CHAPTER 1

INTRODUCTION

Medicinal plants are the local heritage with global importance. The World is endowed with a rich wealth of medicinal plants. Plants have always been the principal form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in concern with the body's own defenses. People in developed and developing nations are consulting trained herbal professionals and are using the plants for medicine. Medicinal plants also play an important role in the lives of rural people in the world.

The World Health Organization (WHO) estimates that 80 % of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of the traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as source of drugs (Fansworth, 1988). Further evidence of the importance of natural products is provided by the fact that almost half of the world's 25 best selling pharmaceuticals in 1991 were either natural products or their derivative (Neill *et al.,* 1993). Thirty percent of the world wide sales of drugs is based on natural products (Grabley and Thiericke, 1999).

1.1 HISTORY OF MEDICINAL PLANTS: A WORLD SCENARIO

In the second century BC trade of many medicinal and culinary herbs between the European, the Middle East and the Asian countries (India and others) had been established. Cloves (*Eugenia caryophyllata*) native of the Philipipines and the Molucca Islands near New Guinea, was imported into China in the 3rd century BC and arrived in Egypt around AD 176. As the century passed, cloves' popularity grew and

by the 8th century AD its strong aromatic flavor and powerful antiseptic and analgesic properties became well known throughout the Europe (Prajapati *et al*., 2003).

Herbs were central to the healing process in the European countries. By the 12th century, trade with Asia and Africa was expanding and new herbs were regularly imported to the European countries. Hildegard of Bingen (1098-1179) the famous German mystic and herbal authority, considered galangal (*Alpinia officinarum*), used in Asia as a warming and nourishing spice for the digestive system, to be the "spice of life", given by God for sound health and to prevent illness.

In Africa the therapeutic use of medicinal plants dates back to the earliest times. Ancient Egyptian writings confirm that herbal medicines have been valued in North Africa for millennia. The Ebers papyrus (c. 1500 BC), one of the oldest surviving medicinal texts includes over 870 prescriptions and formulae, 700 medicinal herbs – including gentian (*Gentiana lutea*), aloe (*Aloe vera*) and opium poppy (*Papaver somniferum*) and covers conditions ranging from chest complaints to crocodile bite. The medicinal arts put forward in this and other Egyptian texts formed the intellectual foundation of African medicine.

Australia is also considered as one of the homes of an ancient herbal tradition. The Aborigines, believed to have settled in Australia over 60,000 years ago, developed a sophisticated empirical understanding of indigenous plants, many of which ,such as eucalyptus *(Eucalyptus globules*) are unique to Australia. While much of this knowledge has vanished with its keepers, currently, there is 2 level of high interest in native herbal tradition.

In India, the use of medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. These systems of

medicine caters the needs of nearly 70 per cent of population residing in the villages. Apart from India, these systems of medicines are prevalent in Korea, China, Singapore, West Asia and many other Countries.

1.2 HISTORICAL OVERVIEW OF INDIAN SYSTEM OF MEDICINE

India has an ancient heritage of traditional medicine. Materia Medica of India provides plenty of information on the folklore practices and traditional aspects of therapeutically important natural products. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others. These traditional system of Indian medicine have their uniqueness. There is a common thread running through these systems in their fundamental principles and practices.

1.2.1 Ayurveda-The ancient traditional Medicine:

In Indian system of traditional Medicine, it is presumed that gods gave the knowledge of Ayurveda. It is accepted as the oldest written medical system that is supposed to be more effective in certain cases than modern therapies. The origin of Ayurveda has been lost in prehistoric antiquity, but its characteristics concepts appear to have been nurtured between 2500 and 500 BC in India (Mukherjee, 2001).

The word ‘Ayurveda’, derived from ‘Ayur’, means ‘life’ and ‘Veda’ means ‘science’. Thus Ayurveda literally means ‘science of life’.

Ayurveda has 8 branch which are called Asatanga Ayurveda as follows-

-Kaya Chikitsa (Medicine)

-Salya Chikitsa (Surgery)

-Bala Chikitsa (Pediatric treatment)

-Jara Chikitsa (Treatment related to Geratics)

-Rasayana Chikitsa (Treatment with chemicals)

-Vajikarama Chikitsa (Treatment with rejuvenation and aphrodisiacs)

-Graham Chikitsa (Planetary effects)

-Visha Chikitsa (Toxicology)

Out of these, “Kaya Chikitsa”, comprises 70 per cent of Ayurveda, and all remaining branches of the system are also rooted from “Kaya Chikitsa” (Mukherjee, 2001).

1.2.2. Siddha system of medicine

The word Siddha comes from ‘siddhi’ which means ‘attainment of perfection’. This system is almost akin to Ayurveda. It is an ancient traditional system of medicine developed by 18 siddhars who glorified human being as the highest form of birth and believed that preserving the human body is essential to achieving eternal bliss (Pillai, 1998).

The Siddha system believes that everything in the universe is made up of five basic elements - earth, water, fire, air and space which constitute the human body and other worldly substances. This system describes 96 principal constituents of human beings, which include physical, physiological, moral and intellectual components of individuals. When there is any imbalance or slight deviation with these 96 units, disease occurs. The diagnostic methodology in Siddha system is eight fold including examination of pulse, tongue, complexion, speech, palpatory findings etc. The Siddha medicine consists of psychosomatic system where attention is given to minerals and metals rather than plant constituents (Mukherjee, 2001).

Thus the formulation in Siddha medicine include the herbal products, inorganic substances and animal products and lead to different formulations like Chendooram-reddish powdered medicine, Choornam powdered drugs, Chunam-medicments prepared by calcinations (Pillai, 1998).

1.2.3. Unani system of medicine

The roots of this system go deep to the times of the well known Greek philosopher Hippocrates who is credited with it. Aristotle Golen (384-322 BC) a Greek- philosopher known as “Father of natural History” also made valuable contributions to it. This system of Greek origin was further carried to Persia (Iran) where it has been improved by Arabian physicians.

This system is based on two theories viz. the Hippocratic theory of four humors and the Pythagorian theory of four proximate qualities. The four humors are blood, phlegm, yellow bile and black bile while the four qualities are states of living human body like hot, cold, moist and dry (Kokate *et al*., 2003).

The Unani system of medicine aims at treating the cause of disease and not its symptoms. History of the patient is recorded in addition to his pulse, urine and stool examinations. The diseased condition is considered to be due to the imbalance between humours and accordingly treatment is given. The drugs are polyherbal formulation and their collective effect is considered (Kokate *et al*., 2003).

1.2.4. Homoeopathic System of Medicine

In comparison to other traditional systems of medicne, Homeopathy is a newer one and has been developed in the eighteenth century by Samuel Hahnemann – a

German physician and chemist. He proposed that the cause of disease itself can be used for its treatment. Hahnemann put forth the Law of Similars which say that like are cure by likes (*Similia similibus curentur*). With this principle, he showed that cincona can produce the symptoms of malaria. He succeeded in getting relevant results with a large number extracts prepared from plants, animals and mineral. He compiled all these observations in 'The Organon of Medine'.

In the homoeopathic system, the drug treatment is not specified, but the choice of drug depends on symptoms and the clinical condition of the patient. This is based on the concept of proving and prover. In a healthy person called prover, the symptoms created by different doses of drug extracts are noted which is called proving, and it specifically considers physical, mental and emotional changes of the prover. Consequently, these symptoms are compared with a patient with similar symptoms and accordingly, same type of extract is given for treatment. During the treatment, the drug extracts are extremely illuted, which is believed to cause potentiation and enhancement of curative effect. The drugs are extracted in the form of mother tinture, which is further diluted in terms of decimal or centesimal potencies. Various medicinal plant used in homoeopathy are *Nux vomica, Thuja occidentalis, Colchicum autumnale, Aconitum napellus,* etc. (Kokate *et al*., 2003).

1.2.5. Yoga and naturopathy

Yoga, which is rooted in Hindu religious principle, has been in practice for the last 5000 years. Derived from the Sanskrit word, Yoga meaning "union", it encompasses a variety of disciplines designed to ultimately bring its practitioner closer to god. 'Dynana yoga', for instance, seeks union through meditation, while 'Janana yoga' entails the study of scriptures and 'Karma yoga' calls for selfless

service to god and mankind. Yoga offers a significant variety of proven health benefits, even though it is not a cure for any medical ailment.

Yoga exercises increase the efficiency of the heart and slow the respiratory rate, improve fitness, lower blood pressure, promote relaxation, reduces stress, and anxiety. It also serves to improve coordination, posture, flexibility, range of motion, concentration, sleep and digestion. It is supplementary therapy for conditions such as cancer, diabetes, arthritis, asthma, migraine and AIDS. It also helps to combat addiction like smoking (Agrawal and Paridhavi, 2007).

1.3 HERBAL MEDICINE SCENARIO IN INDIA

India is a land of immense biodiversity in which two out of 18 hotspots of the world are located in India. India is also one of the 12 mega biodiversity centers having over 45,000 species of plants. Its diversity is unmatched due to the presence of 16 different agro climatic zones, 10 vegetative zones and 15 biotic provinces. The country has 15,000 to 18,000 flowering plants, 23,000 fungi, 2500 algae, 1600 lichens, 1800 bryophytes and 30 million micro-organisms. About 1500 plant species having medicinal properties are mentioned in ancient text and around 800 plants have been used in traditional medicines (Anon, 1998). The turn over of herbal medicines in India as over-the-counter products, ethical and classical formulations and home remedies of Ayurveda, Unani and Siddha systems of medicines is about US $ 1 Billion with a meager export of about US $ 80 million (Kamboj, 2000). It is generally estimated that over 6000 plants in India are used in medicine, folk and traditional medicines, representing about 75 per cent of the medicinal needs of the third world countries (Rajshekharan, 2002). India, with its traditional background, needs to increase its share in the world market.

Natural products remain in a profile source for the discovery of new drugs and drugs leads even from the Vedic period. Recent data suggest that 80 per cent drug molecules are natural products or natural compound inspired (Harvey, 2008). Studies on source of new drugs from 1987 to 2007 reveal that almost half of the drugs approved since 1994 are based on natural products (Butler, 2008). Indian natural products, particularly those from traditional medicinal plants which are reported in the classic texts like Charak Samhita and others books of Ayurveda system of medicine. The Ayurveda system of medicine has contributed towards this 'Boom' in drug discovery. The rich biodiversity of India has remained untouched as far as discovery of new chemical entities is concerned. The role of Indian medicinal plants in the global drugs discovery process, mainly in the disease areas like cardiovascular, metabolic, inflammatory, viral, parasitic and cancer are remarkable.

The traditional Indian system of medicine has a very long term history of usage in a number of diseases and disorders, but lacks recorded safety and efficacy data. However, the main cause for their scientific neglect is due to multi constituent mainstay and the mechanism of action being nuclear. But recently it has been suggested that drug discovery should not always be limited to discovery of a single molecule and the current belief 'One disease one drug' approach may be untenable in the future and that rationally designed polyherbal formulations could also be investigated as an alternative in multi-target therapeutic and prophylaxis (Patwardhan *et al.*, 2009). Development of standardized, safe and effective herbal formulations with proven scientific evidence can also provide an economical alternative in several disease areas. Still, some pro's and con's need attention for improvement of traditional Indian medicines.

1.4 FUTURE STRATEGY FOR MEDICINAL PLANTS

World Bank group have several projects to support the cultivation of medicinal plants through various lending and non lending initiatives. The World Bank is assisting the countries of South Asia to address these needs. Some of these are, the Kerala Forestry Project, the Sri Lanka Medicinal Plants Project, Ritigala Community Based Development and Environment Management Foundation, The India Capacity Building for Food and Drugs Quality Control Project, etc. There is a need to launch number of projects for arid region of India (Prajapati *et al*., 2003).

Although the World Bank has supported some pioneering work in the South Asian region related to medicinal plants or, natural resource management, much remains to be done. In the near future, it will be important to focus on management of medicinal plants and other non-timber forest products in natural resource management and also in other rural development programs.

1.5 PROSPECTIVE OF HERBAL MEDICINE IN MIZORAM

Mizoram is located in the extreme end of Himalayan ranges in the North eastern region of India. It is situated between 21^0 58$^{'}$and 24^0 35$^{'}$N latitudes and 92^0 16$^{'}$ E and $93^0$29$^{'}$ E longitudes and bounded by International boundaries of Myanmar in the East and South west and Bangladesh in South west and Tripura state in the West, and also bounded by Assam in the North and Manipur in the North East. The tropic of cancer passes through the southern periphery of Aizawl town (capital of Mizoram State) at $23^0$30$^{'}$N latitude.

The state of Mizoram has predominantly mountainous terrain of tertiary origin. The mountain ranges run in north to south direction, intercepted by narrow deep valleys and criss-crossed by innumerable small hillocks. The lowest portion is

20 m from sea level and highest peak is 2157 m from sea level, and the average height is about 1000m (Lalramnghinglova, 2003).

Mizoram enjoys a pleasant and moderate climate. The climatic condition is characterized by short winter and long summer with heavy rainfall.

The moderate climatic condition supports five forest types in Mizoram (Anon, 1999) :

i Eastern Himalayan Wet Temperate Forests
ii Cachar Tropical Semi-evergreen Forests
iii Assam Sub-tropical Pine Forests
iv Secondary Moist Bamboo Forests
v Montane Sub-tropical Forests

Medicinal plants diversity occupies a wide range of distribution from narrow ecological rich to diverse ecological systems due to presence of moderate climate throughout the year and rich plant species diversity in forests ecosystems.

Exploration survey of taxonomical plant wealth in Mizoram has certain drawbacks as evidenced by lack of medicinal information through botanical collections conducted by Gage 1889, Parry 1932, Fischer 1938 and Botanical Survey of India till 1989. Lack of scientific investigation on botanical medicines imbalances the rich heritage of ethno-biodiversity of the state. Documentation of local health traditions published by Zoram Upa Pawl 1984 (Mizo version) can be considered as the milestone secondary source of information in Mizoram. Local botanical collection was held since 1990 onwards and an actual ethnobotanical study was conducted since 1994 basically in the tropical wet evergreen forests and extended to the tropical moist deciduous forests, sub-tropical evergreen forests and partly wet temperate forests so far, about 200 medicinal plants have been reported from

Mizoram (Lalramnghinglova 1996 and 1999; Lalramnghinglova and Jha 1996; Lalnundanga *et al*., 1997; Lalramnghinglova and Jha 1997, Jha and Lalnundanga 1998 and Lalnundanga and Jha 2000; Lalramnghinglova 2000 and 2004).

Many species belonging to *Asteraceae, Menispermaceae, Apiaceae, Lamiaceae,* etc. exhibit open habitats as well as secondary successions. The species like *Lasia spinosa, Lasianthus wallichii, anacolossa cressipes, Homalomena* aromatic, etc. grow under dense forests; *Bergenia ciliata, Trapa natans, Pseudodrynaria coronans, Rhodendron arboretum*, etc. are habitat-specific or under restricted distributions, whereas the species like *Picrasma javanica, Dillenia pentagyna, Callicarpa arborea* etc. are distributed in different eco-climatic conditions. Herbceous plants like *Scoparia dulcis, Centella asiatica, Achyranthus aspera, Chromolaena odorata, Cassia tora, Eupatorium glandulosum etc.* grow near human settlements and in damp places. Some valuable medicinal plants survive in cultivation only, *e.g., Curcumorpha longiflora, Kaempferi tarotunda, Catharanthus roseus, Aloe bardadensis* etc. Majority of individual plants are used as herbal medicines for various purposes, some species are used in combination with others (Lalramnghinglova 2004).

1.6 THREAT STATUS OF RARE AND RECORD OF FEW ETHNO-MEDICINAL PLANTS

Factors leading to rate of rarity of species are estimated to be $1/yr^{12}$. The factors effecting rarity are *deterministic* or *man-made* activities, such as deforestation, habitat destruction, road construction, fire, commercial exploitation etc. and *stochastic* or *chance events*, such as natural catastrophes (flood, storm, landslides, earthquakes etc.), demographic variation in individual births and deaths, loss of genetic diversity and heterosis (Given, 1994). Shifting cultivation is the single largest

factor affecting bio-envirodegradation in the northeast India and Mizoram, in particular. De-forestation due to shifting cultivation during 1987-1997 was recorded as 3,800 km^2 or 38,000 ha (SFR, 1999).

Excessive collection of timber, fuel wood, food plants and commercial exploitation of medicinal plants accelerate a great deal of vulnerability to individual species or sub-populations. By nature, forests served as best bio-diversity habitats and best custodians of medicinal plants as they harbour 90% medicinal plants and 10% is attributed to non-forest areas (Lalramnghinglova, 2004).

Some 62 new ethnomedicinal plants have been reported for the first time in Mizoram as new records (Lalramnghinglova & Jha, 1999). New ethno-medicinal plants are understood as those plants whose uses were not known earlier nor reported in the major India medicinal plants literature.

1.7 PHARMACOGNOSY AND ITS STATUS

Herbal medicine, also called as phytomedicines, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medical purposes. These are standardized herbal preparations consisting of complex mixtures of one or more plants, which are used in most countries for management of various diseases. There are numerous herbal products available that claim to treat a wide range of problems. However, incorporation of the traditional medicines in to the healthcare system prior to systematic investigation, standardization and proper formulation may cause problems, such as inaccurate dosage, lack of proof of safety and efficacy and of interaction risk with modern drugs. Standardization and formulation of traditional herbs into modern phytopharmaceuticals shall provide the solution of most of these problems of traditional medicine (Kroll, 2001; Gogtay *et al.*, 2002).

