis* L. MBBT/0039	Oleaceae	Sewali phul	Night jasmine	Sep-Jan, Apr-Jun	Shrub; leaves ovate, scabrous; flowers white with orange tinge at the centre, fragrant, in trichotomous cymes; capsules suborbicular, 1-seeded.	Flower	Malaria, measles, blood purifier, diabetes
288	*Nymphea nouchali* Burm. f. Syn. *N. lotus* L.	Nymphyaceae	Bhet	Indian water lily	Jul-Nov	Aquatic herb; leaves elliptic or orbicular, petiole fleshy; flowers whitish purple on long pduncles; berries globose.	Rhizome	Diarrhoea, dysentery
289	*Nymphoides hydrophyllum* (Lour.) Kuntz.	Mennyanthaceae	—	Crested snowflakes	Jan-Dec	Aquatic floating herb; leaves orbicular, deeply cordate; flowers white, clustered at	Root	Dysentery

	MBBT/0065					nodes; capsules ovoid ellipsoid.		
290	*Ocimum americanum* L. Syn. *O. canum* Sims. MBBT/0006	Lamiaceae	Kola tuloshi	Hoary basil	Jan-Dec	Undershrub; leaves elliptic-lanceolate, aromatic; flowers pale purple, in axillary and terminal, long racemes; nutlets inserted in the calyx tube.	Leaf	Ringworm, skin diseases
291	*Ocimum basillicum* L. MBBT/0103	Lamiaceae	Bon-tuloshi	—	Jan-Dec	Erect aromatic herb; leaves ovate-lanceolate; flowers white, in whorls of simple or branched racemes; nutlets black, ellipsoid.	Leaf	Infected nipple of the breast, cough, bowel complaints of children
292	*Ocimum sanctum* L. MBBT/0277	Lamiaceae	Tuloshi	Sacred basil	Jan-Dec	Erect undershrub; leaves elliptic, oblong, minutely gland dotted; flowers purplish, in elongated racemes; nutlets smooth.	Leaf	Cough, earache
293	*Operculina turpethum* (L.) Silva. Syn. *Merremia turpethum* (L.) Shah & Bhat.	Convolvulaceae		Indian jalap	Oct-Mar	Climbing herb, stem winged, twisted angled; leaves orbicular or ovate-lanceolate; flowers white, in few flowered cymes; fruits capsules, seeds black.	Root	Expel intestinal worms, sores
294	*Opuntia stricta* (Haw.) Haw var. *dillenii* (Kar-Gawl) Benson.	Cactaceae	Sagar-phena	Prickly pear	Apr-Jul	Thorny succulent shrub; phyllocladodes obovate; flowers yellow, long solitary, sessile; berries subglobose, reddish.	Stem	Ulcers, itchy skin, warts
295	*Oroxylum indicum* (L.) Vent.	Bignoniaceae	Bhat-ghila	—	Nov-Mar	Small tree; leaves opposite, leaflets ovate-elliptic; flowers purplish green, in erect long racemes; capsules flat, 2-valved, drooping.	Stem bark	Sour mouth, tongue and throat
296	*Ottelia alismoides*	Hydrocharitaceae	Panikola	—	Jun-Dec	Aquatic herb; leaves radical,	Whole	Carbuncles,

	(L.) Pers.					blade ovate-cordate, long petiolate; flowers white, solitary; fruits winged.	plant	abscesses, burn injury
297.	*Oxalis corniculata* L. MBBT/0119	Oxalidaceae	Tengesi-tenga	Indian sorrel	Jun-Dec	Diffuse herb; stems creeping, rooting at the nodes; leaflets obcordate with long petioles; flowers yellow, in axillary subumbels; capsules subcylindrical.	Whole plant, leaf, root	Dysentery, diarrhoea, scabies, eczema
298	*Oxalis corymbosa* DC. MBBT/0062	Oxalidaceae	Bor tengesi	Wood sorrel	Jan-May	Herb with bulbous rootstock; leaves obcordate, deeply notched at apex; petioles long; flowers pinkish, in subumbellate corymbs; capsules subcylindric.	Whole plant	Dysentery, diarrhoea
299	*Paederia scandens* (Lour.) Merr. Syn. *P. foetida* L. MBBT/0048	Rubiaceae	Bhedai-lota	—	Sep-Dec	Twining foetid smelling herb; leaves ovate-lanceolate; flowers purplish, in axillary or terminal paniculate cymes; fruits ellipsoid, compressed.	Tender leaf and shoot	Dysentery, diarrhoea, abdominal pain flatulence, allergy
300	*Pandanus odoratissimus* (L.) Roxb.	Pandanaceae	Keteki phool	—	Aug-Sep	Large shrub with stilt roots; leaves long, marginal spines ascending; spathes creamy white; spadix with cylindric spikes; fruoits oblong or globose.	Leaf, flower	Scabies, emollient on face
301	*Paspalum scrobiculatum* L. MBBT/0227	Poaceae		—	Aug-Nov	Annual grass; culms long, tufted, erect; leaves linear-lanceolate; spikes 2-6; spikelets elliptic-ovate, in two rows; caryopsis ovoid.	Whole plant	Ulcers
302	*Passiflora foetida* L.	Passifloraceae	Junuka lota	—	Apr-Aug	Climbing tendrilar herb; leaves 3-lobed; flowers white, solitary, axillary; berries globose.	Leaf	Sore feet