Herbal medicines have the ability to affect body systems. The effects are dependent on the chemical constituents present in the plant used. Scientists first started extracting and isolating chemicals from plants in the 18th century, and since that time we have grown accustomed to looking at herbs and their effects in terms of the active constituents they contain. This Encyclopedia is no exception, providing details of all the active constituents of all the medicinal herbs featured and explaining their actions.

The flora on this earth representing an inexhaustible sources of medicinal plants remains and completely unexplored. The conventional rediscovery process aims to identify a single, pure active constituent from an active extract and a method to estimate it in the crude drug. The classical examples of drug discovery like morphine, quinine, dixogin, etc which replaced the extracts of their respective plants were mostly responsible for harbouring the idea that a single active ingredient must have been responsible for the bioactivity. The drawback of this ideology is that it does not look into the synergy or antagonism characteristic present in the mixture. Apart from this some constituents may also process other diverse activities. This factor is corroborate by several examples reported in literature where the described pharmacological activity of the extracts could not be matched with that of the isolated pure compounds. Most of the newer work on medicinal plants is mostly the rediscovery of effect known for a long period of time at cellular and molecular levels. The uncertainty of earlier studies is obvious in case of biological activity because of the lack of standardization techniques or instances where even if present were in a primitive state (Strachey, 1998). Pure and isolated plant constituents are of great importance because they have given various world's most useful drugs. For example, tubocurarine, the most powerful muscle relaxant in existence, is derived from curare

(*Chondrodendron tomentosum*), and the strongest painkiller of all, morphine comes from opium poppy (*Papaver somniferum*). Many anaesthetics are also derived from plants, for example cocaine, which comes from coca *(Erythroxylum coca*). It is hard to think of a world deprived of the antimalarial properties of quinine (derived from *Chincona);* or the heart remedy digoxin (from Digitalis) or the cough – relieving properties of ephedrine (from *Ephedra sinica*), which is present in many prescription and over – the – counter cold remedies. These and many other conventional medicines are all derived from isolated plant constituents. These are most effective of all conventional drugs (Prajapati *et al*., 2003).

The word 'pharmacognosy' had its debut in the early 19th century to designate the discipline related to medicinal plants; it is derived from two Greek words viz., 'pharmakon', 'a drug' and 'gignosco', 'to acquire the knowledge of' and, as recorded' by Dr. K.Ganzinger (sci. Pharm. 1982, 50, 351), the terms 'pharmacognosy' and 'pharmacodynamics' were probably first coined by Johann Adam Schmidt (1759 – 1809) in his hand written manuscript Lehrbuch der Materia Medica, which was posthumously, published in Vienna in 1811. After this publication, 'pharmacognosy' appears again in 1815 in a small work by Chr. Aenotheus Seydler entitled 'Analecta Pharmacognostica' (Trease and Evans, 2002).

Pharmacognosy may be defined as a branch of bioscience which treats in detail medicinal and related products of crude or primary type obtained from plant, animal and mineral origins. In short, Pharmacognosy is study of crude drugs from natural sources treated scientifically and it also encompasses the knowledge of the history, distribution, cultivation, collection, processing for market and preservation, the study of sensory, physical, chemical and structural characters and the uses of crude drugs. Pharmacognosy also includes study of other materials used in pharmacy

such as suspending, disintegrating and flavouring agents, filtering aids etc and substances like antibiotics, allergens, hallucinogens, poisons of plants and toxins of micro-organisms, immunizing agents, pesticides, steroidal materials for the production of oral contraceptives etc. (Kokate *et al.*, 2003).

The unexplored medicinal plants provide the most challenging aspect of pharmaceuticals and medicinal science to scientist in search of new potential plant species having medicinal properties. The medicinal plants have been explored and desired information are already documented in the State of Mizoram. Although studies on phytochemistry of medicinal plants of Mizoram state are in infancy stage. Lalnundanga (2000:Ph.D thesis unpublished, NEHU) studied phytochemistry of medicinal plants [(i) *Garcinia sopsopia* Mabb. (ii) *Mallotus roxburghianus* Muell-Arg and (iii) *Vitex peduncularis* Wall.] of Mizoram. His contribution may be recognized as pioneer in the Mizoram state.

The present study has been undertaken to screen out the medicinal plants having potential for phytochemistry studies in future and to identify one candidate medicinal plants for in depth phytochemistry study with below mentioned objectives :

1. Study the relative efficacy of the ethno medicinal plants based on its uses by the tribes of Mizoram.
2. To screen out the potential medicinal plants for biological screening**.**
3. Isolation and to characterize active biochemical compound present in the candidate ethno-medicinal plants.

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CHAPTER 2

REVIEW OF LITERATURE

The medicinal plants continue to receive attention of scientists from chemical, pharmacological and clinical angles in India and abroad. The studies on folk medicine through ethnobotanical survey are gaining importance. In discussing the role of ethnobotany in our search for new drug plants, we must constantly bear in mind the widespread exaggeration of the usefulness of ethnobotanical data. Nevertheless, we cannot afford to pre-judge reports of aboriginal uses of plants simply because they seem to fall beyond our limits of credence. Since primitive man does have some knowledge as yet unknown to us, there is no reason to suppose that man in primitive society possesses nothing more than a very limited intuition about the properties of plants. It, therefore, behooves us to push forward, along with ethnobotanical investigation, studies on the flora in general (Schultes 1962; Lalramnghinglova & Jha 1999).

2.1.ETHNO-BOTANICAL RESEARCHES FOR SCREENING OF POTENTIAL MEDICINAL PLANTS FOR PHYTOCHEMISTRY STUDIES.

The systematic study of medicinal plants under ethnobotanical researches by different workers which have been serving as sole source for identification of potential medicinal plants for phytochemistry studie**s**, are reviewed here.

2.1.1 Abroad

Ethnobotanical investigations on medicinal plants in ethnobotanically rich human societies have been undertaken by various ethnobotanists abroad. A few significant contributions are mentioned below.

The evolution of the modern approach to the science of ethnobotany started in the United States, and the foremost center is the Botanical Museum of Harvard University in Massachusetts. Ethnobotanists such as E.Wade Davis, Richard Gorden Wasson, Richard Evans Schultes, Sir Von Reis Altschul, Timothy Plowman, and others have contributed in various fields of ethnobotany from this center (Jha & Lalnundanga 1998). The best studied area for these purposes is the South-West of the United States of America (Ford, 1985).

El rayah (1993) documented a brief account of herbal medicines prescribed for various ailments in Sudan.

Barrett (1994) has investigated 152 plants used by the people of Nicaragua's Atlantic Coast for the treatment of various diseases. The diversity and prevalence of medicinal plant used for this region has been reported for the first time.

The traditional and modern uses of 48 native plants which grow in the First Yukon region, Alaska, have been documented and the medicinal and edible material used by the Guich in athabaskan and Caucasian residents have been identified. The present and past values of these plants in Guich's culture are discussed (Holloway & Alexander 1990). George (1995) has reported pharmacopoeia of 108 medicinal species from 52 families. Fifty percent of the pharmacopoeia is composed of species indigenous to Tonga, 30 percent of the species introduced by Polynesian settlers, and 20 percent are species of post – European introduction.

Joshi and Edington (1990) reported medicinal plantrs of Central Region of Nepal. Ethnobotanical observation on 7 plant species from Tharu tribe of Chitwan District, and 86 plant species from Makawanpur District of Nepal were reported by Dangol and Gurung (1991) and Bhattarai (1990), respectively.

Huyin *et al.,* (1998) have reported that *Baphicacanthus cusia* plays a very

important role in the traditional life of the Hani and other ethnic groups in Jinpin country, Yunnan province. Indigo obtained from aerial parts is used to dye their traditional clothes, and the roots and leaves are used as medicine.

2.1.2 India

The use of plants as medicine for human beings as well as animals in India dates back to the earliest times. Scripture of the Hindus *viz*. the *Rigveda* (4500 – 1600 BC), *Ayurveda*, the indigenous systems of medicine from the Vedic ages (1500 – 800 BC), has been an integral part of India culture (Lalramnghinglova & Jha 1999). The Vedic Aryans were familiar with medicinal plants. Several plants are described in the *Atharva Veda*. This was followed by monumental ancient treatise on the subject like *Charak Samhita* (1000 – 800 BC), *Sushrut Samhita* (800 – 700 BC) and *Vaghatta's Astanga Hridaya*. The Yunani system which originated in Greece in about 400 BC came to India, through Arab Physicians, who accompanied Mogul invaders, and came to be known as Yunani - Tibb. The Siddha system, with a record history from about 2000 BC, is believed to have originated from Lord Shiva and to have been passed on through his wife Parvati to a number of disciplines. Its use became common in Dravidian civilization. The texts of each of these three systems deal with herbs used in these systems only. Books in English, written usually include plants from all these systems (Jain, 1994).

The Indian system of herbal medicine had caught the attention of the west during the colonial days and since 1563, books on these have been published. The important contributions are : (a) *Colloquies on the Simples and Drugs of India* in 1563 by the personal physician of the then Portuguese Governor in India, (b) 12 volumes work on Kerala Medicinal Plants (1678 – 1703) from Amsterdam, (c) *A catalogue of Indian Medicinal Plants and Drugs* (Fleming 1810) and (d) *Materia*

Medica of Hindoostan (Ainslie, 1813).

Studies on ethnobotany in India was initiated by the economic botany section of Botanical Survey of India since 1954. Dr. E.K. Janki Ammal (1956) had published a paper on subsistence economy of India. Dr. S.K. Jain started intensive field studies among the tribals of Central India in 1960 and published a good number of papers on ethnobotany (Jain 1963 a-c; 1964 a-b; and 1965 a-b). The last two decades have been different works carried on to record information on different medicinal plants from different regions of the country (Jain & Mitra 1997). Mudgal and Pal (1987) gave a synoptic treatment on ethnobotanical works in India. Binu et al (1992) compiled an outline of ethnobotanical work carried in India. Ved Prakash (1998) reviewed status of Indian medicinal plants. Through the reports of ethnobotanists who had surveyed States and U.Ts of India, different medicinal plants prevalent in particular regions among different people of different states, and even some less well known medicinal plants have been recorded and informations provided.

Hemadri *et al*., (1987) recorded 211 species of the medicinal plants wealth of the state of Andhra Pradesh. The medicinal plants wealth of Karimnagar districts was documented by Hemadri (1990, 1991). Reddy *et al.,* (1989) investigated plant based crude drugs of Anantpur and Chitoor districts. They reported 64 plant drugs. Aruneekumar & Nisteswar (1990) documented 188 medicinal plant species of Kakinada district. The medicinal plants used for family planning and birth control is reported by Vedavathy *et al.,* (1991).

In Bihar State Boddings (1925 & 1927) documented Santhals taboos, medicines and folklore customs. The plants of ethnomedicinal value are reported by Srivastva and Verma (1981). The medicinal plants used by Santhals, Mundas, Orans, Birhors, Bedia of Chotanagpur plateau are reported by Tarafder (1983 a-b, 1984 a-b).

Jha *et al.,* (1989) reviewed folk medicine of Mithila zone of Bihar. Other important contributions of importance are ethnomedicinal plant species of 'Paaharia' tribe (Singh *et al.,* 1992); Sauri Paaharia tribe (Jha & Verma 1996); Santhal and Paharia tribes (Kaushal & Goel 1998) and Hazaribagh forest mines regions (Prasad *et al.,* 1998). Verma and Pandey (1990) reported 32 species having medicinal value from Lohardaga district.

The ethomedicinal information along with other uses of 133 plant species used by tribals of Saurashtra are recorded by Shah *et al*., (1981). The folk medicines of Dangs are reported by Joshi *et al.,* (1980). Shah and Gopal (1982) reported 145 plant species having medicinal uses.

Lal and Yadav (1983) recorded 69 species having medicinal importance and 66 prescriptions for therapeutic does were also mentioned. Medicinal application of each species was presented. Jain (1984) documented 26 medicinal plant species of Morni and Kabasar hills in Ambala district of Haryana.

The medicinal plants of Chamba Forest Division and Kangra Forest Division (Uhal valley) in Himachal Pradesh are recorded by Gupta (1964) and Uniyal and Chauhan (1971), respectively. 50 plant species having ethnomedicinal importance along with part of plants used and mode of administration of each species are reported by Kapahi (1990).

The medicinal plants used by Amchis of Ladakh have been recorded by Srivastva *et al., (1981).* Other important studies on medicinal plants are survey on the ethobotany of Kashmir Sind Valley (Dar *et al.,* 1983); herbal drugs traditionally used in Ladhakh (Uniyal & Issar 1988); traditionally important medicinal plants of Dudu Valley (Kapur 1991).

Important contributions on medicinal plants are medicobotany of Mysore (Rao

1977) cited by Binu *et al.,* 1992; medicobotany of Tumkur district (Yoganarsimhan *et al.,* 1982); folk medicine of Bangalore district (Pushpalata *et al.,* 1990); ethobotany of Soligas in Biligiri Betta (Hosagaudar & Henry 1996) and the ethnobotany of Gowlis of Uttara Kannada district (Bhandary *et al.,* 1996).

The ethomedicinal investigations containing information about the medicinal plants in Kerala are reported by Mooss (1952, 1976 & 1978); Kolammal (1979); Manilal (1981); Ramachandran and Nair (1981), Nambiar *et al.,* (1986) and Sivarajan and Balachandran (1994) and Radhakrishnan *et al.,* (1996).

Pandey *et al.,* (1991) reported folk medicine of Baiga tribes and mentioned medicinal uses of 25 species. The flowering plants (233 numbers) of high medicinal value of the state recorded by Oommachan and Masih (1991). The medicinal plants commonly used by 'Sahariya' tribe recorded by Jain (1992).

Ethnomedico-botany of some sacred plants of Western Maharashtra (Upadhye *et al.,* 1997) and ethnomedico-botany of genus *Mucuna* from Western Maharashtra (Upadhye *et al.,* 1997).

Ethnomedicine of Bhills in Rajasthan : plants used in diarrhoea (Khandelwal 1998); ethnomedicinal observations from certain watershed areas of Rajasthan (Katewa & Sharma 1988).

Important contributions are native medicine of Jaunsari tribe (Singh 1997); ethno-medico-botanical studies on the fungi of Kumaun Himalaya (Joshi *et al.,* 1997) and native plant remedies for liver disorder among the tribals of Uttar Pradesh (Singh & Prakash 1998).

2.1.3 North Eastern States

The ethnomedicinal studies have been recorded and reports and information

on the medicinal plants employed by the people of these regions have been provided by the various ethnobotanists.

Some of the notable works are medicinal plants used by the Karbi Anglong of Mikir hills (Borthakur 1976); ethnomedicinal surveys of Miris (Hajra & Baishya 1981); medicinal plants from Tezpur (Puri 1987); ethnobotany of Miris and Mishings of Assam (Borthakur 1996); herbal remedies of the Nepalese of Assam (Borthakur *et al.*, 1996) and plant used to cure jaundice in Golaghat district (Pandey *et al.*, 1996).

The most relevant contributions are medicinal plants of Arunachal Pradesh (Hajra 1977); ethnomedicinal plants of Tirap district (Tiwari *et al.*, 1978); plants used by the Monpas tribes of Kameng district (Dam & Hajra 1981); ethnobiological records on 171 plant species of lower Subansiri district (Gangwar & Ramakrishnan 1990); medicinal plants of Lohit district (Bhuyan 1989); medicinal plants of the tropical, sub-tropical and temperature regions of Siang, Subansiri and Tirap districts (Tiwari and Tiwari 1996) and notes on the ethnobotany of the Monpa tribe of Tawwang district (Rawat *et al.*, 1997).

Some of the important contributions are : medicinal uses of 36 species used by Naga tribes in Ukhrul district (Elangbam *et al.*, 1989). Ehtnobotanical uses of 931 medicinal plants (Sinha 1987); folk medicines used to cure twenty five diseases by the Manipuris (Sinha 1990). Singh (1996) reported aphrodisical plants used by Meitei community. Medico-botany of Meitei community in Manipur state is recorded by (Singh & Huidrom 1997).

The main contributions are : ethnobotany of Khasi and Jaintia tribes (Joseph & Kharkongor 1981); ethnobotany of Khasi and Garo (Rao & Neogi 1980) and medicinal plants used by Garo (Rao and Shampru 1997; Rao 1989).

Some of the notable works are medicinal plants used by Nagas (Rao & Jamir

1982 a, b); medicinal plant species used by the Angamis of Kohima district (Megoneitso & Rao 1983); medicinal plants used by Zealang sub-tribes (Jamir & Rao 1990); ethnobotany of the Ao and Angami Nagas (Rao & Jamir 1990) and the medicinal herbs utilized by the Naga tribes (Jamir 1997).

The contributions made by Hajra and Chakraborty (1982), Bennet (1983) and Uniyal (1980) in the field of ethnomedicinal plants are important.