303	*Peperomia pellucida* L. MBBT/0053	Peperomiaceae	Ponow-nowa	—	Aug-Jan	Succulent small herb; leaves alternate, broadly ovate; flowers minute, unisexual, in axillary, terminal, or short spikes; fruits ribbed.	Whole plant	Burn injury
304	*Phlogacanthus thyrsiformis* (Hardw.)Mabb. Syn. *P. thyrsiflorus* (Roxb.) Nees. MBBT/0099	Acanthaceae	Ronga-bahok, Tita phul	—	Jan-May	Shrub; leaves opposite, elliptic, or ovate-lanceolate; flowers deep red, curved, in elongated terminal panicles; capsules linear.	Flower	Rheumatism*, anaemia, cough
305	*Phyla nodiflora* (L.) Greene. MBBT/0160	Verbenaceae	Goda bon	Bank mat	Jan-May	Prostrate herb; rooting at nodes; leaves opposite, obovate, spathulate; flowers purplish white, in dense globose, axillary peduncled spikes; drupes globose.	Whole plant	Diuretic, febrifuge
306	*Phyllanthus emblica* L Syn. *Emblica officinalis* Gaertn. MBBT/0161	Euphorbiaceae	Amlokhi	—	May-Nov	Deciduous tree; leaves oblong, obtuse to subacute; flowers greenish yellow, in axillary clusters; drupes globose, seeds 3-angled.	Fruit	Allergy, anti-dandruff shampoo, skins diseases
307	*Phyllanthes fraternus* Webst. *P. niruri* L. MBBT/0014	Euphorbiaceae	Pani amlakhi, Bhui amlakhi	—	Jul-Dec	Erect herb; leaves alternate, pinnate, leaflets 10-20 pairs; flowers minute, unisexual, on underside of the rachis; drupes pale green, drooping.	Root, tender shoot	Jaundice, dysentery
308	*Phyllanthes virgatus* G.Forst. Syn. *P. simplex* Retz. MBBT/0180	Euphorbiaceae	Pani amlakhi	—	Jun-Nov	Erect herb; leaves linear-oblong; rounded at base; Flowers white, unisexual, in axillary clusters or solitary, capsules globose, long stalked.	Root, tender shoot	Same as above
309	*Physaslis minima* L. MBBT/0049	Solanaceae	Kopalphuta	Sunberry	Oct-Mar	Erect herb; leaves ovate, toothed; flowers pale yellow, solitary, axillary; berries	Leaf	Diuretic

						globose.		
310	*Piper betle* L. MBBT/0133	Piperacaeae	Pan	Betel	Mar-May	Root climber; leaves broadly ovate-cordate, unequal at the base; flowers greenish white	Leaf	Cuts and wounds, piles, laxative
311	*Piper longum* L. MBBT/0246	Piperaceae	Pipoli	Long pepper	Aug-Dec	Creeping herb; leaves ovate-rounded or suborbicular, cordate at base; flowers greenish yellow, unisexual, in spikes; fruits crowdwd on fleshy spikes.	Root, dried spike	Cough, stomachache, asthma, bronchitis, fever, dyspepsia
312	*Piper nigrum* L. MBBT/0006	Piperaceae	Jaluk	Black pepper	Aug-Sep	Root climber; rooting at nodes; leaves ovate, 5-7 nerved; spikes cylindrical, pedunculate, berries black, globose.	Fruit	Cold, cough, influenga, galactogue to nursing mothers
313	*Pisum sativum* L.	Fabaceae	Motor mah	Garden pea	Oct-Jan	Herbaceous climber; leaves pinnately compound; flowers white or pink; pods long, curved; seeds yellowish green, 6-9 per pod.	Seed	Emollient on face, measles
314	*Plantago erosa* Wall. Syn. *P. major* L. MBBT/0129	Plantaginaceae	Sing-apat	—	Apr-July	Herb; leaves radical, ovate-oblong, long petioled; flowers greenish white, in long axillary spikes; fruits capsule.	Leaf	Cuts and wounds
315	*Plumbago rosea* L. Syn. *P. indica* L.	Plumbaginaceae	Ronga agiachita	Fire plant	Apr-Nov	Perennial herb; roots stout, cylindrical, irregularly bent, yellowish brown; leaves elliptic-ovate, tapering to a short petiole; floweres bright red, in long terminal spikes.	Root	Useful in leucoderma
316	*Plumbago zeylanica* L.	Plumbaginaceae	Boga agiachita	—	Aug-Nov	Perennial undershrub; leaves ovate-acute, base tapering; floweres white, in long racemes; capsules oblong.	Root	Pneumonia, skin diseases

317	*Plumeria rubra* L. MBBT/0081	Apocynaceae	Gulanchi	—	Aug-Dec	Small tree with milky juice; leaves oblong or obovate, lanceolate; flowers white with yellow center, fragrant, in umbelliform cymes; follicles linear-oblong, rigid, seeds winged.	Leaf, latex	Rheumatic pain
318	*Pogostemon benghalense* L. MBBT/0100	Lamiaceae	Shookloti	—	Jan-Jun	Shrub; leaves ovate-orbicular; flowers yellowish white.	Whole plant	Wounds, elephantitis*
319	*Polyathia longifolia* Benth.	Annonaceae	Debodaru	Mast tree	July-Sep	Evergreen tree; leaves with undulate margin, shining; flowers yellowish green, in fascicles; berries ovoid, small.	Bark	Scabies
320	*Polygonum chinense* L. MBBT/0056	Polygonaceae	Modhu-soleng	—	Sep-Mar	Climbing herb; leaves ovate or oblong-lanceolate; flowers pinkish white, in terminal corymbose heads; nuts trigonous.	Tender shoot	Dysentery
321	*Polygonum hydropiper* L. MBBT/0156	Polygonaceae	Pothorua bihoongoni	Water pepper, Pepperwort	Aug-Mar	Erect herb; leaves linear-lanceolate, stipules ochreate; flowers pinkish white, in slender racemes; nutlets trigonous.	Whole plant	Anthelmentic, insect repellent
322	*Pongamia pinnata* (L.) Pierre. Syn. *P. glabra* Vent. MBBT/0231	Fabaceae	Korosh	Indian beech	Apr-Jun	Tree with spreading branches; leaves imparipinnate; leaflets 5-7, ovate-elliptic; flowers pinkish white, in axillary racems; pods obliquely oblong.	Leaf, flwer	Expels intestinal worms
323	*Portulaca oleracea* L.	Portulacaceae	Malbhog saki	Common garden Pursslane.	May-Sep	Prostrate succulent herb; leaves opposite, oblong, fleshy; flowers yellow, in terminal heads; capsules obovoid.	Whole plant	Dysentery, wounds, burns