Deb (1968) has recorded medicinal plants of Tripura. Ethnomedico-botanical studies in Tripura reported by Singh *et al.*, (1997).

2.1.4 Mizoram

In the state of Mizoram, scientific researches on ethnobotany had been properly carried out. Although, phytochemistry studies of medicinal plants are still at its infant stage. Mention was made by Lorrain (1940) about few traditional medicinal plants, after which some diseases ailments along with medicinal treatments from plants were mentioned by Irish (1975) and Thangchuanga (1979). The “Zoram Upa Pawl Thurawn Bu” (Anon, 1984) documented a total of 228 cases of human diseases and 27 animal diseases along with herbal medicine used for their treatments. This document may be regarded as spearheading medicinal survey in the state of Mizoram.

Herbal medicine used for treatment of 97 diseases has been reported by Darlianthanga (1989). Saptawna (1990) had reported 58 plant species used as medicine whereas Lallianthanga (1990) reported 128 ethnomedicinal plants. Vailinga (1991) also documented 165 diseases and their ethnomedicine. Lalramnghinglova (1991) documented 437 plant species on the basis of the secondary information.

Some of the notable contributions (based upon actual ethnobotanical survey)

have been made by Lalramnghinglova (1996); Lalramnghinglova and Jha (1996); Lalnundanga *et al*., (1997); Lalramnghinglova and Jha (1997), Jha and Lalnundanga (1998) and Lalnundanga and Jha (2000) Lalramnghinglova (2004),and Sawmliana (2003).

2.2 ETHNO-PHYTOCHEMICAL STUDIES OF POTENTIAL MEDICINAL PLANTS

2.2.1 Abroad

The phytochemical analysis of ethnomedicinal plants to see their physiological effectiveness is the need of the day. This analysis may result in the discovery of new chemical compounds and drugs for modern medicine. There is a vast emporium of unknown chemical compound awaiting discovery from the flora which is yet to be studied. A few significant contribution on phytochemical analysis of medicinal plants are antibacterial substance in seed plants against *Tubercle bacilli* (Gottshale *et al.,* 1950); constituents from Muira-Puama; the roots of *Ptychopetalum olacoides* (Ito *et al.,* 1995); antifungal properties of the leaf oils (Zygadlo *et al.,* 1994); antifungal activity of dihydrodiosocrine extracts from *Dioscorea bulbifera* (Adeleye & Ikotum 1989), antiasthmatic effect of onion extracts (Dorsch *et al.,* 1985); anti-asthmatic principles of *Allium cepa* (Wagner *et al.,* 1988); phytochemical analysis of Aloe vera (Rowe & Parks 1941), screening of anti-radical, anti-lipoperoxidant and hepatoprotective effects of plants extract (Joyeur *et al.,* 1995); identification of Melatonin and its effect on plasma melatonin levels (Hattori 1995); screening of Diphenylamine (Karawva 1986); determination of 1, 8-dihydroxyanthracine derivatives in vegetable drugs (Zwaving & Elama 1976); biologically active substance of *Allium* species (Pobozsny *et al.,* 1979; Wagner *et al.,* 1988). Herz *et al.,* (1981)

have reported presence of Damsinic acid and Ambrosonolids from *Ambrosic hispida*. Quantitative analysis of Garlic essential oil (Bekdairava & Klysher 1982). Oxalate content of some leafy vegetables (Gad *et al.,* 1982). Haq and Hannan (1981) analysed leaves of *Aloe vera* and reported presence of Glucogalacto-nannan. The cancer cell growth inhibitory constituents of *Terminalia arjuna* (Pettit *et al.,* 1996); anti-inflammatory and anti-arthritic properties of Terminalia ivorensis (Iwu & Anyanwu 1982); effect of amino acid in *Aloe* extract (Yagi *et al.,* 1987); phytochemical analysis of Terminalia catappa (Diyabalanoya *et al.,* 1997); natural products as inhibitors of Human Immuno Deficiency Virus Type I (Tan *et al.,* 1991); pharmacological screening of plant decoctions used in Cuban folk medicine (Carbajal *et al.,* 1991) and Chebulanin from *Terminalia chebula* as an anticancer agents. (Tokura & Kagawa 1993); Ross (1999) has reported 26 common medicinal plant of the world their chemical constituents and pharmacological activities.

Glabresin, a daphnane diterpenoid, neoboutonin, a degraded diterpenoid with a novel skeleton and neoglabresins A and B two rhamnofolane derivatives have been isolated from the stem and bark of *Neoboutunia glabrescens*. (Tchinda *et al*., 2003).

Four new oleanane-type saponins and a known one were isolated from the leaves and stem of *Meryta lanceolata* (Melek *et al*., 2003).

Four triterpene saponins, agrostemmosides A-D were isolated from the methanol extract of *Agrostemma gracilus*. (Koz *et al.,* 2010).

The principal components of *Centella asiatica* are a bitter principle vallerin and a mixture of triterpenoid saponins, the most active of which is asiaticoside (Trease, 2002).

In phytochemical analysis of extracts of *Echinacea angustifolia*, assays for

caffeic acid derivatives revealed that 60% ethanol extract contained 0.16 mg per milliliter of cynarine; however echinacoside was not detectable in any extract (Turner *et al.,* 2005).

Two pentacyclic triterpenes, D-friedomadeir-14-en-3 β-yl acetate and D; C friedomadeir 7-en-3β-yl acetate, named madeiranyl acetate and isomadeiranyl acetate respectively,were isolated from the leaves of *Euphorbia stygiana* (Lima *et al.,* 2002).

Seven oloenane-type saponins were isolated from the leaves and stems of *Oreapanax guatemalensis*, together with known saponins of lupine and oleanane type (Melek *et al.,* 2002).

Eight Isoquinone alkaloids- tetrahydropalmatine, isocorypalmine, stylopine, corydaline, columbamine, coptisine, 13-methylpalmatine and dehydrocorybulbine was isolated from *Corydalis yanhusuo* (Zhong-Ze, 2008).

Five triterpene never reported before, hederifoliosides A-E and four known triterpene saponins were isolated from *Cyclamen hederifolium* (Altunkeyik *et al.,* 2011).

The aqueous and ethanol extract of the leaves of *Cymbopogon citrates* showed antimicrobial activity against different microbes (Oloyede, 2009). The extract of leaves showed more antibacterial activities than the extracts of fruits in *Ficus auriculata* Lour (El-Fishawy, 2011).

2.2.2 India

A few significant phytochemical contributions are (i) flavone from *Terminalia arjuna* (Nagar *et al*., 1979); (ii) a new potential antitumor alkaloid from *Tylophora asthmatica* (Mulchandani *et al.*, 1971); (iii) a calcium oxalate as a source of oxalic acid in barks of seven species of *Terminalia* (Bharadwaj & Chandra 1983);

(iv) antiasthmatic principles of *Allium cepa* (Handa *et al.,* 1983). (v) Gallic acid from Myrobalans (Grampurohit 1986); (vi) Triterpenoids and their Glucosides from *Terminalia bellirica* (Nandy *et al.,* 1989); (vii) hexahydroxydiphenic acid ester in *Terminalia bellirica* fruits (Ali & Bhutani 1991). (viii) Pentacyclic triterpenoid sapogenols and their glycosides from *Terminalia bellirica* (Mahato *et al.,* 1992); (ix) Alkaloid, antraquinone glycoside, saponins, flavonoids, polysaccharides, Steroid, Tannin in the fruits of *Terminalia bellirica* (Meena *et al.,* 2010); (x)a triterpene glycoside from *Terminalia arjuna* (Tripathi *et al.*, 1992); (xi) triterpenoid constituents of the seed of *Diospyros melanoxylon, Tecomella undulata* and *Terminalia bellirica* (Singh & Sharma 1997) and (xii) a tannin anti-cancer promoter from *Terminalia arjuna* (Kandil & Nassar 1998).

The phytochemical analysis of ethanolic leaf extract of *Phyllanthus amarus* showed the presence of alkaloids, cyanogenic glycosides, saponins, tannins and oxalates (Adegoke *et al.,* 2010).

The chemical constituents of the methanol extract of *Eupatorium edenophorum* were investigated. From preliminary phytochemical analysis, it was found that the extracts showed the presence of flavonoids, tannins, steroids, triterpenoids, gums and reducing sugars (Mandal *et al.,* 2005).

Passiflora quadrangularis contains alkaloids, glycosides and flavonoids. It is used as antibacterial, antialgal and antihypertensive drug (Dhanabal *et al.,* 2005). *Ocimum sanctum* contain bright yellow volatile oil which contains eugenol 70% as major constituents (Jha *et al.,* 2005).

The five new phyto constituents *viz.* n-Docosane, henicos-11-ene-8-one, stigmast-5-ene-3β, 4α-diol, stigmast-5-ene-3β-benzylol-12β-ol and n-entacos-4 ene-3-one-18, 23-diol were isolated from the flowers of *Hibiscus rosasinensis* (Siddiqui *et*

al., 2006).

Phytochemical analysis of *Crataeva nurvala* leaves resulted in the isolation of compounds like dedaconoic anhydride, methyl pentacosanoate, kaempferol-3-O-α-D-glucoside and quercitin-3-O-α-D-glucoside (Gagandeep *et al.,* 2006).

Isolation of isomeric furocoumarins from the seeds of *Psoralea corylifolia* is being reported from the variety found in western Rajasthan they have been identified as 2H-furo [3', 2'-g] [1] benzopyran –2- one (1) and 2h-furo [2',3'-h] [1-] benzopyran –2- one (2) (Pramilla *et al*., 2006).

A new flavone glycosides, echioidinin 5-glucoside along with its known aglycone, echioidinin have been isolated from the whole plant of *Andrographis alata* (Damu *et al*., 1998).

The lignans phyllanthin and hypophyllanthin were reported from *Phyllanthus niruri* and the former is responsible for hepatoprotective activity of plant (Aeri *et al.,* 2005).

Syzygium aromaticum contain volatile oil, fixed oil, a peculiar tannin, gum, resin, fibre, water and two crystalline principles called caryophyllin and eugenin (Jha, 2007). The phytochemical study of *Ocimum sanctum* shows the presence of Alkaloids, glycosides, steroids and tannins. *Eugenia caryophyllata* shows the presence of Glycosides, tannins and reducing sugars. *Achyranthes bidentata* show the presence of alkaloids, glycosides terpenoids and steroids and reducing sugars. *Azadirachta indica* shows the presence of alkaloids, glycosides, terpenoids and steroids and tannins (Joshi *et al.*, 2011).

The study of antimicrobial activity of *Amomum subulatum* Roxb revealed that

this plant possessed broad spectrum anti microbial activity (Agnihotri, 2010). The study of ethanol extract of *andrograhis paniculata* showed antimicrobial activity (Mishra *et al.*, 2009). Jain *et al.*, (2010) worked on anti bacterial activity of important arid zone plants of Rajasthan. Muanda *et al.*, (2011) also worked on anti microbial activities of *Desmodium adscendens* leaves.

The supercritical fluid extract, steam distilled oil and petroleum ether extract have superior antibacterial activity to that of the ethanolic extract of leaves of *Vitex negundo* Linn.(Nagarsekar *et al.,* 2010). Berberine an isoquinoline alkaloid, present in the roots and the stem bark of Berberine species showed antimicrobial activity (Singh *et al*., 2010).

2.2.3 Mizoram

Garcinia sopsopia contains, terpenoids and polyphenols, *Mallotu*s *roxburghianus* contain flavonoid, *Vitex peduncularis* contains sitosterol and glucoside (Lalnundanga, 2000).

A study of *Mallotus roxburghianus* shows the presence of alkaloids which is having an IUPAC name of 1-(1-(pyrrolidine-1-carbonyl)pyrrolidine-3-yl) - dihydropyrimidine-2,4 (1H,3H) - dione (Lalhlenmawia, 2008).

Three different compounds compound A, B and C were isolated from *Melothria heterophylla* they were identified by comparing their melting points, UV, IR, Mass and NMR spectral datas from the literatures. The compounds were found to be dicotyl phthalate (di-(2-ethylhexyl)phthalate), apigenine-7-O-glucoside (2-(4-hydroxy-phenyl) -5-hydroxy-7- (3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy) -chromen-4-one) and luteolin 7-O-glucoside (2-(3, 4-Dihydroxy-phenyl)-5)-hydroxy-7- (3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-chromen-

4-one) respectively. The molecular formula and molecular weight of the three compounds were found to be $C_{24}H_{38}O_4$, $C_{21}H_{20}O_{10}$, $C_{21}H_{22}O_{11}$ and 390,432 and 448 respectively (Lalhriatpuii, 2010).

The anti-microbial activity of *Syzigium cumini* Linn. by using disc diffusion method was reported (Thanzami *et al.*, 2011).

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CHAPTER 3

METHODOLOGY

3.1 LITERATURE SURVEY OF ETHNO-BOTANICAL RESEARCHES FOR SCREENING OF POTENTIAL MEDICINAL PLANTS FOR PHYTOCHEMICAL STUDIES

All relevant literature will be studied to identify a set of 50 plant species having ethnomedicinal value .The natural habitat will be surveyed for verification of place of distribution and thereafter ,medicinal uses will be confirmed by interviewing villagers and herbal practioners.Five percent population of the study area will be surveyed and all 50 etnobotanical plant species would be ranked bases on score obtained by each ethnomedicinal plants. And out of 50 etnomedicinal plants top 20 will be identified as potential medicinal plants for phytochemial analysis in near future. Out of 20 ethnomedicinal plant, one will be considered as candidate etnomedicinal plant for in depth study of habitat, taxonomy, uses and phytochemical analysis.

3.2 COLLECTION AND IDENTIFICATION OF PLANT

The potential ethno medicinal plants will be collected identified and ranked. Before processing for phytochemical analysis, it is necessary to authenticate the potential ethno-medicinal plant. Identification will be carried with the help of taxonomist, Mizoram University, and with the help of floras such as : (i) *Forest flora of British Burma* (Kurz 1877), (ii) *A Botanical Tour in the South Lushai Hills* (Gage 1889), (iii) *Indian Trees* (Brandis 1906), (iv) *Flora of Assam* (Kanjilal *et al.*, 1934 – 1940 ; Bor 1940), (v) *Flora of Lushai Hills* (Fischer 1938), (vi) *Flora of Tripura State* (Deb 1981 & 1983), (vii) *Forest Flora of Meghalaya (*Haridasan & Rao 1985 &

1987) and (viii) *Flora of Mizoram Vol I* (Singh *et al.,* 2002) (ix) *The Book Of Mizoram Plants* (Sawmliana 2003).

Besides these floras , in order to match the specimens for confirmation and to identify the unidentified species, plant specimens were taken to the Botanical Survey of India (Kolkata) and Botanical Survey of India (Eastern Circle), Shillong. The local herbarium of parent Department had also been consulted for confirmation and identification.

3.3 PHYTOCHEMICAL ANALYSIS

The Phytochemical investigation of plant involves different technique like extraction of plant material, separation and isolation of target chemical compounds and characterization of isolated compound.

The fresh plant tissues are considered most ideal stock for phytochemical analysis. The plant material under investigation should be plunged into boiling alcohol within minute of collection. Alternatively, plants may be dried before extraction under controlled conditions or in shade to avoid occurrence of any chemical changes. It should be dried as quickly as possible, without using high temperature preferably in a good air draft. Indeed analysis of flavonoids, steroids, alkaloids, quinines and terpenoids has been successfully carried out on herbarium plant tissue dating back many years.

The plant tissue free from contamination (with other plants or its part) and diseases (free from viral, bacterial or fungal infection) are selected. Infected plants may seriously alter plant metabolism and unexpected products may be found. Contamination may also occur while collecting lower part of plant material for analysis. Sometimes, parasitic fungi contaminate the tree tissues. Mosses often grow in close association with the higher plants and it is sometimes difficult to obtain plant

samples free from contamination or infections.

The collected plant sample will be washed with distilled water and dried in ventilated room under shade, pulverized by grinder and passed through 60-mesh sieve to get the fine powder.

3.3.1 Extraction

The extraction is done by Soxhlet apparatus using methanol as a solvent. The extraction is done for forty (40) hours. The extracts are evaporated to obtain a dark-brown coloured semi solid mass. The concentrated extracts are refrigerated at 4˚C. Thereafter extracts are subjected to preliminary phytochemical analysis to identify the presence of phytoconstituents.

3.3.2 Preliminary phytochemical analysis of plant extract

Preliminary phytochemical group testing of the crude extracts is carried to observe the presence of the following chemical constituents:

i) Alkaloids
ii) Flavonoids
iii) Steroids and triterpenoids
iv) Amino acids
v) Reducing sugars
vi) Tannins
vii) Saponins
viii) Gums

3.3.3 Reagents used for the different chemical group tests (Kokate, 1994).

i) *Mayer's reagent:* 1.36gm of mercuric iodide in 60ml of water

mixed with a solution which contains 5gm of potassium iodide in 20ml of water.

ii) *Liberman-Burchard reagent*: 5gm of acetic anhydride is carefully mixed under cooling with 5ml of concentrated sulfuric acid, this mixture is added continuously to 50ml of absolute ethanol under cooling condition.

iii) *Dragendroff's reagent*: 1.7gm basis bismuth nitrate and 20gm tartaric acid are dissolved in 80ml of water. This solution is mixed with a solution containing 16gm potassium iodide and 40ml of water.

iv) *Fehling's solution A*: 34.64gm copper sulphate is dissolved in a mixture of 0.5ml of sulphuric acid and water is added to produce 500ml.

v) *Fehling's solution B*: 176gm of sodium potassium tartarate and 77gm of NaOH are dissolved in sufficient water to produce 500ml. Equal volumes of above solutions are mixed at the time of use.

vi) *Benedict's reagent*: 1.73gm of cupric sulphate, 1.73gm of solution citrate and 10gm anhydrous sodium carbonate are dissolved in water and the volume is made up to 100ml with water.

vii) *Molish's reagent*: 2.5gm of pure a-naphthol is dissolved in 25ml of ethanol.