324	*Pouzolzia zeylanicca* (L.) Benn. Syn. *P. indica* Gaud. MBBT/0105	Urticaceae	Borali-bokua	—	May-Jan	Erect or prostrate herb; leaves opposite, ovate-lanceolate; flowers white, in axillary clusters; achenes 2-winged.	Whole plant	Sprain, cuts and wounds
325	*Premna latifolia* Roxb.	Verbenaceae	Goniori	—	May-Sep	Small tree; leaves elliptic or ovate, rounded at base; flowers white, in terminal corymbs; drupes subglobose, black when ripe.	Leaf	Stomach pain
326	*Psidium guajava* L. MBBT/0163	Myrtaceae	Modhuri am	Guava	Apr-Nov	Small tree; leaves opposite, elliptic-oblong; flowers white, in axillary peduncles; berries globose.	Stem bark, Leaf	Blood dysentery, piles, diarrhoea
327	*Punica granatum* L. MBBT/0147	Punicaceae	Dalim	Pome-granate	Mar-Jan	Shrub, leaves opposite, oblong-lanceolate, flowers reddish, axillary, solitary; berries globose, seeds juicy.	Root, tender shoot and fruit	Urinary trouble, diarrhoea, conjunctivitis
328	*Quamoclit pinnata* (Desr.) Boj.	Convolvulaceae	Kunjalata		Jul-Dec	Slender twiner; leaves deeply pinnatisect; flowers red, long, solitary, in few flowered peduncled cymes; capsules globose.	Root	Skin diseases
329	*Quisqualis indica* L.	Combretaceae	Malati phul	Rangoon creeper	Mar-Aug	Woody climber, leaves opposite, oblong-lanceolate; flowers pinkish white, fragrant, in terminal drooping spikes; fruits shortly 5-winged.	Root	Anthelmintic
330	*Randia dumetorum* (Retz.) Poir. Syn. *Catunaregam spinosa* (Thunb.) Tirven	Rubiaceae	Bitmora, Bihmona	—	May-Oct	Spiny shrub, spines axillary; leaves fasciculated on brnches, obovate; flowers creamy white, solitary or 2-3 together; berries subglobose.	Fruit, Bark	Fish poison, pneumonia

331	*Ranunculus scleratus* L. MBBT/0104	Ranunculaceae	Pani dhania	—	Jan-Apr	Erect succulent herb; stem hollow, ribbed; leaves 3 partite, sessile or stalked above; flowers yellow, solitary, terminal; achenes turgid.	Leaf	Skin diseases
332	*Raphanus sativus* L. MBBT/0086	Brassicaceae	Moola	Radish	Dec-Jan	Annual herb; stem short, condensed; tap root tuberous; basal leaves long, lyrately pinnate, upper leaves petiolate; flowers white or lilac, in lax racemes; pods yellow or pale purple,	Root	Liver, gall blader trouble
333	*Rawvolfia serpentina* (L.) Benth. ex. Kurz. MBBT/0143	Apocynaceae	Sarpa-gandha	—	Apr-Aug	Undershrub; leaves 3 whorled, elliptic-lanceolate; flowers fragrant, white, in axillary corymbose cymes; follicles 3-ribbed, 3-6 seeded.	Root	Hypertension, pimples, absceses, scabies
334	*Rhynchostylis retusa* (L.) Bl. MBBT/0130	Orchidaceae	Kopou-phul	—	Apr-Jun	Epiphytic herb with stout, leafy stems; leaves strap-shaped, spreading and recurved, flower pinkish-white in long dense racemes	Flower	Emollient on face*
335	*Ricinus communis* L. MBBT/0041	Euphorbiaceae	Era	Castor	Jan-Dec	Evergreen shrub; leaves long petiolate, 6-11 lobed, lobes ovate-lanceolate; flowers pale yellowish, monoecious, in terminal panicles; capsules 3-lobed.	Root, leaf	Stomachache, carbuncles
336	*Rorippa indica* (L.) Hiern. Syn. *Nasutium indicum* (L.) DC.	Brassicaceae	Bon horioh	Wild mustard	Feb-Aug	Erect herb; leaves radical, pinnatifid; flowers yellow, in terminal racemes; siliqua cylidric	Leaf	Diuretic
337	*Rubus alceifolius* Poir. Syn. *R.*	Rubiaceae	Jetuli-poka, Jejeli-poka	—	Aug-Dec	Prickly shrub; leaves ovate or suborbicular, lobed; flowers	Root, tender	Dysmenorrhea*, cough,

	moluccanus Hk.f. MBBT/0224					white, in axillary or terminal racemes; fruits globose, scarlet	shoot, fruit	pneumonia*, fungal infection of tongue
338	*Rumex acetosella* L. MBBT/0167	Polygonaceae	Chuka-sak	—	Dec-Apr	Fleshy annual herb; leaves elliptic-ovate or oblong, cordate; flowers greenish pink, in racemose whorls; nutlets ovate-oblong	Whole plant	Blood purifier
339	*Rumex nepalensis* L. MBBT/0157	Polygonaceae	Lo-borua	—	Nov-Apr	Erect herb; stem deeply grooved; leaves lanceolate, narrowed to the base; flowers greenish white, in racemose whorls; nutlets ovate-oblong	Leaf	Eczema
340	*Saccharum officinarum* L	Poaceae	Kuhiar	Sugarcane	—	Shrubby grass; leaves rigid, linear-lanceolate, spreding or drooping at the tip; panicles pyramidal, very large, dense, spreading, silky white	Stem juice	Expectorant. abdominal pain, jaundice
341	*Saccharum spontaneum* L.	Poaceae	Kohua	—	Sep-Dec	Perennial grass, culms erect; leaf blades linear acuminate; inflorescence a lax panicle, silky hairy; spikelets 1-flowered.	Root	Diuretic
342	*Sapindus mukorossi* Gaertn. f.	Sapindaceae	Moni-chal, ritha	Soap-nut tree	Apr-Dec	Tree; leaves paripinnate; leaflets oblong-lanceolate; flowers white, in axillaryor terminal panicles; fruits globose drupes, 1-seeded	Seed	Tonsillitis, pharyngitis, scabies
343	*Saraca asoka* (Roxb.) de wilde. Syn. *S. indica* L. MBBT/0165	Caesalpiniaceae	Ashok goch	—	May-Aug	Evergreen tree; leaves pinnate having 2-3 pairs of lanceolate leaflets; flowers orange or orange yellow, in dense corymbs, very fragrant; pods lathery, compressed.	Bark, seed	Pyorrhea, urinary complaints