3.3.4 Phytochemical group test : (Kokate,1994; Trease and Evans, 1972 and Ali 1998).

A. Tests for alkaloids

1. **Mayer's test for alkaloids**: 1 to 2ml of the extract is treated with 0.2ml of dilute hydrochloric acid and filtered. The filtrate was treated with 0.1ml of Mayer's reagent. Formation of yellowish buff colored precipitate indicates the presence of alkaloid.

2. **Dragendorff's test for alkaloids**: 2ml solution of the plant extract is added with 0.1ml of dilute hydrochloric acid and filtered. The filtrate is treated with 0.1ml of Dragendorff's reagent .Development of orange brown precipitate indicates the presence of alkaloid.

3. **Wagner's test for alkaloids**: 2ml solution of the plant extract is treated with few drops of dilute hydrochloric acid and filtered. The filtrate is treated with 0.1ml of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

4. **Hager's test for alkaloids**: 2ml of the plant extract is treated with 0.2ml of dilute hydrochloric acid and filtered. The filtrate is treated with 1ml of Hager's reagent. A yellowish precipitate indicates the presence of alkaloids.

B. Tests for flavonoids

1. 1. 5 ml of extract solution is hydrolyzed with 10% v/v Sulphuric acid and cooled. Then extracted with diethyl ether and divided into three portions in three separated test tubes. 1ml of dilute ammonia,1ml of dilute sodium carbonate and 1ml of 0.1(M)sodium hydroxide added to the first, second and third test tubes respectively. In each test tubes development of yellow colour indicates the presence of flavonoids.

2. The extract is dissolved in alcohol. One piece of magnesium followed by concentrated hydrochloric acid was added drop wise to that and heated (Shinoda's test). Appearance of magenta colour indicates the presence of flavonoids.

C. Tests for steroids and triterpenoids

1. Salkowski test: Concentrated Sulphuric acid is added to the chloroform solution of the extract of the plant (10 mg of extract in 1ml of

chloroform), a reddish-blue color in the chloroform layer and green fluorescence in acid layer indicates the presence of steroids.

2. Libermann- Burchard reaction: 10 mg of the extract of the plant is dissolved in 1ml of chloroform; 1ml of acetic anhydride is added to the mixture followed by 2ml of concentrated sulphuric acid. Presence of a reddish violet ring at the junction of the two layers indicates the presence of triterpenoids and steroids.

D. Tests for amino acid

Small quantities of the extract of the plant are dissolved in a few ml of distilled water and treated with Ninhydrin at the pH range of 4-8. The development of purple colouration suggests the presence of amino acids.

E. Tests for reducing sugars

1. 5ml of the plant extract solution is mixed with 5ml of Benedict's reagent in a test tube and --heated for a few minutes. Formation of a brick red color precipitate confirmes the presence of reducing sugars.

2. 5ml of the plant extract solution is mixed with 5ml of Benedict's reagent in a test tube and heated for a few minutes. Development of a brick red color precipitate confirms the presence of reducing sugars.

F. Tests for tannins

1. 5ml of the extract solution is allowed to react with 1ml of 5% ferric chloride solution. Greenish black coloration indicate the presence of tannins.

2. 5ml of the extract is treated with 1 ml of 10 % aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggested the presence of tannins.

3. 5ml of the extract is treated with 1ml of 10% lead acetate solution in water. Yellow color precipitation gave the test for tannins.

G. Tests for saponins

1. 1ml solution of the extract is diluted with distilled water to 20ml and shaken in a graduated cylinder for 25 min. development of stable foam suggestes the presence of saponins.

2. 1ml extract is treated with 1% lead acetate solution. Formation of white precipitate indicates the presence of saponins.

H. Tests for gums :

2ml of concentrated sulphuric acid is added to 2ml of extract solution. Then it is treated with 15% α-naphthol in ethanol (Molisch's reagent) Formation of red violet ring at the junction of two layers indicates the positive test for gums (Molisch's test).

3.4 ISOLATION OF THE ACTIVE CONSTITUENTS

3.4.1 Thin Layer Chromatography Study

3.4.1.1 Introduction :

Thin layer chromatography (TLC) is one of the universal analytical technique in chemical analysis for organic and inorganic matter. It is an open bed techniques in which substances are separated by the differential migration that occurs when a solvent flows along a fine powder spread on a glass or plastic plate. The principle of separation is adsorption. The components are separated based on the affinity towards the stationary phase. One or more compound are spotted on a thin layer of adsorbent coated on a chromatographic plate. The compound travels to different distances along with the mobile phase depending on the Partition coefficient of each molecules.

The substance most frequently used as a coating materials are silica gel, alumina and cellulose and to give stable layers they often contained binders such as calcium sulphate (gypsum) or starch

The different solvents are like petroleum ether, cyclohexane, carbon tetrachloride, benzene, chloroform, diethylether, ethylacetate, pyridine, acetone, alcohol, water, dichloromethane, organic acids, mixture of acids or bases with ethanol (Stahl, 1969).

3.4.1.2Preparation of Plates :

Readymade plate made of aluminium sheet and silica gel (Merck & Co. Ltd.) are used. The plate is cut in to 3X10 cm .The plates are activated at 120˚ C for 30 minutes (Stahl, 1969).

3.4.1.3 Development of Chromatogram

2-5 µl of the material extract is spotted on the plates with the help of capillary tube. The spot is kept 1cm above the bottom of the plates. The development chamber is pre-saturated by using the respective solvent systems to avoid edging or tailing effect where the solvent front in the middle of the TLC plate moves faster than that of the edge. The chromatography plate is placed at an angle of 70%.The solvent front is allowed to rise to a distance of about $3/4^{th}$ from the base line and the plate is removed from the chamber and allowed to dry in the air. The plate is then kept in Iodine chamber. Since the rate of migration of compound on a given adsorbent depends upon the solvent used, the solvent system is arranged in order of elutive power (Skoog, 1988; Fried *et al.*,1994). Mixtures of two or three solvents of different polarities gave better separation than the chemically homogenous solvent. By several trial and error methods suitable solvent system is selected for thin layer chromatography.

3.4.1.4 Solvent system :

The choice of the mobile phase depends on the nature of substance to be separated and the adsorbent material to be used. It is preferable to use an organic solvent mixture of as much low polarity as possible. Polarity of solvents and

substance to be separated play an important role in selection. Highly polar solvent are generally avoided to minimize adsorption of any component of the solvent mixture. While selecting a solvent “eluotropic series” is consulted. The soluble mixing gives mobile phases of intermediate cluting power.

The following solvent systems in varying proportions are used on trial and error basis as shown in Table No. 3.

1.Chloroform : Ethyl acetate

2. Petroleum ether : chloroform

3. Chloroform : Ethyl acetate : Methanol

4. Dichloromethane : Methanol

5. Chloroform : Methanol

6. Dichloromethane : Acetone

7. Dichloromethane : Ethyl acetate

8. Dichloromethane : Benzene : Acetone

9. Chloroform : Acetone

3.4.2 Column Chromatography

3.4.2.1 Introduction :

The term Column Chromatography is used to those methods in which the separation takes place within a packed column. The packing material is a stationary phase and is solid with adsorptive or exclusion capabilities. A liquid mobile phase is used as the effluent. In 1941 Martin and Synge developed a liquid chromatographic process in which they used as a packed column containing water saturated with silica gel and a mobile phase of butanol and chloroform (Agrawal and Paridhavi, 2007).

3.4.2.2 Column :

A glass column of about 3.5 cm internal diameter and 45 cm in length fitted with stopcock is used.

3.4.2.3 Adsorbent used

Silica Gel 60-120 mesh size (E. Merk & Co. Ltd.) is activated by heating at 120°C for one hour and is used as stationary phase in the column.

3.4.2.4 Solvent System used

Dichloromethane : Methanol (9:1, 8:2, 7:3)

3.4.2.5 Preparation of column :

The bottom portion of the column is plugged with cotton wool above which the column of adsorbent using silica gel (60 – 120 mesh) packed. The column is packed using wet packed technique. The stationary phase settles uniformly in the column and there is no entrapment of air bubbles (Lala, 1981). After packing the column, it is kept undisturbed for at least 2 hr.

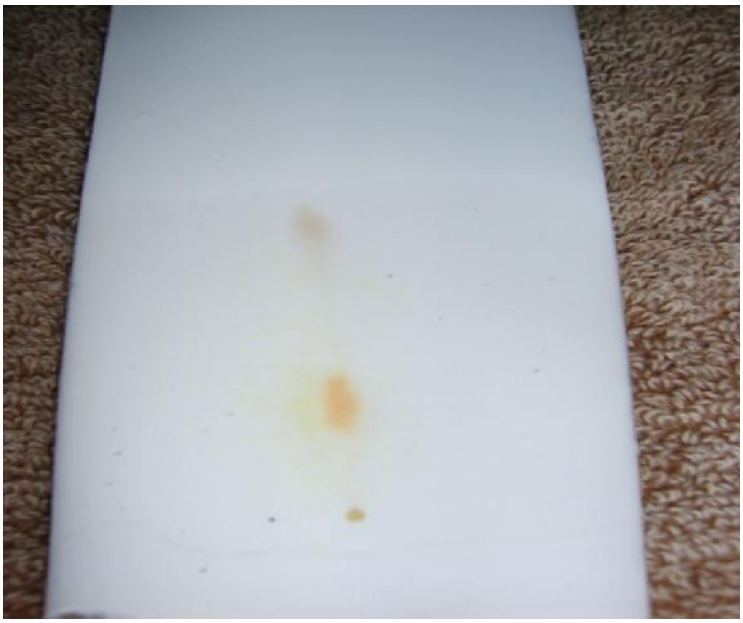

Photo 1: Photograph of TLC

Photo 2 : Photograph of Column Chromatography

3.4.2.6 Separation and Isolation by column chromatography :

10g of crude methanol extract is dissolved in a minimum amount of methanol, adsorbed with activated silica gel (60 - 120 mesh) to form a slurry, air dries till free flowing and pack over the layer of silica gel inside the column and eluted successively with dichloromethane and methanol of increasing order of polarity (9:1, 8:2, 7:3). The solvent elution rate is 45 drops per minute. The solvent eluting through the column is collected in a test tube with a time gap of 5 minutes per fraction and all the eluted fraction is monitored by TLC on silica gel G plates with dichloromethane : methanol (9:1) as mobile phase. The chromatogram is kept in Iodine chamber and their R_f value is calculated. More than 200 fractions are collected in which fractions 31-49 shows a single spot in TLC plate at R_f value of 0.52- 0.56 and are combined together and concentrated to $1/10^{th}$ of its volume and transferred to small beaker. The concentrated material is evaporated at room temperature and ethanol (99.9%) is added to the material. Again it is evaporated and kept in a refrigerator for 12 hours to enhance crystallization and purification.

3.5 STRUCTURAL ELUCIDATION OF THE ISOLATED COMPOUND

In most cases of extraction and isolation of natural products, the end point is the identification of the compound or the conclusive structure elucidation of isolated compounds. However, structure elucidation of compounds isolate from plants fungi, bacteria, or other organisms is generally time consuming and sometimes can be “bottleneck” in the natural product research. There are many useful spectroscopic methods of getting information about chemical structures. The following spectroscopic techniques are used for the structure elucidation of natural products:

3.5.1 Infrared spectroscopy (IR) : Determines different functional groups, e.g., -C=O, -OH, -NH_2, aromacity, and so on, present in the molecules.

3.5.2 Mass spectrometry (MS) : Mass spectroscopy technique has been used for the structural characterization and identification of alkaloids (Wang *et al.,* 2004) it gives information about the molecular mass, molecular formula, and fragmentation pattern. Most commonly used techniques are: electron impact mass spectrometry (EIMS), chemical ionization mass spectrometry (CIMS), electronspray ionization mass spectrometry (ESIMS), and fast atom bombardment mass spectrometry (FABMS).

3.5.3 Nuclear Magnetic Resonance (NMR) spectroscopy : Reveals information on the number and types of protons and carbons (and other elements like nitrogen, fluorine, etc.) present in the molecule, and the relationships among these atoms. The NMR experiments used today can be classified into two major categories:

(a) One dimensional techniques :- ^{1}H NMR, ^{13}C NMR, ^{13}C DEDPT.

(b) Two-dimensional techniques : 1H-1H, COSY, 1H-1H, DQF-COSY, 1H-1H COSY-Ir, 1H-1H NOESY, 1H-1H ROESY, 1H-1H TOCSY, 1H-13C HMBC, 1H- 13C HMQC, 1H-13C HSQC, HSQC-TOCSY.

3.5.4 Elemental analysis : It is for knowing the component of carbon, hydrogen and Nitrogen in percentage in an isolated crystal.

3.5.5 Determination of Melting point : Determination of melting point by using melting point apparatus.

3.6 ANTIMICROBIAL ACTIVITY

The methanol extract of the plant sample is studied for its antimicrobial activity as it is used traditionally for treating different kinds of microbial infection.

3.6.1 Microorganisms : The microorganisms used for the present study are *Escherichia coli* (MTCC-40), *Micrococcus luteus* (MTCC- 106), *Pseudomonas aeruginosa* (MTCC-424) and *Klebsiellla pneumonia* (MTCC- 39), which are obtained from Institute of Microbial Technology (IMTECH), Chandigarh, Punjab. The microorganisms were subcultured in nutrient broth and incubated at 37˚C for 24 hrs prior to the experiment.

3.6.2 Preparation and extraction of the plant material :- The methanol extract of the plant which is extracted earlier for other tests was used.

3.6.3 Disc diffusion method: The antimicrobial activity of the crude extract is tested on the different test microorganisms mentioned by disc diffusion method (Bauer *et al.*, 1966).

Preparation of stock extract solution :- The dried methanolic extract of the plant is weighed and dissolved in Dimethyl Sulfoxide (DMSO) made by GR, Merck Specialities Private Limited, Mumbai making 20mg/ml and 10 mg/ml concentrations.

Preparation of test microorganisms: The turbidity of the sub cultured microorganisms is adjusted with sterile distilled water using 0.5 Mc Farland as standard (~1.5 X 10^8 microorganisms/ml).

Preparation of plant extract disc : Paper disc of 5 mm diameter is prepared using Whatman filter no 3. The paper discs are sterilized before applying the extracts. Then, 10 µL of the plant extracts of different concentrations are applied to the sterilized paper discs so that the discs contain 200 µg and 100 µg of the extract respectively, after which the discs are air dried.

Preparation of media : 200 mL of Nutrient Agar (HiMedia) is prepared by dissolving readymade nutrient agar powder in distilled water. Then the dissolved nutrient agar is sterilized.

Evaluation of antimicrobial activity of plant extract : Antimicrobial activity of plant extract is assessed by disc diffusion method. Agar plates are prepared which are inoculated with the test microorganisms by pour plate method and allowed to dry at room temperature. Then, the paper disc containing two different concentrations of plant extract and antibiotic disc containing 10 µg of tetracycline (HiMedia) are kept carefully on the surface of the agar plate. In addition, paper disc containing DMSO is also kept as negative control to make sure that the solvent used for dissolving the extracts do not have antimicrobial activity. Then, the plates are incubated at 37˚C for 24 hours in inverted position. After incubation is over, the plates are observed for antimicrobial activity. If the extract possesses such activity, the zone of inhibition was measured and compared with the standard antibiotic.

3.6.4 Minimum inhibitory concentration: The minimum inhibitory concentration of the crude alcoholic extract of *Hiptage benghalensis* (L.) Kurz. is determined on the test organisms where the extract is found to be active by disc diffusion susceptibility test method (Mendoza,1998).

Preparation of stock extract solution: The dried methanolic extract of the plant is weighed and dissolved in Dimethyl Sulfoxide (DMSO) making 20mg/ml as stock solution. Then, the extract is diluted by two fold dilution making concentrations ranging from 20 mg/ml to 0.3125 mg/ml.

Preparation of test microorganisms: The turbidity of the sub cultured microorganisms is adjusted with sterile distilled water using 0.5 Mc Farland as standard (~1.5 X 10^8 microorganisms/ml).

Preparation of plant extract disc: Paper disc of 5 mm diameter is prepared using Whatman filter no 3. The paper discs are sterilized before applying the extracts. Then, 10 µL of the plant extracts of different concentrations are applied to the

sterilized paper discs so that the discs contain 200 µg, 100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg and 3.12 µg of the extract respectively, after which the discs are air dried.

Preparation of media: 100 mL of Mueller Hinton Agar (HiMedia) is prepared by dissolving readymade powder in distilled water. Then the dissolved Mueller Hinton Agar is sterilized.