344.	*Sarcochlamys pulcherima* (Roxb.) Gaud.	Urticaceae	Mechaki	Dogal tree	Jun-Dec	Shrub; leaves lanceolate, narrowed down to the base; flowers creamy, clustered in spikes; achenes oblique.	Tender shoot	Dysentery
345	*Sauropus androgyns* (L.) Merr.	Euphorbiaceae	Bari-sundari	Star goose berry	Jan-Dec	Undershrub; branches with two prominent longitudinal lines; leaves distichous ovate-lanceolate, membranous; inflorescence axillary cymes; fruits globose, pinish	Fruit	Anthelmintic
346	*Schumanianthus dichpotomus* (Roxb.) Gagnep Syn. *Clinogyne dichotoma* Roxb.	Marantaceae	Patidoi	—	May-Sep	Tall shrub; leaves elliptic-oblong or bolong-lancolate; flowers white, large, paired in terminal panicles; fruits subglobose.	Flower	Carminative
347	*Scindaspus officinalis* Schott	Araceae	Hati-kuhiar	—	—	Epiphytic climber; aerial roots adventitious, growing on trees an rocks; leaves large; flowers densely arranged in the spadix; spathe green outside and yellow inside.	Inflorescence	Cough, bronchitis
348	*Scoparia dulcis* L. MBBT/0025	Scrophulariaceae	Cheni-bon, bon dhania	Sweet broomweed	Jul-Oct	Erect herb; stem ribbed; leaves ovate-lanceolate, rhomboid or elliptic, flowers white, 3-6 in axillary whorls; capsules globose	Stem	Gastritis
350	*Sesamum orientale* L. Syn. *S. indicum* L. MBBT/0011	Pedaliaceae	Til	Sesame	Aug-Oct	Hirsute herb; leaves alternate or opposite; flowers axillary, solitary, pinkish purple; fruits quadriangular, seeds compressed, black.	Leaf, seed	Hair-wash to prevent dandruff, hair oil
351	*Sesbania grandiflora* (L.) Pers.	Fabaceae	Bok-phul	Sesban, Swamp pea	Mar-Aug	Tree; leaves abruptly pinnate, leaflets 41-61, linear-oblong; flowers fleshy white or pink, showy; pods flat and 4-	Leaf	Sore throat and mouth

						cornered.		
352	*Shorea robusta* Gaertn. f.	Dipterocarpaceae	Shal	Sal	Jul-Aug	Deciduous tree; bark grey or reddish brown, smooth or longitudinally fissured; leaves ovate-oblong, base cordate; flowers yellow, in lax axillary or terminal panicles; fruits ovoid with 5 equal wings.	Bark	Wounds, abscesses
353	*Sida acuta* Burm. f. Syn. *S. carpinifolia* Mast. MBBT/0177	Malvaceae	Sonborial	Snake's tongue	Sep-Nov	Undershrub; leaves linear-lanceolate; flowers yellow, axillary, solitary or in 2-3 flowered clusters; fruits capsules.	Root	Stomach pain
354	*Sida rhombifolia* L. MBBT/0125	Malvaceae	Soru sonborial	Sida hemp	Nov-Apr	Erect or diffuse undershrub; leaves rhomboid or obovate, 5-nerved; floweres yellow, solitary.	Seed	Fever
355	*Smilax perfoliata* Lour. Syn. *S. prolifera* L. MBBT/0145	Smilaceae	Tikoni-borua	—	Mar-Aug	Climbing prickly shrub; leaves elliptic-lanceolate or oblong-ovate; petiole stout; flowers white, in axillary umbels; berries subglobose or globose, red when ripe.	Stem, tender shoot	Strengthens the gums, useful as blood purifier
356	*Solanum indicum* L. Syn. *S. violaceum* Ortega. MBBT/0052	Solanaceae	Tita bhekuri	Indian nightshade	May-Oct	Prickly undershrub; leaves ovate-oblong, prickly along nerves; flowers violet, in extra axillary short cymes; berries globose.	Fruit	Blood purifier
357	*Solanum myriocanthum* Dunal. Syn. *S. khasianum* Clarke. MBBT/0107	Solanaceae	Kutahi-bengena	Horse nettle	May-Nov	Prickly undershrub; leaves ovate, lobed or angled, prickly on both surfaces and petioles; flowers white, in few flowered lateral cymes; berries globose.	Fruit	Abscesses
358	*Solanum nigrum* L.	Solanaceae	Loch-	Black	Mar-Nov	Erect glabrous herb; leaves	Tender	Skin diseases

	MBBT/0005		kochi, Pokmow	nightshade		ovate-oblong or oblong-lanceolate; flowers white, in extra axillary drooping umbellate cymes; berries globose, when ripe.	shoot	
359	*Solanum torvum* Sw. MBBT/0024	Solanaceae	Hati-bhekuri	—	May-Sep	Shrub; prickles scattered; leaves ovate-elliptic, midrib sparsely prickly; flowers white, in corymbose cymes; berries globose.	Fruit	Stomach problems
360	*Spilanthes acmella* (auct. non L.) Merr. Syn. *S. Clava* DC	Asteraceae	Suhuni bon	Para cress	Jan-Dec	Erect or ascending herb; leaves opposite, ovate or ovate-lanceolate; heads yellow, long peduncled; florets tubular; achenes black, cilliate.	Infloresc ence	Inflammation of throat
361	*Spinacea oleracea* L. MBBT/0091	Chenopodiaceae	Paleng sak	Spinach	Sep-Feb	Soft herb; leaves ovate-lanceolate, dark green; flowers white, in racemes	Leaf	Blood purifier
362	*Sphaerostephnos unitus* L. Syn. *Nephrodium cucullatum* Baker	Thelypteridaceae	Bihlongoni dhekia	—	Fertile: Jul-Oct	Terestrial fern; stipes slender, scaly; lamina simple pinnae, 15-30 pairs, linear, oblanceolate; sori small, in apices of veins.	Leaf	Sprain
363	*Spondius pinnata* (L.f.) Kurz. Syn. *S. mangifera* Willd.	Anacardiaceae	Amora	Hog plum, wild mango	Mar-Dec	Medium sized tree; leaves imparipinnate, leaflets 3-6 pairs, elliptic-oblong; flowers greenish white, in terminal panicles; drupes ovoid, fleshy.	Stem bark, leaf, fruit	Blood dysentery, otalgia, chronic dysentery
364	*Stellaria media* (L.) Villars. MBBT/0046	Caryophyllaceae	Morolia sak, Thutoni bon	—	Mar-Sep	Diffuse herb; leaves ovate, cordate at base; lower ones long petioled, upper sessile; flowers white, solitary or cymose or racemose; fruits a capsule.	Whole plant	Piles

365	*Stephania japonica* (Thunb.) Miers. Syn. *S. hernandifolia* (Willd.) Walp.	Menispermaceae	Tubuki lota	—	Apr-Dec	Climber; roots tuberous; leaves ovate, peltate, rounded at base, long petiolate; flowers greenish yllow, in axillary umbels; drupes ovoid or subglobose.	Leaf	Septic ulcer
366	*Sterculia villosa* Roxb.	Sterculiaceae	Udal	—	Mar-Jul	Tree; leaves long, crowded at the ends of the branches, deeply palmately 5-7 lobed, cordate; flowers yellow, polygamous; follicles brownish.	Bark	Constipation
367	*Streblus asper* Lour.	Moraceae	Saura	Toothbrush tree, seamese rough bush	Jan-Jun	Small or medium sized tree; leaves elliptic-obovate or rhomboid; male flowers pale yellow, in globose heads; female flowers solitary; drupes 1-seeded, covered by enlarged perianth.	Stem	Toothache
368	*Symplocos cochinchinensis* (Lour.) Moore. Syn. *S. laurina* Wall. Ex Rehd. ; *S. spicata* Roxb.	Symplocaceae	Bhoomloti	—	—	Evergreen tree; leaves lanceolate, elliptic or oblong; flowers yellowish white, fragrant, in close clusters; drupes globose, ribbed, purple.	Bark	Febrifuge
369	*Syzygium cumini* (L.) Skeels. Syn. *Eugenia jambolana* Lamk. MBBT/0108	Myrtaceae	Jamuk	—	Mar-Aug	Evergreen tree; leaves oblong-elliptic; flowers greenish white, in lateral or terminal panicles; beries purple black, 1-seeded.	Bark, fruit	Diarrhoea, dysentery, bleeding piles, diabetes
370	*Syzygium jambos* (L.) Alston. Syn. *Eugenia jambos* L.	Myrtaceae	Bogi-jamu	Rose-apple	Mar-jun	Small tree; leaves lanceolate, narrowed into short petioles; flowers greenish white, in short terminal racemose cymes; fruits pale yellow to	Leaf, fruit	Epistaxis

						pinkish white.		
371.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem.et Schult. MBBT/0028	Apocynaceae	Kothona phul	Crepe jasmine	May-Nov	Small shrub; leaves elliptic-lanceolate; flowers fragrant, white, in axillary corymbose cymes; follicles 3-ribbed.	Root,	Malaria
372	*Tagetes patula* L.	Asteraceae	Narji phool	Marigold	Nov-Jan	Slender erect herb; leaves deeply incised and sharply toothed; heads solitary, long stalked; flowers pale yellow or brownish yellow,	Leaf	Skin cuts
373	*Talauma hodgsonii* Hook.f. Thoms	Magnoliaceae	Borhomothuri	—	Apr-Jul	Tree with grey wood; leaves oblanceolate, red and erect when young; flowers pink, fragrant, terminal, solitary; Fruits ovoid, beaked, woody, indehescent carpels.	Stipule with bud	Strengthens gums and teeth*
374	*Tamarindus indica* L.	Casalpiniaceae	Teteli	Tamarind	Apr-Jan	Tree; leaves paripinnate, leaflets 10-20 pairs, small, linear-oblong; flowers yellow, in few flowered terminal racemes; pods compresed, seeds dark brown.	Leaf, fruit	Vermifuge, headache, laxative
375	*Telanthera ficoidea* Mog. Syn. *Alternanthera ficoides* R.Br. ex Griseb.	Amaranthaceae	Brindadbon	Joy weed	Mar-May	Herb; leaves oposite, close, often coloured red or purplish; flower in axillary clusters.	Tender shoot	Cuts and wounds
376	*Terminalia arjuna* (Roxb.) Wt. Et. Arn. MBBT/0003	Combretaceae	Arjun	—	Aug-Feb	Tree; leaves opposite, elliptic-oblong, glands at base; flowers yellowish white, in axillary and terminal spikes; fruits ovoid or oblong, fibrous, 5-winged.	Bark	Asthma, diarrhoea, dysentery, bone fracture
377	*Terminalia bellerica*	Combretaceae	Bhumura	—	Mar-Feb	Deciduous tree; leaves	Fruit	Septic ulcer, sore

	(Gaertn.) Roxb. MBBT/0247					crowded at the ends of the branches, elliptic or ovate-orbicular; floweres greenish yellow, foetid, in slender interrupted spikes; drupes ovoid or ellipsoid.		eyes
378	*Terminalia chebula* (Gaertn.) Retz.	Combretaceae	Silikha	—	Apr-Jan	Deciduous tree; leaves ellipting-oblong or ovate-elliptic, petioles short with 2 glands; flowers creamy, sessile, in terminal cymes; drupes ovoid-ellipsoid, faintly ridged.	Fruit	Constipation, mouth inflammation, sweelings of mumps
379	*Thladiantha cordifolia* (Bl.) Cogn. Syn. *T. calcarata* Cl.	Cucurbitaceae	Belipoka	—	Sep-Oct	Climber; leaves deeply cordate; male flowers with bracts; female flowers single; fruits oblong, longitudinally 12-15 nerved.	Root	Sinusitis
380	*Thunbergia grandiflora* Roxb.	Acanthaceae	Nil lota	—	Nov-Mar	Woody climber; leaves ovate or orbiculr, irreguarly toothed; flowers white or bluish, in dense terminal racemes; fruits a capsule.	Leaf	Dyspepsia
381	*Tinospora cordifolia* Miers.	Menispermaceae	Shaguni lota	—	Jan-May	Succulent climber; leaves ovate or orbicular, cordate at base; flowers greenish yellow, in axillary or terminal racemes; drupes globose.	Leaf	Leprosy
382	*Torenia flava* Ham. MBBT/0169	Scrophulariaceae		Torenia	May-Sep	Erect herb; leaves serrated, ovate; flowers violet, small; capsules ridged.	Leaf	Pain and swellings
383	*Trapa bispinosa* Roxb.	Trapaceae	Pani-singori	—	Aug-Dec	Aquatic floating herb; floating leaves in rosettes, rhomboid, crowded in the upper part of the stem, submerged ones	Fruit	Appetizer, diuretic

						dissected; floweres white, solitary, axillary; fruits angled nuts.		
384	*Trichosanthes wallichiana* (Seringe) Wight	Cucurbitaceae	Kowa-bhaturi	—	Jul-Nov	Scandent climber; tendril trifid; leaves palmately 3-9 lobed; floweres white; fruits ovoid-oblong.	Fruit	Diabetes
385	*Tridax procumbens* L. MBBT/0223	Asteraceae		Mexican daisy	Jan-Dec	Procumbent herb; leaves opposite, ovate or lanceolate; heads yellow, solitary, on long peduncles; achenes brown.	Leaf, flower	Wounds
386	*Trigonella foenum-graceum* L. MBBT/0112	Fabaceae	Methi	—	Mar-May	Small herb; leaves trifoliate, leaflets obovate; flowers stalkless, yellow located in axils; pods compressed.	Leaf, seed	Anaemia, indigestion, flatulence
387	*Triumfettta rhomboidea* Jacq.	Tiliaceae	Bon ogora	—	Sep-Nov	Undershrub; leaves variable, often 3-lobed, rhomboid or orbicular-ovate; flowers yellow, in dense, lateral, terminal cymes; fruits globose capsule, hooked spiny.	Stem bark, Leaf	Urinary trouble, diarrhoea
388	*Typhonium trilobatum* (L.) Schott. MBBT/0117	Araceae	Somahu, soma kochu, adolia kochu	—	Apr-May	Herb; tuber subglobose; leaves 3-lobed; spathes long, pinkish green below and dark red above; spadix dark red; flowers unisexual; berries ovoid.	Tuber, latex	Stomach pain, pimples, abscesesses
389	*Urena lobata* L. MBBT/0031	Malvaceae	Bor-sonborial	Aramina, Cadilla	Jul-Dec	Hairy undershrub; leaves variable; upper ones small, elliptic, oblong-ovate, lower ones orbicular, 3-5 lobed; flowers bright pink, axillary, solitary; fruits capsule.	Leaf	Sores
390	*Verbena officinalis*	Verbenaceae		Vervain	Mar-Sep	Erect herb; leaves various	Leaf	Healing of