Evaluation of antimicrobial activity of plant extract: The MIC of plant extract is assessed by disc diffusion method. Agar plates are prepared which are inoculated with the test microorganisms by pour plate method and allowed to dry at room temperature. Then, the paper disc containing seven different concentrations of plant extract and antibiotic disc containing 10 µg of tetracycline (HiMedia) are kept carefully on the surface of the agar plate. Negative control is also kept which was DMSO . Then, the plates are incubated at 37°C for 24 hours in inverted position. After incubation is over, the plates are observed for antimicrobial activity and the lowest concentration of the extract inhibiting the growth of microorganism is noted and considered as the MIC for each test microorganism.

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CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 LITERATURE SURVEY OF ETHNO-BOTANICAL RESEARCHES FOR SCREENING OF POTENTIAL MEDICINAL PLANTS FOR PHYTOCHEMICAL STUDIES

All relevant published and unpublished literatures were studied in depth to identify a set of 50 plant species having ethno medicinal value. Fifty ethno medicinal plants were identified. The natural habitats were surveyed for verification of range of distributions. Ranking of all 50 ethno medicinal plants were done on the basis of responses of five percent population of the study area and herbal doctors. The score was allocated on the basis of the responses of the villagers with reference to popularity of ethno medicinal plants among the villagers, pressure over the natural growing stock, ex-situ conservation, and propagation in the kitchen garden and stocking of the dried plants or its formulation in families. All 50 etnobotanical plant species were allocated scores and top 20 ethno medicinal plants were ranked as potential medicinal plants for phytochemial analysis (Table-1). Out of 20 potential ethno medicinal plant, *Hiptage benghalensis* (L.) Kurz scored maximum score and accepted as candidate ethno medicinal plants for photochemical analysis.

The voucher specimen was identified and authenticated by Botanical Survey of India, Kolkata (Reference No: CNH/21/2012/Tech.II/661 Dated: 16-02-2012) and kept in the herbaria of Dept. of Forestry Mizoram University Aizawl.

Candidate ethnomedicinal plants : *Hiptage benghalensis* (L.) Kurz

Ethnomedicinal uses : Almost all the household store root bark in a powdery form prepared indigenously during emergency. Decoction of the root bark is consumed orally for stomachache, chewed in a raw for diarrhoea and the powdered root bark

mixed with water for dysentery (Lalnundanga, 2000).

Distribution

World : *Hiptage benghalensis* is a native of India, Southeast Asia and the Philippines. Found in Florida, Hawaii, Mauritius, Western Australia, Malaysia, China, Pacific Islands, South-east Queensland

India : It is found throughout India

Mizoram : South eastern and southern part of Mizoram (Cherhlun, Ngharchhip, Thenzawl and S. Lungrang).

Laltitude and longitude at place of collection: Lunglei district

(i) Cherhlun : 22˚59′58.14″ N latitude and 93˚ 05′ 29.15″E longitude.

(ii) Ngharchhip :22˚58′50.42″ N latitude and 93˚ 06′ 07.52″ E longitude.

Altitude at place of collection: Lunglei district

(i) Cherhlun : 1311.25metres.

(ii) Ngharchhip : 1047.25 metres

Description: A large, woody, evergreen, straggling or climbing shrub with young branches being grey tomentose it belongs to the family Malpighiaceae. The opposite and entire leaves are oblong to ovate-lanceolate, 9-21 cm long and 4-9 cm wide, acute or acuminate, glabrous, and have petioles of *c*. 1 cm length. White and fragrant flowers of 2-3 cm diameter are borne in erect, pubescent racemes of 10-20 cm length, the pedicels being 15-20 mm long. Flowers have a yellow centre and orbicular to elliptic petals that are hairy outside. Fruits are samaras with three wings each, the middle wing being 4-6 cm long and the lateral wings 2-3 cm long

Habitat : Habitat variable. Prefers climates ranging from warm temperate to tropical. Dry and moist areas from sea level to 1000m

Phenology: **Flowering :** September – December

Fruiting : January – March

Table 1 Potential Ethno-medicinal plants*

Sl. No.	Botanical name	Local Name	Family	Uses
1	*Achyrantes aspera* Linn.	Ui-hlo	Amaranthaceae	Juice of the pounded leaves is used in piles, cough, boils, sores, wounds.
2	*Adhatoda vasica* Nees.	Chhawldai	Acanthaceae	Ashes of the dried leaves is use for treatment of toothache. Decoction of the leaves is used for treating urinary tract infection.
3	*Benincasa hispida* Thunb.	Maipawl	Cucurbitaceae	Juice of the fruit is recommended for cholera, diarrhoea and vomiting. Infusion of the leavea and fruits are used externally in snake-bite.
4	*Blumea lanceolaria* Roxb.	Buarze	Compositae	Decoction of the leaves is used in stomach ulcer, indigestion and dysentery. Juice of the leave is use for treatment of scabies, skin disease and dandruff.
5	*Centella asiatica* Linn.	Lambak	Umbelliferea	Leaves are used for stomachache, diarrhoea, high blood pressure and skin disease.
6	*Chukrasia tabularis* A.Juss	Zawngtei	Meliaceae	Juice of the leaves is applied to fresh cuts.
7	*Costus speciosus* Koen ex.Retz.	Sumbul	Zingiberaceae	Juice of the crushed roots is used in diseases of kidney, fever, bronchitis, indigestion, rheumatism.
8	*Croton caudatus* Geisel.	Ranlungdam dawi	Euphorbiaceae	The leaves are crushed and the juice is applied as a poultice to sprains.
9	*Dillenia pentagyna* Roxb.	Kaihzawl	Dilleniaceae	Decoction of the leaves or bark are used for curing gastric trouble, asthma, dysentery, cancer etc.
10	*Elaeagnus caudata* Schltdl.	Sarzuk	Elaeagnaceaea	Decoction of the root is used for expelling pieces of retained placenta after child birth and also for stopping the menses.

11	*Eupatorium odoratum* Linn.	Tlangsam	Compositae	Juice of the leaves is applied to new cuts.
12	*Euphorbia hirta* Linn.	Midum-an/Hnutetui tamna	Euphorbiaceae	The boiled juice of the whole plant is used for the treatment of stomachache, dysentery, stone in kidney.
13	*Hedyotis scandens* Roxb.	Laiking tuibur	Rubiaceae	Decoction of the roots and leaves are used in the treatment of fever, urinary complaints, inflamed kidneys, and womb troubles. Juice of crushed leaves is used externally for sores, rheumatism and eye disease.
14	*Hiptage benghalensis* (L.) Kurz	Raisentur	Malpighiaceae	Decoction of the root bark taken orally for stomachache, chewed in a raw for diarrhoea and the powdered root bark mixed with water is use for dysentery.
15	*Hodgsonia macrocarpa* Cogn.	Khaum	Cucurbitaceae	The seed is boiled and the water is drink for urinary tract infection.
16	*Lobelia angulata* G.Forst	Choakathi	Campanulaceae	Juice of the crushed leaves and berries are taken against diarrhea, tonsillitis, tootache.
17	*Mimosa pudica* Linn.	Hlonuar	Mimosaceae	Decoction of the root is used for treating stone in kidney and bladder. Crushed root is used for the treatment of swells.
18	*Phyllanthus urinaria* Linn.	Mitthi-sunhlu	Euphorbiaceae	Juice of the whole plant is used for liver problems and jaundice. The stem and leaves are also eaten raw for diabetis and sore-throat.
19	*Scoparia dulcis* Linn.	Perhpawng chaw	Scrophulariaceae	Juice of the pounded leaves, stem and roots are used in diabeties, stomach trouble, diarrhoea, dysentery.
20	*Stephania glandulifera* Miers.	Chaihchun	Menispermaceae	Juice of pounded tuber is used in fever, colic and diarrhoea.

***Plants are arranged in alphabetical order of their names**

Photo 3 : ***Hiptage benghalensis*** **(L.) Kurz**

Photo 4 : Flower bud of ***Hiptage benghalensis*** **(L.) Kurz**

Figure 1 : *Hiptage benghalensis* (L.) Kurz

4.2 PHYTOCHEMICAL ANALYSIS

4.2.1 Phytochemical group testing

The result of the phytochemical group tests for methanol extract of *Hiptage benghalensis* (L.) Kurz has been given in table 2. The result of the phytochemical group test revealed the presence of alkaloids, reducing sugar and tannins. The curative properties of medicinal plants are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc*. The presence of alkaloids may be the main reason why the plant extract showed therapeutic action in the traditional use since alkaloids have traditionally been of great interest to humans because of their pronounced physiological and medicinal properties

Table : 2. Preliminary phytochemical group tests of the methanol extract of *Hiptage benghalensis* (L.) Kurz

Sl No.	Phytoconstituents	Inference
1	Alkaloids	+
2	Flavonoids	–
3	Steroids	–
4	Triterpenoids	–
5	Amino acids	–
6	Reducing sugar	+
7	Tannins	+
8	Saponins	–
9	Gums	–

+ (Positive) = present **- (Negative) = absent**.

4.2.2 Isolation of the active constituents

Results of Thin Layer Chromatography

The results of Thin Layer Chromatography are presented in Table 3. As given in table 3 a definite single spot shows light brown colour in two solvent systems i.e., Dichloromethane: Methanol (9:1) and Dichloromethane : Acetone (8:2) after keeping inside the iodine chamber. But Dichloromethane : Methanol (9:1) shows better results than the other one. It was then selected as a solvent system for further uses.

Table : 3 Thin Layer Chromatographic study of methanol extract

Sl.No.	Solvent System	Results
1	Chloroform : Ethyl acetate (7:3)	No definite spots,tailing exist
2	Petroleum ether : Chloroform (7:3)	No definite spots,tailing exist
3	Chloroform: Ethyl acetate : Methanol (7:2:1)	No definite spots,tailing exist
4	Dichloromethane : Methanol (10:0,9:1,8:2,7:3,6:4,5:5,4:6,3:7, 2:8,1:9, 0:10)	**A s**ingle spot which is light brown in colour was seen in 9:1 ratio with Rf value of 0.53
5	Chloroform: Methanol (9:1)	No definite spot
6	Dichloromethane : Acetone (8:2)	A single spot with Rf value of 0.26
7	Dichloromethane : Ethyl acetate (7:3)	No definite spots,tailing exist
8	Dichloromethane :Benzene : Acetone (7:2:1)	No spots (Sample do not move)
9	Chloroform : Acetone (1:1)	No definite spots

Result of Column Chromatography

From the column chromatography of methanol extract of *Hiptage benghalensis* (L.) Kurz a pale yellow crystal was isolated. It is readily soluble in methanol. The isolated material was further process to elucidate the structure and other properties.

4.2.3 Structure elucidation of the isolated compound:

Alkaloids: Occurrence, functions, structure and nomenclature

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral (Naught & Wilkinson, 1997) and even weakly acidic properties (Manske, 1965). Also some synthetic compounds of similar structure are attributed to the alkaloids. In plants it is found as secondary metabolites. True alkaloids are of rare occurence in lower plants (Trease and Evans, 2002). Many, probably most, alkaloids are derived, at least partly from various amino acids as their direct precursors. The most common ones are phenylalaline, tyrosine, lysine, omithine, histidine, tryptophan and anthranilic acid.

As an exception some alkaloids (e.g., steroidal alkaloids) are in their biogenesis, more directly derived from isoprenoid or other precursor compounds of carbohydrates.

Alkaloids are found in various parts of the mature plants like seeds, roots, rhizomes, corm, leaves, fruits and barks. The major distribution of the alkaloids occurs in the angiosperms (Kokate, *et al.*, 2003). The same plant can sometimes produce enough alkaloids in more than one organ to warrant the production of more than one commercial entry, example belladonna (leaves and roots) or colchicum (seeds and corm) (Agrawal and Paridhavi, 2007).

The functions of alkaloids are :-

i) They act as reservoirs for protein synthesis.

ii) They may act as protective substances against animal or insect attacks.

iii) Like hormones, they may have functions as stimulants or regulators in activities like growth, metabolism and reproduction.

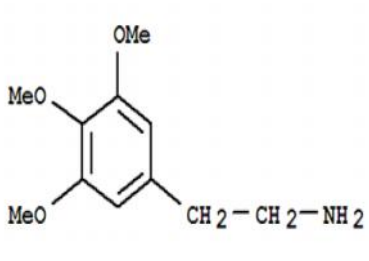
iv) They may function as de-toxicating agents by methylating, condensing and cyclising the compounds whose accumulation might otherwise cause damage to the plant. But, it is also very important to note as much as 85-95% of the plants manage very well without any alkaloids (Agrawal and Paridhavi, 2007).

v) Waller and Nowacki distinguished the role of alkaloids in plants growth stimulatora and inhibiters and also as protective agents and reservoirs of nitrogen (Aniszewski, 2007).

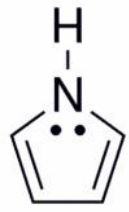
vi) It has been suggested that alkaloids may have a role in the defence of the plant against singlet oxygen, which is damaging to all living organisms and is produced in plant tissues in the presence of light (Larson et al., 1984; Larson, 1988)

Alkaloids include literally thousands of bitter, nitrogenous compounds found throughout the plant kingdom. They often contain one or more rings of carbon atoms, with a nitrogen atom in the ring. The position of the nitrogen atom in the carbon ring and peyote-type (mescaline). Some of these have remarkable structural similarities with neurotransmitters in the human central nervous system, including dopamine, serotonin and acetylcholine.

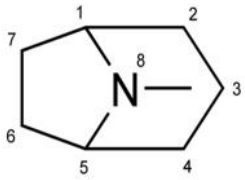
Because of the complex structure and other historical reasons, no attempt has been made for the systematic nomenclature for alkaloids. A large number of alkaloids



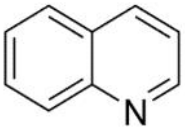
Mescaline



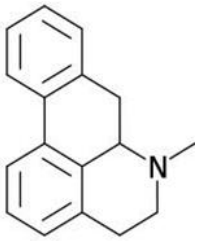
Pyrrole



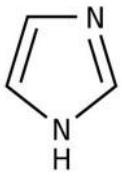
Tropane



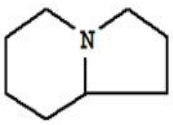
Quinoline



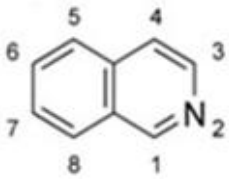
Aporphine



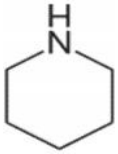
Imidazole



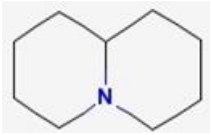
Indolizidine



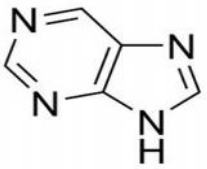
Isoquinoline



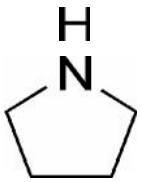
Piperidine



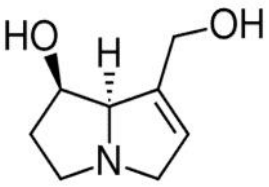
Nor-Lupinane



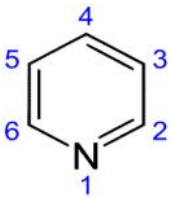
Purine



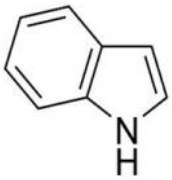
Pyrrolidine



Pyrolizidine



Pyridine



Indole

Figure 2 : Skeletal structure of Alkaloids

have been named according to the plants from which they are obtained, viz., papaverine from *Papaver somniferum*. A few alkaloids have been named because of their physiological action viz., morphine (German : Morphine god of dreams), emetine (Greek*: emetekos*- to vomit).

A single alkaloid group pelleteirine from pomegranates has been named after its discoverer P J Pelletier. The names of minor alkaloids are derived by adding a prefix or suffix to the name of the principal alkaloid.

An alkaloid end with the suffix 'ine'. This is to discriminate alkaloids from other classes of secondary metabolites (Agrawal and Paridhavi, 2007).

4.2.3.1 Infrared spectral analysis

The IR spectra of the isolated compound was taken by using IR Prestige-21, FTRI Spectrophotometer . The Spectra was recorded in the region of 4000cm^{-1} to 400 cm^{-1}. The spectrum is given in Fig 3.

IR (KBr) n_{max} : The IR spectrum showed strong absorption band at 1633.76 cm^{-1} indicating the presence of conjugated carbonyl group (Al Mutagabani, 2003). Also it showed an absoption band at 2943.47cm^{-1} for C-H stretching and 1610.61 cm^{-1} for C=C stretching (Ahlam El-Fishawy *et al.*,2011). The IR spectrum also showed two strong absorption band at 1456.30cm^{-1} and 1446.66 cm^{-1} indicating the presence of methoxy group (OCH_3) (Furnises *et al.*,2005).

4.2.3.2 Mass spectral analysis

The mass spectra of the isolated compound was recorded by using A Time of Flight –Mass Spectrophotometer: QT of Micro Y A263 (Waters co. USA). The ESI-Mass spectra of sample (full scan positive mode) showed molecular ion peak at m/z 356 $[M+H]^+$ and m/z 354$[M-H]^+$.