	L. MBBT/0164					lobed; floweres bluish white, in long spikes; fruits ribbed.		wounds
391	*Vernonea cineria* L. MBBT/0050	Asteraceae	Sahadevi	Ash coloured fleabane	Oct-Dec	Erect herb; leaves ovate to lancolat; heads pinkish, in terminal corymbs; achens 4-5 angled, hairy.	Leaf	Eczema, ringworm, conjunctivitis
392	*Vigna mungo* (L.) Hepper. Syn. *Phaseolus mungo* L.	Fabaceae	Mati-mah	Black gram	Nov-Jan	Hispid trailing herb; flowers yellow; pods hairy, erect; seeds usually black, sometimes greenish.	Seed	Scabies and ringworm
393	*Vitex negundo* L. MBBT/0016	Verbenaceae	Pochotia	Chinese chaste tree	Apr-Aug	Aromatic shrub; leaves digitately 3-5 foliate, opposite, leaflets lanceolate, glabrous above, shining beneath; flowers purplish blue, in terminal panicles; drupes globose, black when ripe.	Leaf	Scabies, pneumonia
394	*Withania somnifera* Dunal. MBBT/0150	Solanaceae	Aswa-gandha	Winter cherry	Mar-Apr	Undershrub; leaves ovate; flowers grennish yellow, in axillary fascicles; berries globose, orange coloured when mature.	Root, leaf	Carbuncles, ulcers, painful swellings
395	*Wedelia chinensis* (Osbeck) Merrill. Syn. *W. calandulacea* Less. MBBT/0013	Asteraceae	Bhringaraj	—	Nov-Jan	Procumbent perennial herb; rooting at the lower nodes; leaves linear-oblong, oblanceolate; flowers yellow, in axillary or terminal heads; achns compressed or tubercled.	Whole plant	Diabetes*, uterine haemorhage, hair oil
396	*Xanthium strumarium* L. MBBT/0101	Asteraceae	Ogora	Cocklebur	Nov-Jan	Undershrub; leaves ovate-triangular, palmately lobed; male heads white, many flowered, female heads 2-flowered in axillary racemes; fruits achene.	Leaf	Sores infested with worms

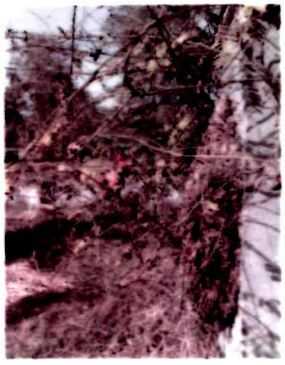
397	*Zanthoxylum budrunga* (Roxb.) DC. Syn. *Z. rhesta* (Roxb.) DC	Rutaceae	Bajar-nail, Bajramoni	—	Mar-May	Deciduous tree; stem armed with conical spines; branches armed with prickles; leaves paripinnate or imparipinnate, clustered at the ends of the branches; flowers white, in terminal panicles; follicles globose, aromatic, red.	Tender shoot	Blood purifier
398	*Zanthoxylum nitidum* (Roxb.) DC Syn. *Z. hamiltonianum* Wall.ex.Hook	Rutaceae	Tezmui, Tezmuri	—	Mar-May	Scandent shrub with recurved prickles; leaves imparripinnate, leaflets opposite, eliptic; flowers dull white, in axillary, fasciculated cymes; follicles subglobose.	Root, stem, fruit	Pneumonia, toothache, Pyorrhea, siallagogue, fish poison
399	*Zanthoxyllum oxyphyllum* Edgew. MBBT/0233/0248	Rutaceae	Mezenga	—	Mar-Jul	Scrambling shrub with aromatic smell and hooked prickles; leaves, paripinnate, leaflets variable in shape and size, pointed, dazzling, shrill and pungent; flowers greenish white, in axillary panicled cymes; fruits tubercled, small	Tender shoot, fruit	Stomach trouble, leucoderma*, blood purifier*, bleeding piles*, stomachic
400	*Zingiber officinale* Rosc.	Zingiberaceae	Ada	Ginger	—	Herb with tuberous, creeping rhizome; rhizome aromatic, thick, pale yellow; leaves sub-sessile, distichous; flowers yellowish with dark-purple, creamy, mottled tip.	Rhizome	Stomach trouble, expectorant, allergy

* new information

Annexure II

Photographs of some recorded medicinal plants

Abelmoschus esculentus
(Fam. Malvaceae)



Abrus precatorius
(Fam. Fabaceae)

Abutilon indicum
(Fam. Malvaceae)

Acacia fernesiana
(Fam. Mimosaceae)

Achrus zapota
(Fam. Sapotaceae)

Achyranthus aspera
(Fam. Amaranthaceae)

Acorus calamus
(Fam. Araceae)

Alocasia macrorhiza
(Fam. Araceae)

Aloe barbadense
(Fam. Liliaceae)

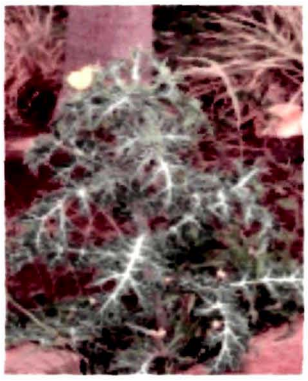
Plate 4

Alpinia galanga
(Fam. Zingiberaceae)

Alpinia nigra
(Fam. Zingiberaceae)

Alstonia scholaris
(Fam. Apocynaceae)

Andrographis paniculata
(Fam. Acanthaceae)



Argemone mexicana
(Fam. Papavaraceae)

Argyreia nervosa
(Fam. Convolvulaceae)