Photo 5 : Powdered Crude Drug

Photo 6 : Photograph of Isolated Compound

4.2.3.3 ^{1}H NMR and ^{13}C NMR spectral analysis

^{1}H NMR and ^{13}C NMR spectra of the isolated compound was recorded in Bruker-DPX-300 (Switzerland; 300mHz) Spectrophotometer at CD3OD using TMS as internal standard. The spectra are recorded and tabulated.

^{1}H NMR (300 MHz,CD3OD): δ 6.92, 6.95 (2 X 1H, d, J =8.4 Hz), δ 6.72, δ 6.89 (1H, s), δ 4.22 (1H, d, J=15.6 Hz), δ 3.82, δ 3.84, δ3.85 (12H, s, 4X OCH_3) The ^{1}H NMR spectrum indicates singlets at δ 6.72, δ 6.89 (1H, s) as well as two doublets δ 6.92, 6.95 (2 X 1H, d, J=8.4 Hz), at the downfield shift. As expected for aromatic compounds, the spectrum indicates the presence of two sets of aromatic protons but at two entirely different environments respectively. Signals at δ 3.82, δ 3.84, δ 3.85 (12H, s, 4X OCH_3) in upfield attributes the presence of Methoxy (-OMe) groups in the ortho and meta positions of the two aromatic rings. δ 4.22 (1H, d, J=15.6 Hz), a doublet signal implies the presence of N-nitro atoms in the environment, characteristic of donating lone pairs shielding the vicinal proton slightly downfield. Doublet signals like δ2.61 to 3.34 (see spectra) indicates germinal environment and since the spectral studies do not indicate quarternary ammonium, it can roughly be concluded as rings with hetero –atoms sandwitch by the two di-substituted aromatic rings.

^{13}C NMR (300 MHz, CD3OD): δ 151.8 (C-10), δ 149.3 (C-3), δ 149.1 (C-2), δ 146.2 (C-9), δ 130.7 (C-14(a)), δ 128.9 (C-12(a)), δ 128.7 (C-8(a)), δ 127.8 (C-4(a)), δ 125.2 (C-12), δ 113.0 (C-4), δ 112.7 (C-11), δ 110.4 (C-1), δ 60.8 (C-14), δ 60.6 (9-OCH3), δ 56.7 (3-OCH3), δ 56.4 (2-OCH3), δ 56.3 (10-OCH3), δ 54.8 (C-8), δ 52.7 (C-6), δ 36.5 (C-13), δ 29.4 (C-5).

^{13}C spectral shift for genus *Corydalis* alkaloid at105.5 ppm and 108.2 ppm for C-1 and C-4 are close to the spectral signal observed at 110.4 ppm and 113.0 ppm. Further δ 146.3 and δ 145.6 at C-2 and C-3 respectively of the same alkaloid also falls

in accordance with our spectra of δ 149.1 and δ 149.3 for one of the aromatic ring. In comparison with this alkaloid of the genus *Corydalis* having the parent skeleton of phenanthrene, the ^{13}C NMR spectral signals of our isolated compound all fall closely with each other which enhanced our conclusion to be as per the parent phenanthrene skeleton of *Corydalis*.

Expected specific rotation still needs to be determined to assign the absolute configuration or its stereochemistry but nevertheless the spectral signals all conform to the alkaloid tetrahydropalmatine.

^{13}C signal of δ 29.4, δ 52.7, δ 54.8 and δ 36.5 all corresponds to the presence of CH_2 and signals at δ 110.4, δ 113.0, δ 128.7, δ 151.8, δ 146.2, δ 112.7, δ 125.2 and δ 128.4 gives the signature shift for olefenic carbons as well as Aromatic carbons.

The addition signals at δ 56.40, δ 56.7, δ 60.6 and δ 56.35 corresponds to the chemical shifts of carbon bonded to electronegative oxygen (methoxy, OCH_3). OCH_3 signal is found at δ 158-179.

4.2.3.4 Elemental analysis of the isolated compound:

The elemental analysis of the isolated compound was done at Indian Association for Cultivation of Science (IACS), Jadavpur, Kolkata by using Perkin Elmor precisely series II CHNS/O Analyser, Model 2400.

The elemental analysis value is given below :

Calculated: C 70.96,H 7.09,N 3.94 for $C_{21}H_{25}NO_4$ and

C 67.54,H 7.29,N 3.75 for $C_{21}H_{25}NO_4.H_2O$

Found: C 67.86, H 7.16, N 2.76

4.2.3.5 Physical properties of the isolated compound

Melting point: Melting point were determined on a melting point apparatus (DSGW, Model-3046).