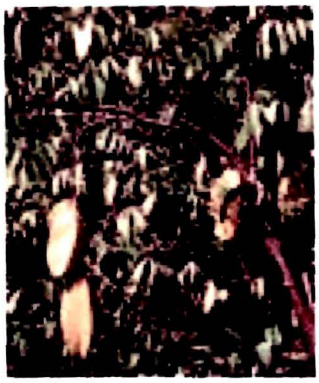
Artocarpus heterophyllus
(Fam. Moraceae)

Artocarpus lakucha
(Fam. Moraceae)

Arundo donax
(Fam. Poaceae)

Plate 5

Asparagus racemosus
(Fam. Liliaceae)



Averrhoea carambola
(Fam. Averrhoeaceae)

Azadirachta indica
(Fam. Meliaceae)

Bacopa monnieri
(Fam. Scrophulariaceae)

Bambusa balcooa
(Fam. Poaceae)

Bambusa tulda
(Fam. Poaceae)

Basella alba
(Fam. Basellaceae)

Begonia roxburghii
(Fam. Begoniaceae)

Benincasa hispida
(Fam. Cucurbitaceae)

Plate 6

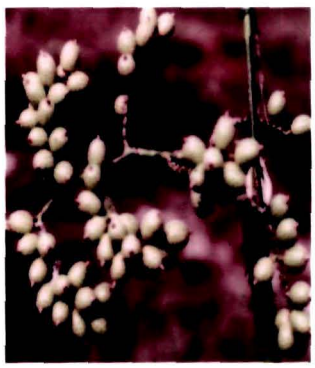
Bischofia javanica
(Fam. Euphorbiaceae)

Bixa orellana
(Fam. Bixaceae)

Boerhaevia diffusa
(Fam. Nyctaginaceae)

Caesalpinia bonduc
(Fam. Caesalpiniaceae)

Caesalpinia pulcherima
(Fam. Caesalpiniaceae)



Calamus tenuis
(Fam. Arecaceae)

Calotropis procera
(Fam. Ascleipiadaceae)

Cardiospermum halicacabum
(Fam. Vitaceae)

Cassia alata
(Fam. Caesalpiniaceae)

Plate 7

Cassia tora
(Fam. Caesalpiniaceae)

Catheranthus roseus
(Fam. Apocynaceae)

Centella asiatica
(Fam. Apiaceae)

Citrus grandis
(Fam. Rutaceae)

Citrus limon
(Fam. Rutaceae)

Clerodendron colebrookianum
(Fam. Verbenaceae)

Clitorea ternatea
(Fam. Fabaceae)

Cordia dichotoma
(Fam. Cordiaceae)

Crotalaria pallida
(Fam. Fabaceae)

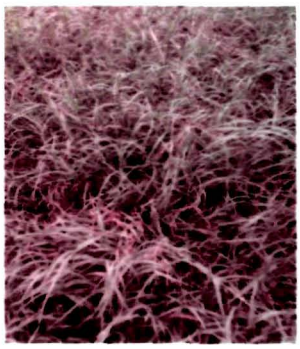
Plate 8

Curculigo orchioides
(Fam. Hypoxidaceae)

Curcuma amada
(Fam. Zingiberaceae)

Curcuma aromatica
(Fam. Zingiberaceae)

Curcuma caesia
(Fam. Zingiberaceae)



Cynodon dactylon
(Fam. Poaceae)

Cynoglossum glochidiatum
(Fam. Boraginaceae)

Datura metel
(Fam. Solanaceae)

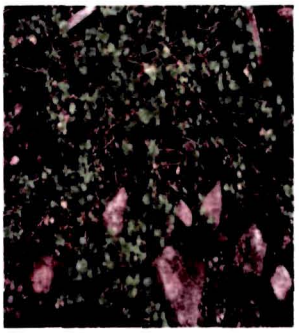
Derris eliptica
(Fam. Fabaceae)

Dioscorea bulbifera
(Fam. Dioscoreaceae)

Plate 9

Diospyros malaberica
(Fam. Ebenaceae)

Dracena angustifolia
(Fam. Agavaceae)



Drymaria cordata
(Fam. Caryophyylaceae)

Duchesnea indica
(Fam. Rosaceae)

Eclipta alba
(Fam. Asteraceae)

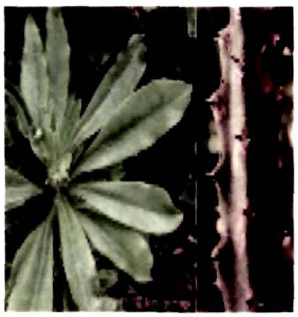
Elaegnus conferata
(Fam. Elaegnaceae)

Eryngium foetidum
Fam. Apiaceae

Euphorbia antiquorum
(Fam. Euphorbiaceae)

Euphorbia hirta
(Fam. Euphorbiaceae)

Plate 10

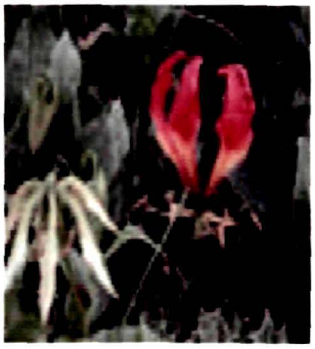


Euphorbia ligularia
(Fam. Euphorbiaceae)

Ficus auriculata
(Fam. Moraceae)

Flacourtia jangmons
(Fam. Flacourtiaceae)

Flemingia strobilifera
(Fam. Fabaceae)



Gloriosa superba
(Fam. Liliaceae)

Helitropium indicum
(Fam. Boraginaceae)

Hibiscus cannabinus
(Fam. Malvaceae)

Hibiscus mutabilis
(Fam. Malvaceae)

Hibiscus sabdariffa
(Fam. Malvaceae)

Plate 11

Hauttuynia cordata
(Fam. Saururaceae)

Hydrocotyl sibthropioides
(Fam. Apiaceae)

Impatiens balsamina
(Fam. Balsaminaceae)

Jasminium sambuc
(Fam. Oleaceae)

Jatropha curcus
(Fam. Euphorbiaceae)

Jatropha gossipifolia
(Fam. Euphorbiaceae)

Kaemferia galanga
(Fam. Zingiberaceae)

Lasia spinosa
(Fam. Araceae)

Leucus plukenitii
(Fam. Lamiaceae)