Table 4 : Physical properties of isolated compound

Melting Point	141-144˚C
Colour	Pale Yellow
Nature	Crystal

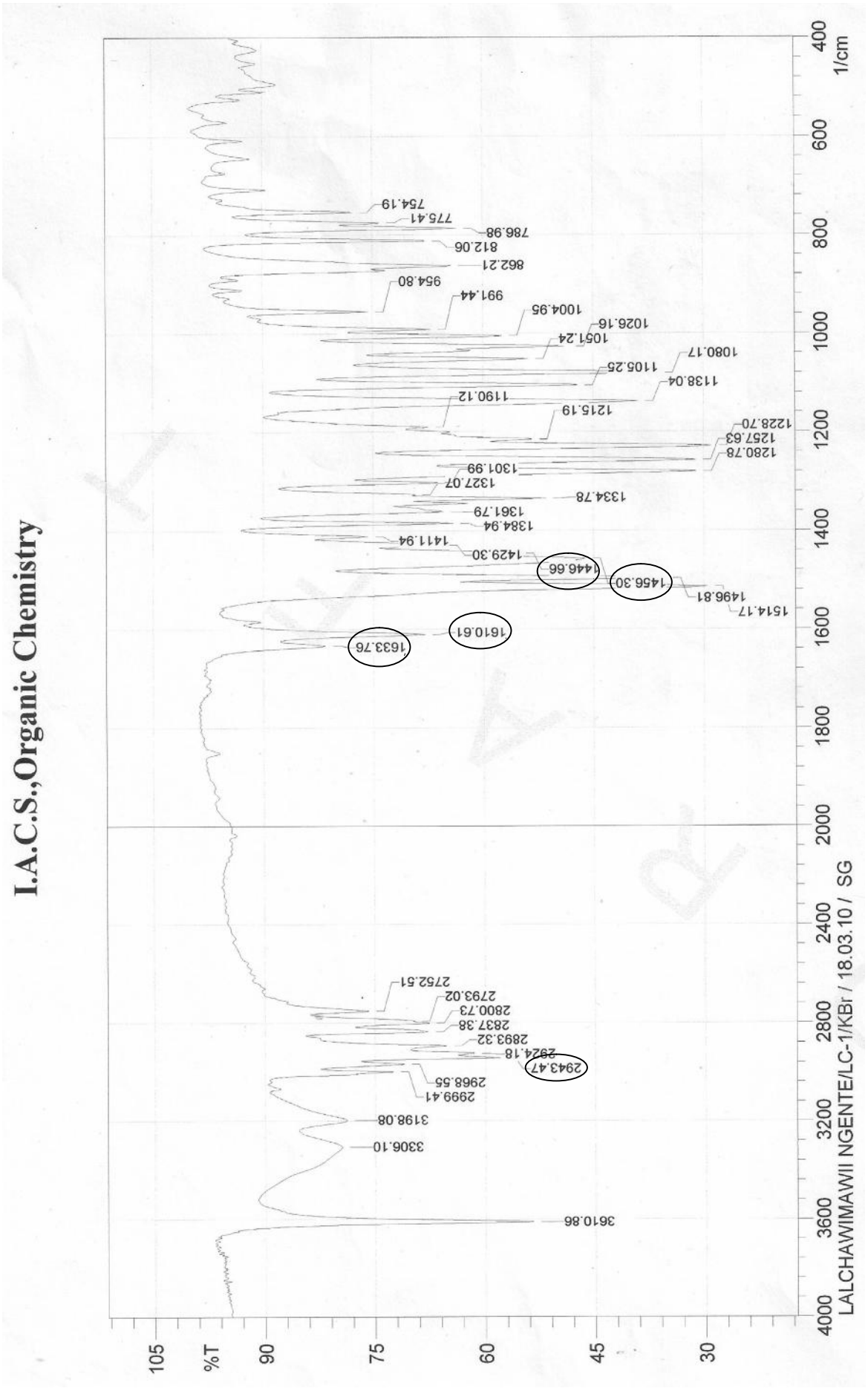


Fig: 3 IR Spectrum of Isolated Compound

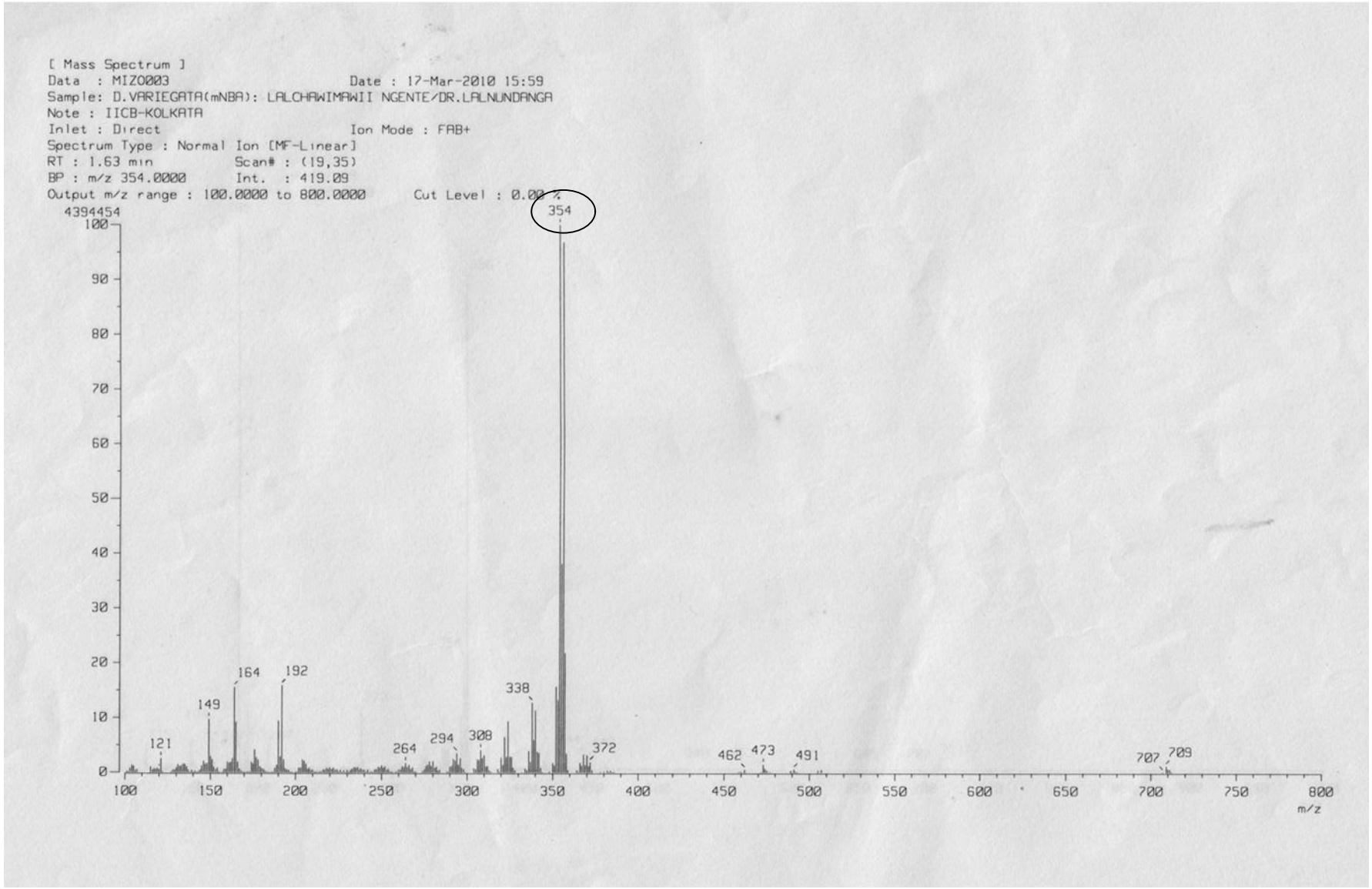


Fig: 4 Mass Spectrum of Isolated Compound

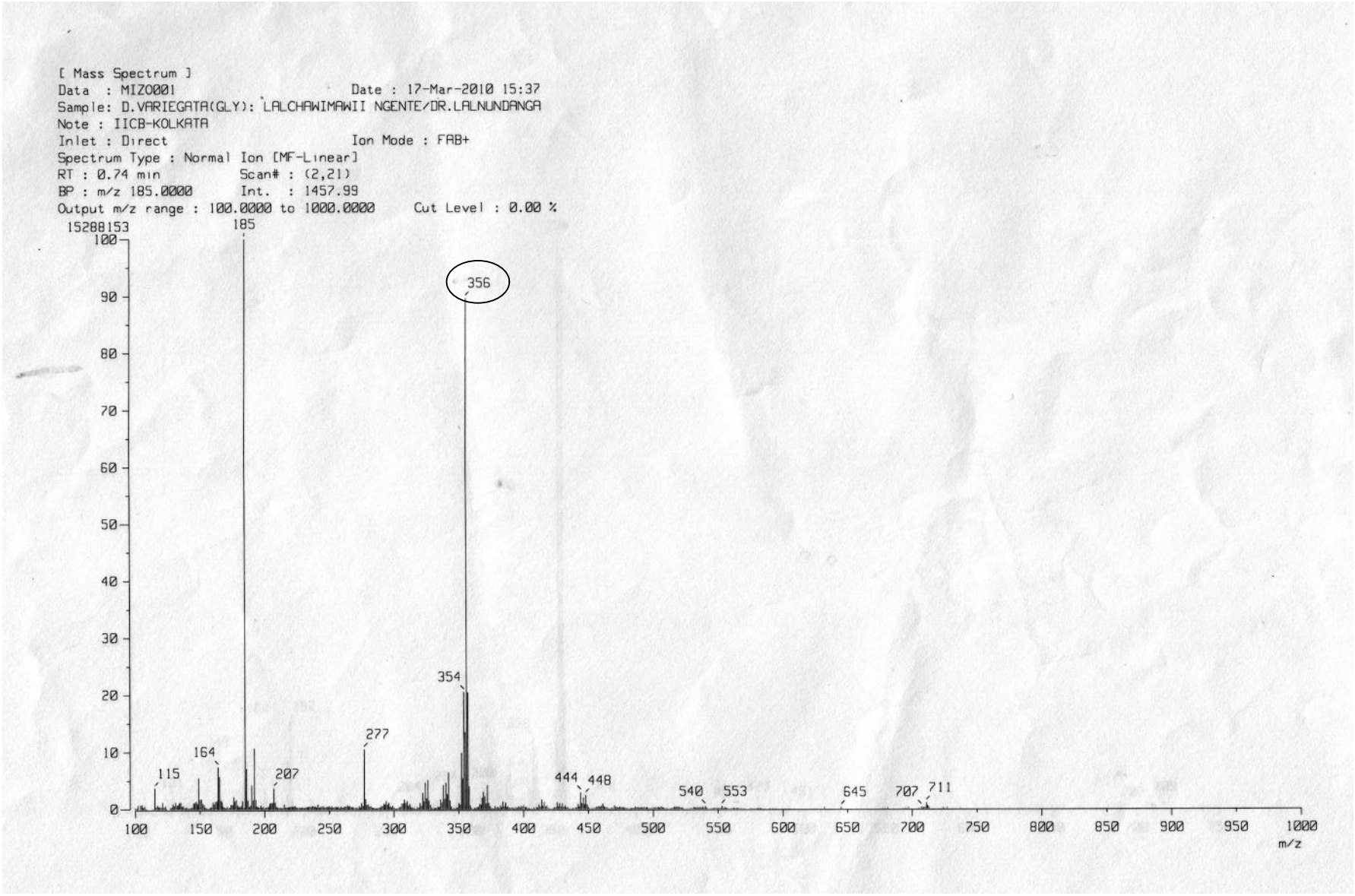


Fig: 5 Mass Spectrum of Isolated Compound

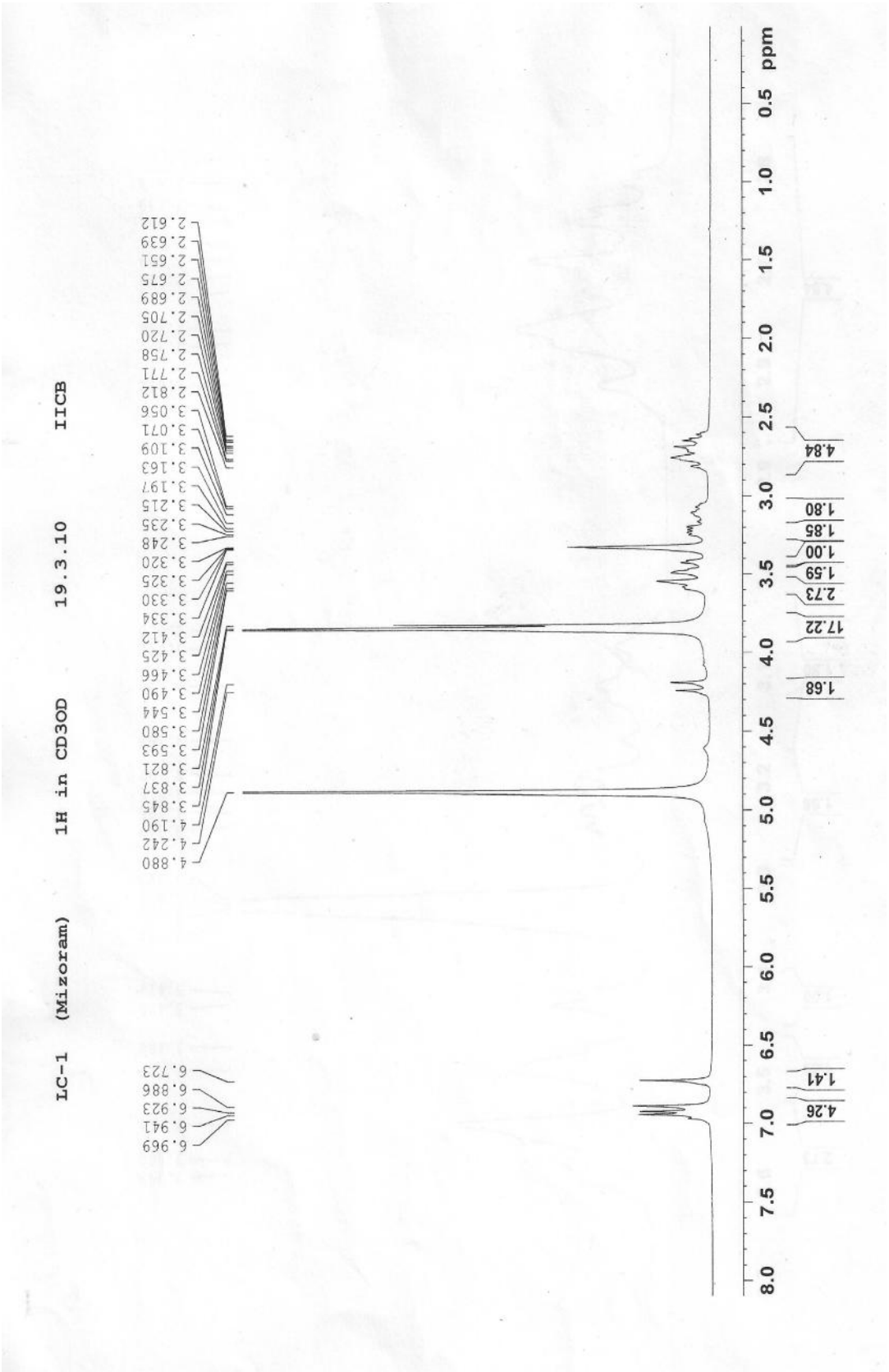


Fig: 6 ^{1}H NMR of Isolated Compound

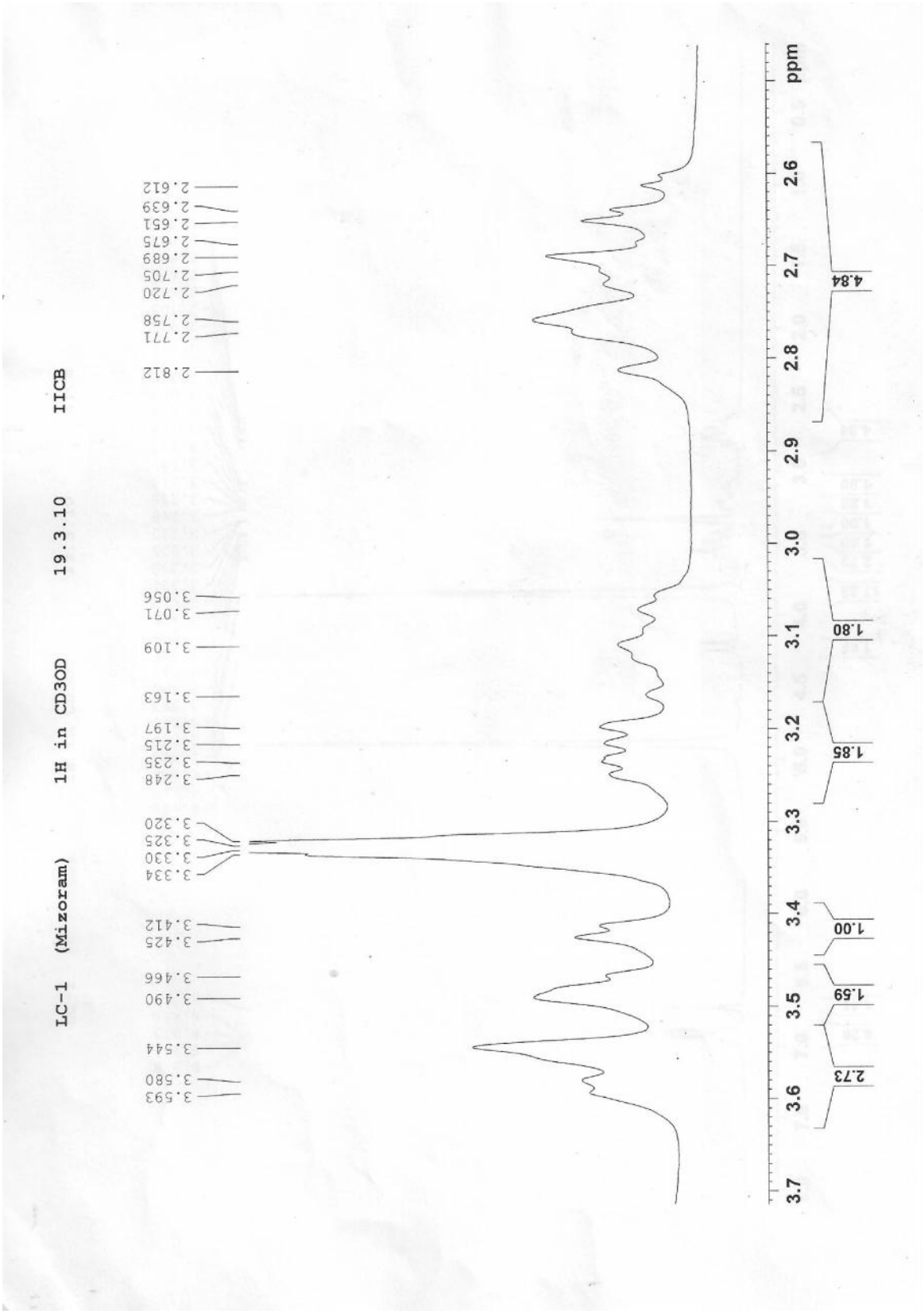


Fig: 7 ^{1}H NMR of Isolated Compound

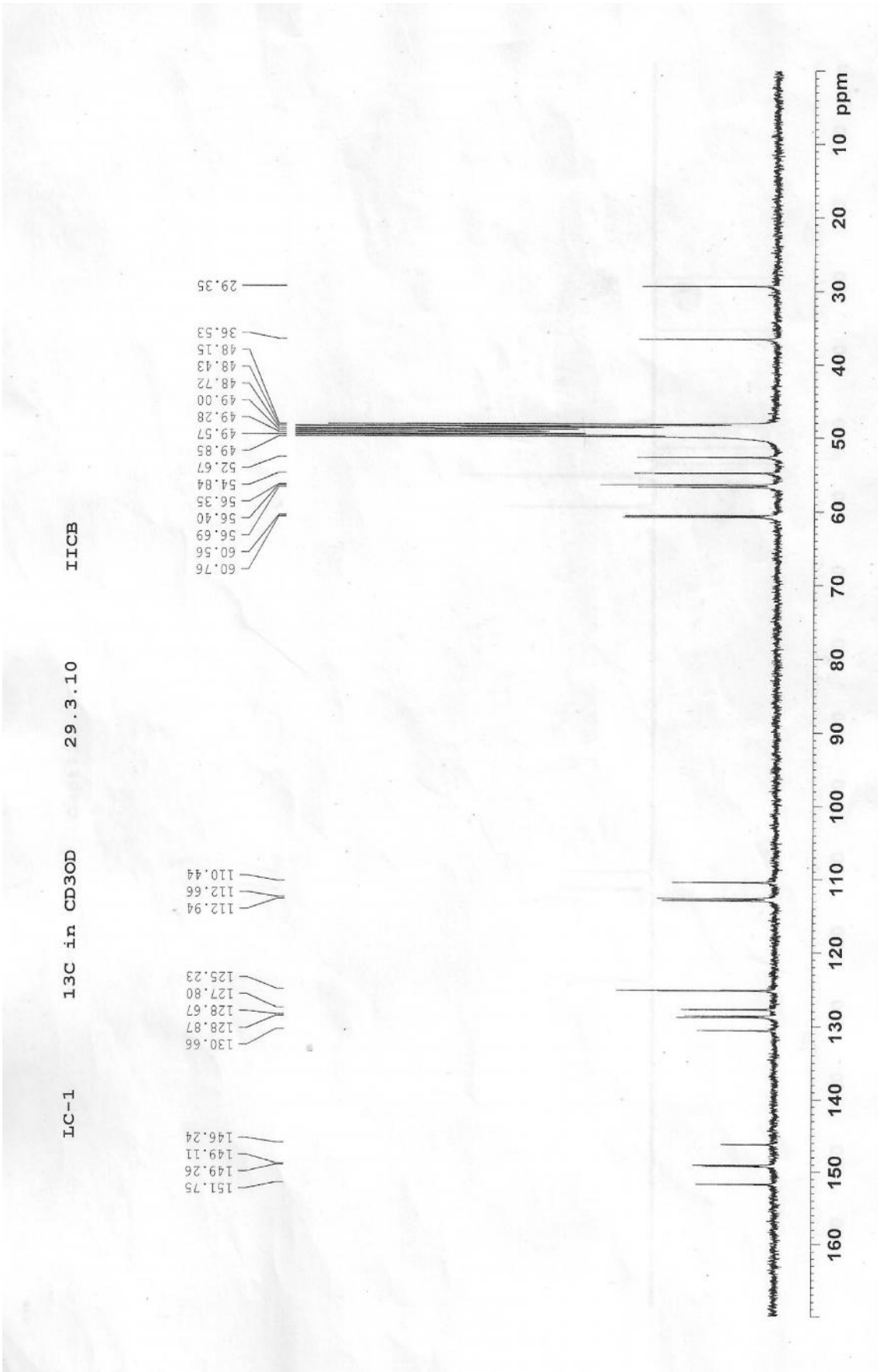


Fig: 8 ^{13}C NMR of Isolated Compound

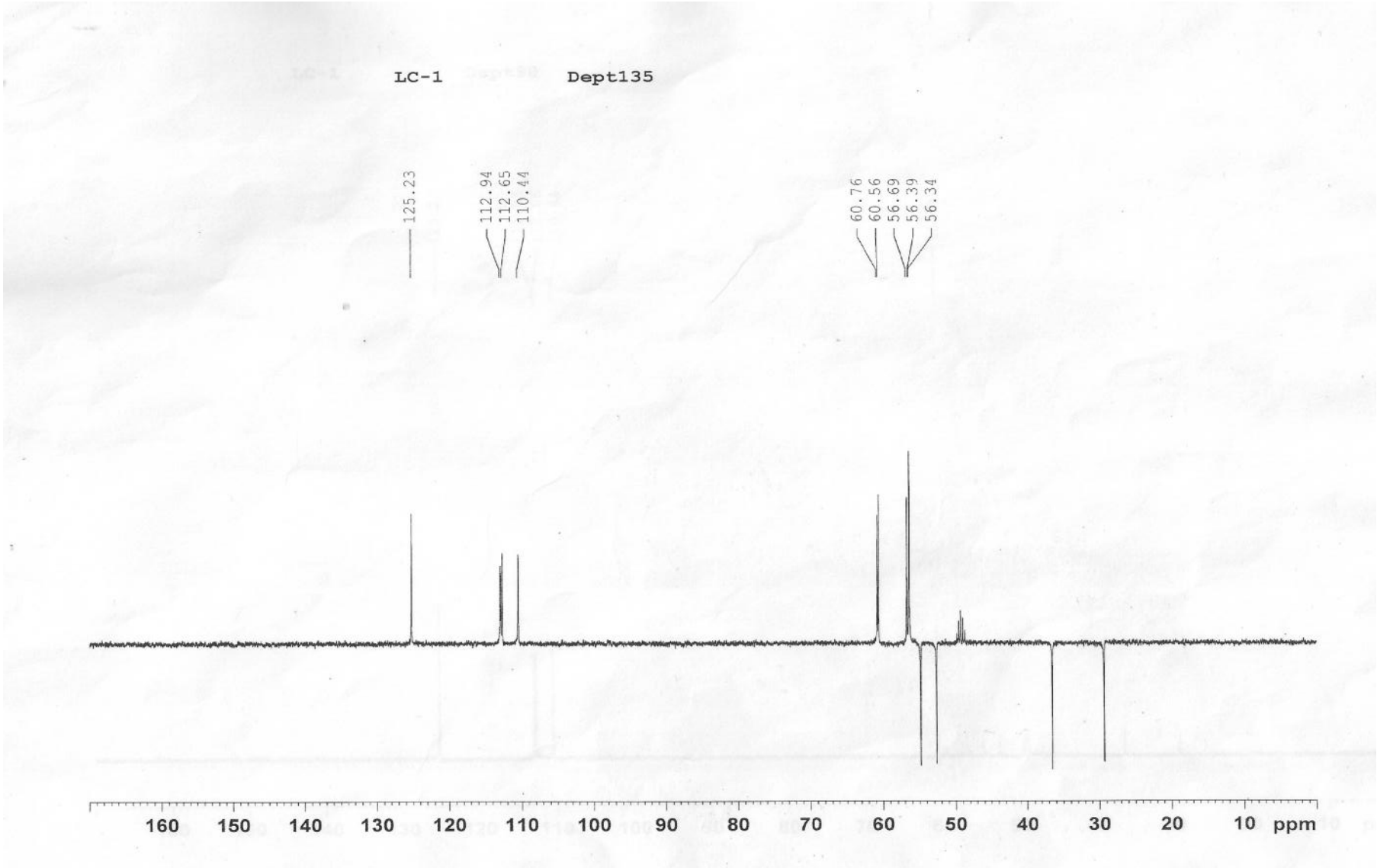


Fig: 9 ^{13}C NMR of Isolated Compound (Dept. 135)

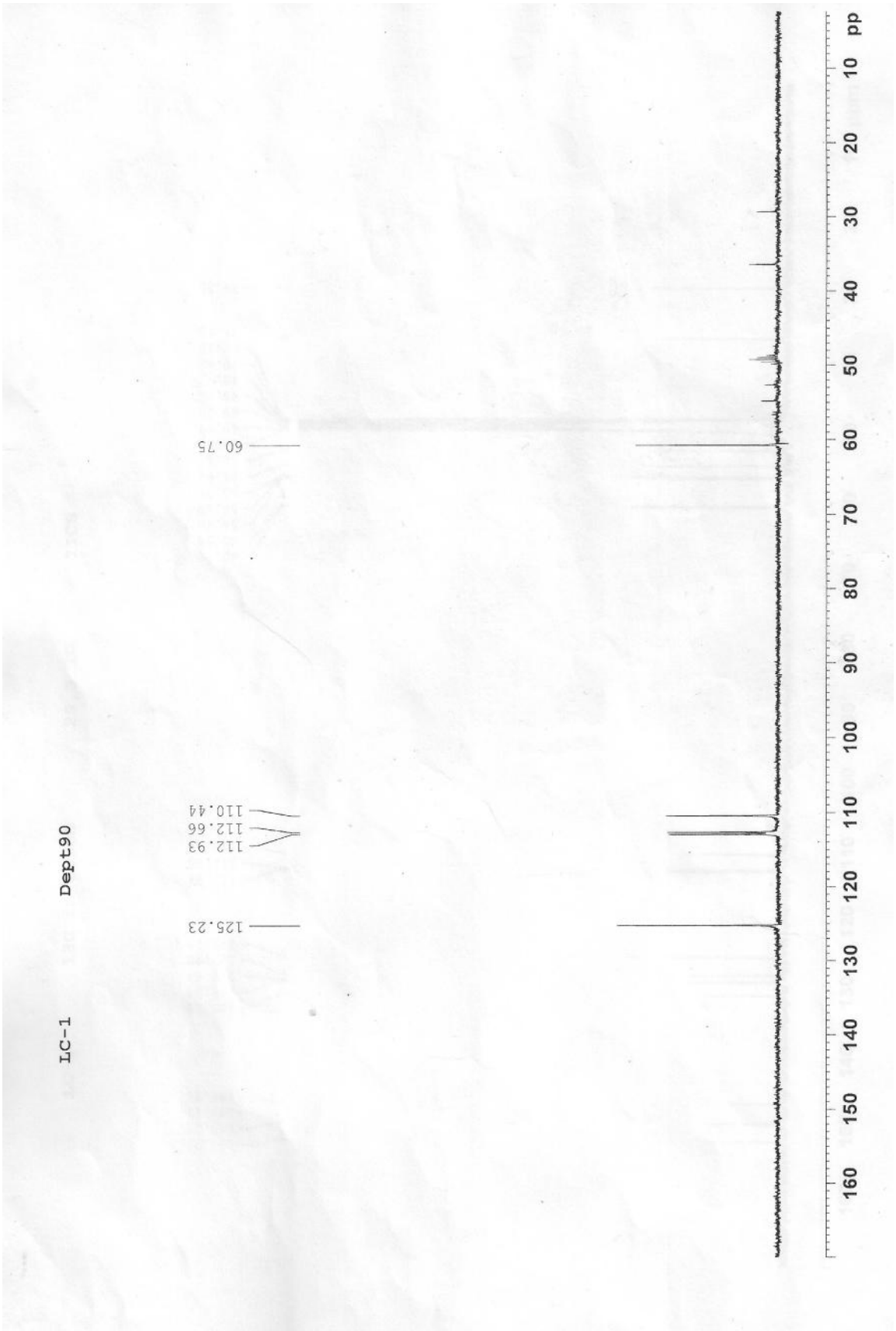


Fig: 10 ^{13}C NMR of Isolated Compound (Dept.- 90)