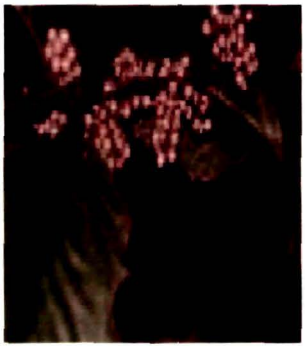
Plate 12

Limnophila indica
(Fam. Scrophulariaceae)

Litsea cubeba
(Fam. Lauraceae)

Litsea glutinosa
(Fam. Lauraceae)

Luffa aegyptica
(Fam. Cucurbitaceae)



Maesia indica
(Fam. Myrsinaceae)

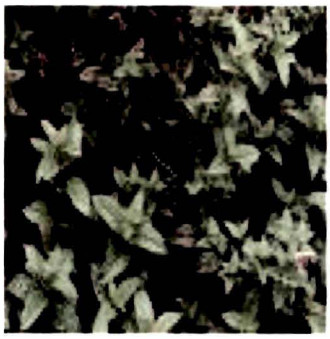
Mallotus phillipinensis
(Fam. Euphorbiaceae)

Meliotus alba
(Fam. Fabaceae)

Melisoma pinnata
(Fam. Sabiaceae)

Melochia corchorifolia
(Fam. Tiliaceae)

Plate 13



Mentha spicata
(Fam. Lamiaceae)

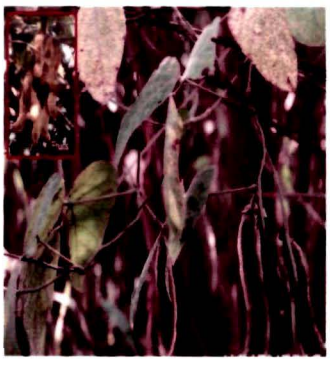
Merremia umbellata
(Fam. Convolvulaceae)

Mimosa pudica
(Fam. Mimosaceae)

Mirabilis jalapa
(Fam. Nyctaginaceae)

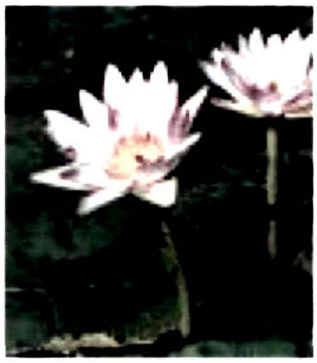
Momordica charantia
(Fam. Cucurbitaceae)

Morus australis
(Fam. Moraceae)



Mucuna pruriens
(Fam. Fabaceae)

Nelumbo nucifera
(Fam. Nelumbaceae)



Nymphaea nouchali
(Fam. Nympheaceae)

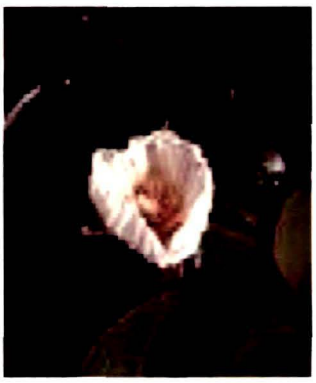
Plate 14

Nerium indicum
(Fam. Apocynaceae)

Ocimum basillicvum
(Fam. Lamiaceae)

Ocimum sanctum
(Fam. Lamiaceae)

Oldenlandia corymbosa
(Fam. Rubiaceae)



Ottelia alismoides
(Fam. Hydrocharitaceae)

Oxalis corniculata
(Fam. Oxalidaceae)

Phyllanthus fraternus
(Fam. Euphorbiaceae)

Phyllanthus virgatus
(Fam. Euphorbiaceae)

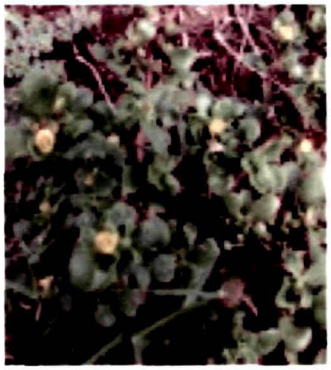
Physalis minima
(Fam. Solanaceae)

Plate 15

Plumbago rosea
(Fam. Plumbaginaceae)

Plumbago zeylanica
(Fam. Plumbaginaceae)

Plumeria rubra
(Fam. Apocynaceae)



Portulaca oleracea
(Fam. Portulacaceae)

Pouzolzia zeylanica
(Fam. Urticaceae)

Quisqualis indica
(Fam. Combretaceae)

Randia dumetorum
(Fam. Rubiaceae)

R icinus communis
(Fam. Euphorbiaceae)

Rauvolfia serpentina
(Fam. Apocynaceae)

Plate 16

Rhynchostaylis retusa
(Fam. Orchidaceae)

Sapindus mucrossi
(Fam. Sapindaceae)

Saraca asoka
(Fam. Caesalpiniaceae)

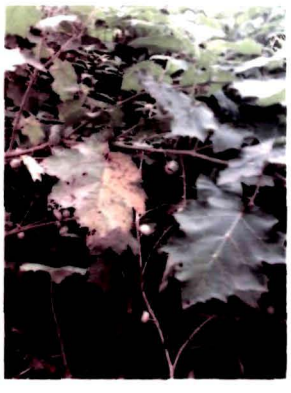
Saurupus androgynous
(Fam. Euphorbiaceae)

Scoparia dulcis
(Fam. Scrophulariaceae)

Sesbania grandiflora
(Fam. Fabaceae)

Sida acuta
(Fam. Malvaceae)

Solunum indicum
(Fam. Solanaceae)



Solanum myriocanthum
(Fam. Solanaceae)

Plate 17

Solanum nigrum
(Fam. Solanaceae)

Solanum torvum
(Fam. Solanaceae)

Spilanthus acmella
(Fam. Asteraceae)

Stephania japonica
(Fam. Menispermaceae)

Stellaria media
(Fam. Caryophyllaceae)

Syzygium cumini
(Fam. Myrtaceae)

Syzygium jambos
(Fam. Myrtaceae)

Tabernaemontana divaricata
(Fam. Apocynaceae)

Tamarindus indica
(Fam. Caesalpiniaceae)

Plate 18

Terminalia arjuna
(Fam. Combretaceae)

Terminalia bellerica
(Fam. Combretaceae)

Tinospora cordifolia
(Fam. Menispermaceae)

Triumfetta rhomboidea
(Fam. Tiliaceae)

Urena lobata
(Fam. Malvaceae)

Vitex negundo
(Fam. Verbenaceae)

Wedelia chinensis
(Fam. Asteraceae)

Withania somnifera
(Fam. Solanaceae)

Xanthium strumarium
(Fam. Asteraceae)

Plate 19