On comparision with the literature, it was revealed that the physical and spectral data (IR, Mass, NMR spectrum) of the chemical compound of the present study were in good agreement with tetrahydropalmatine (an alkaloid) which was isolated earlier from *Corydalis yanhusuo* (Qu *et al.*,2007) and *Rhizoma corydalis* (Xu XH *et al.,* 2002). Therefore, it was concluded that the isolated compound is **Tetrahydropalmatine**

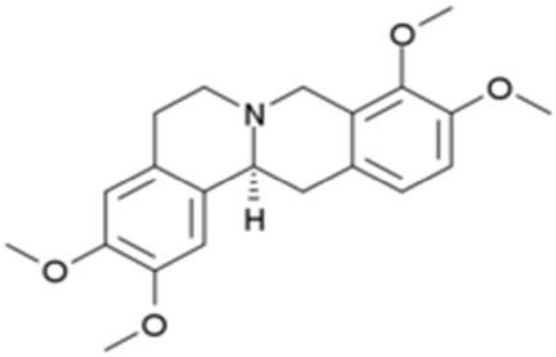


Fig. 11 Chemical structure of Tetrahydropalmatine

IUPAC Name : -2,3,9,10-tetramethoxy-6,8,13,13a-tetrahydro-5H-isoquinolino[2,1-b]isoquinoline

Molecular formula: $C_{21}H_{25}NO_4$

Molecular Weight: 355.428g/mol

DISCUSSION:

Tetrahydropalmatine (THP) is an alkaloid found in several different plant species mainly in the *Corydalis* family (Chen *et al*., 1999, Ma *et al.,* 2006), but also in other plants such as *Stephania rotunda* (Chea *et al.,* 2007). These plants have traditional uses in Chinese herbal medicine. The pharmaceutical industries has synthetically produced the more potent enantiamer Levo-tetrahydropalmatine (Levo THP), which has been marketed worldwide under different brand names as an

alternative to anxiolytic and sedative drugs of the benzodiazepine group and anelgesics such as opiates. It is also sold as a dietary supplement.

Tetrahydropalmatine has been demonstrated to possess analgesic effects and may be beneficial in the treatment of heart disease and liver damage (Wu *et al.,* 2007, Min *et al.,* 2006). It is a blocker of voltage activated L-type calcium channel active potassium channels. It has also shown potential in the treatment of drug addiction to both cocaine and opiates,and preliminary human studies have shown promising results.(Mantsch *et al.,* 2007, Chu *et al*., 2008, Yang *et al.,* 2008) *dl*-Tetrahydropalmatine (*dl*-), one of the major active alkaloids, has been found to be a neuroactive alkaloid (Hong *et al.,* 2005). It has been listed in the Chinese Pharmacopoeia since1977 as an analgesic with sedative and hypnotic effects. Recent studies have demonstrated that *l*- tetrahydropalmatine inhibits opiate tolerance and withdrawal syndromes in rats (Jin *et al.,*1998, Ge *et al.,*1999). It was also reported that *l*- tetrahydropalmatine significantly inhibits cocaine- or methamphetamine-induced conditioned place preference (Ren *et al.,* 2000, Luo *et al.,* 2003). In addition, *l*- tetrahydropalmatine inhibited cocaine-triggered reinstatement (Manstch *et al*., 2007) and the rewarding effects of cocaine in rats as measured by cocaine self-administration (Manstch *et al*., 2007, Xi *et al.,* 2007) and intracranial self-stimulation (Xi *et al.,* 2007).

Animal experiment have shown that the sedative effect of tetrahydropalmatine results from blocking dopaminergic neurons in the brain .Dopamine is an important neurotransmitter in the central nervous system where it occurs in several important signaling systems that regulate muscular activity and attention, as well as feelings of joy, enthusiasm and creativity .Therfore, tetrahydropalmatine causes no feeling of euphoria, and has been seen as an alternative to addictive drugs for people suffering

from anxiety and pain, and as a possibility for relief for people not helped by existing drugs. Several cases of poisoning related to tetrahydropalmatine have been reported (Lai *et al.*,1999). In some countries it is treated as a controlled substance and license is required to sell it.

4.3 ANTIMICROBIAL ACTIVITY

Disc diffusion method: The antimicrobial activity of the methanol extract of *Hiptage benghalensis* (L.) Kurz was assessed by measuring the zone of inhibition and comparing with that of the standard antibiotic, i.e., 10 µg of tetracycline.

Table: 5 Antimicrobial activity of the methanol extract of *Hiptage benghalensis* (L.) Kurz on different test microorganisms.

Sl. No	Test Organisms	Zone of Inhibition (in mm)*		
		200 µg of plant extract	100 µg of plant extract	Standard (10µg Tetracycline)
1.	*Klebsiella pneumoniae* (MTCC No. 39)	9.5	9	16.5
2.	*Escherichia coli* (MTCC No. 40)	8	8.5	17.5
3.	*Micrococcus luteus* (MTCC No. 106)	9	8.5	17
4.	*Pseudomonas aeruginosa* (MTCC No. 424)	9.5	8	21

*** The zone of inhibition shown above is the mean of three readings and includes the diameter of the paper disc, i.e., 5 mm.**

It is shown in Table 5 that the extract is active on all microorganisms *Klebsiella pneumonia* (MTCC – 39), *Escherichia coli* (MTCC – 40) *Micrococcus luteus* (MTCC – 106) and *Pseudomonas aeruginosa* (MTCC – 424) from the study, it is seen that DMSO, the solvent used for dissolving the extract does not show any antimicrobial activity towards the test organism.

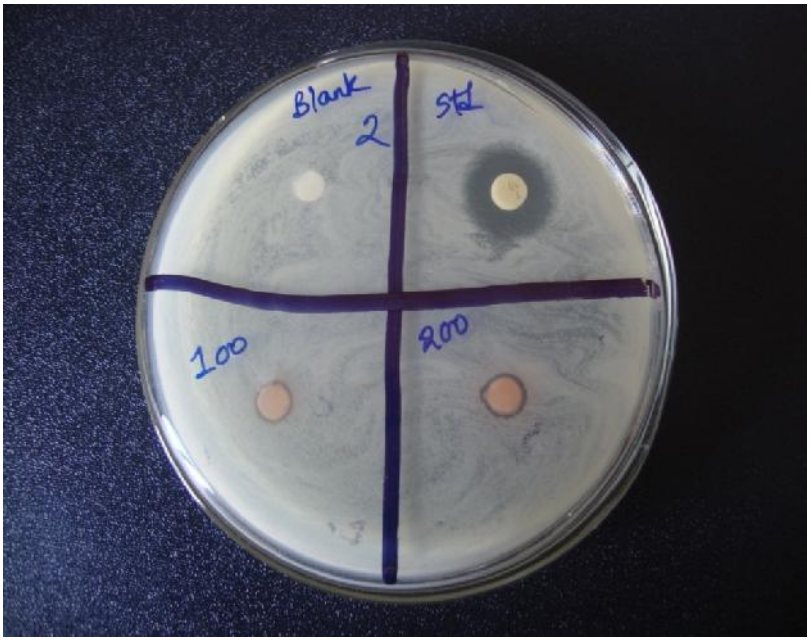


Photo 7 : Antimicrobial activity of the plant extract on *Klebsiella pneumonia*

Photo 8 : Antimicrobial activity of the plant extract on *Escherichia coli*

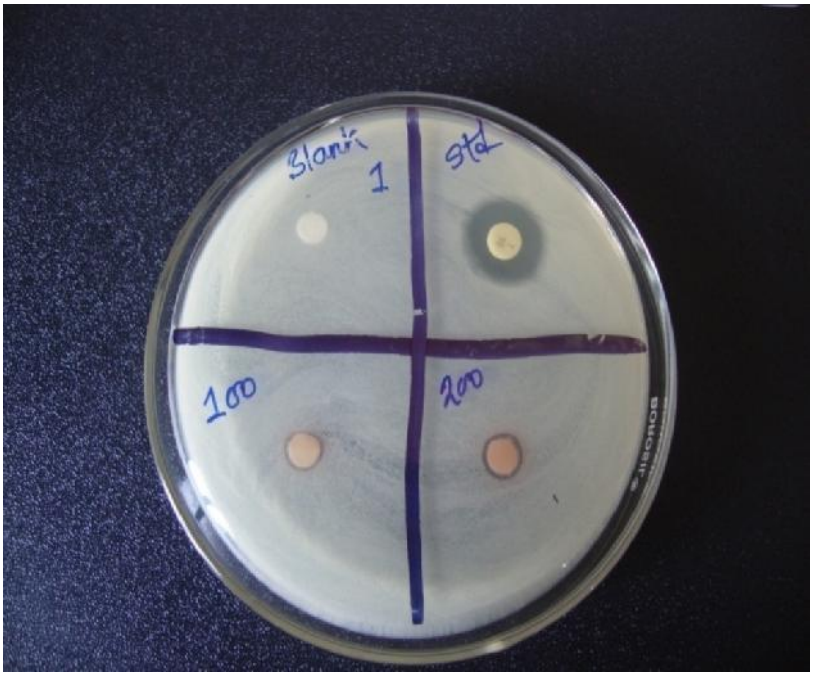


Photo 9: Antimicrobial activity of the plant extract on *Micrococcus luteus*

Photo 10 : Antimicrobial activity of the plant extract on *Pseudomonas aeruginosa*

Table 6: Comparison of the antimicrobial activity of the different concentrations of the methanol extract of *Hiptage benghalensis* (L.) Kurz. on selected test microorganisms.

Sl. No.	MTCC No.	Zone of inhibition (in mm)							
		20mg/ml (200 µg/ disc)	10mg/ml (100 µg/ disc)	5mg/ml (50 µg/ disc)	2.5mg/ml (25 µg/ disc)	1.25mg/ml (12.5 µg/ disc)	0.625mg/ml (6.25 µg/ disc)	0.3125mg/ml (3.125 µg/ disc)	Tetracycline (10 µg/disc)
1.	39	9.5	9	8	6.5	7	6.5	-	16.5
2.	40	8	8.5	8.5	7.5	7	6.5	6	17.5
3.	106	9	8.5	7.5	8	7.5	6.5	-	17
4.	424	9.5	8	9	7.5	7.5	7	-	21

Minimum inhibitory concentration*:* The zone of inhibition exhibited by the different concentrations of the extract on the four test organisms was measured (Table 6).

The minimum concentration of the crude extract that inhibits the growth of the test microorganism was determined and recorded as MIC of the extract on that particular organism (Table 7).

Table 7: Minimum inhibitory concentration (MIC) of the alcoholic extract of *Hiptage benghalensis* (L.) Kurz on different test microorganisms.

Sl. No.	Microorganism	MIC
1.	*Klebsiella pneumoniae* (MTCC No. 39)	0.625 mg/ml
2.	*Escherichia coli* (MTCC No. 40)	0.3125 mg/ml
3.	*Micrococcus luteus* (MTCC No. 106)	0.625 mg/ml
4.	*Pseudomonas aeruginosa* (MTCC No. 424)	0.625 mg/ml)

It is seen from the table 7 that the plant extract is quite active at low concentrations, even though the standard antibiotic has a larger zone of inhibition, it is evident that the extract contains compound which is highly active against the test

organisms, which when isolated and purified may lead to the discovery of new compound better than the standard antibiotic. Since some antibiotics have become almost obsolete because of drug resistant and consequently new drugs must be sought, the antimicrobial activity of the methanol extract of the root bark of *Hiptage benghalensis* (L.) Kurz may pose as one of the answers to the present status on treating diseases caused by antibiotic resistant microorganisms.

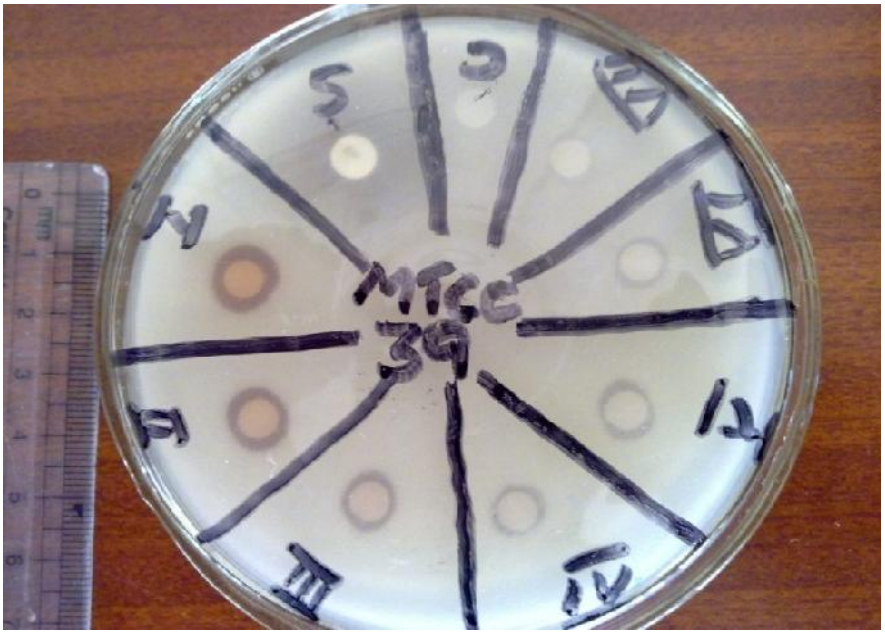


Photo 11 : Determination of MIC of the plant extract on *Klebsiella pneumoniae*

I = 200 µg/disc II = 100 µg/disc III = 50 µg/disc IV = 25 µg/disc
V = 12.5 µg/disc VI = 6.25 µg/disc VII = 3.125 µg/disc S = 10 µg tetracycline/disc C = control

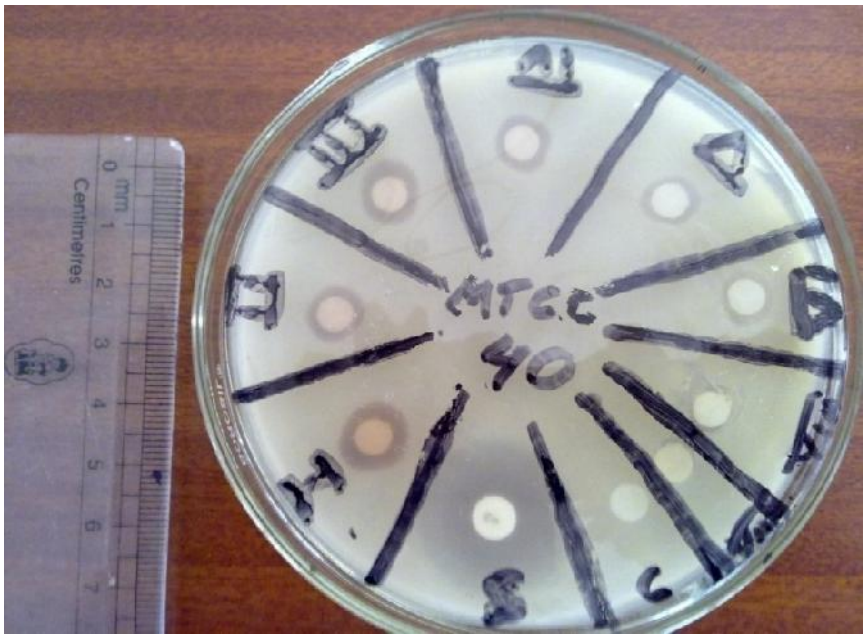


Photo 12 : Determination of MIC of the plant extract on *Escherichia coli*

I = 200 µg/disc II = 100 µg/disc III = 50 µg/disc IV = 25 µg/disc
V = 12.5 µg/disc VI = 6.25 µg/disc VII = 3.125 µg/disc S = 10 µg tetracycline/disc C = control

Photo 13 : Determination of MIC of the plant extract on *Micrococcus luteus*
I = 200 µg/disc II = 100 µg/disc III = 50 µg/disc IV = 25 µg/disc
V = 12.5 µg/disc VI = 6.25 µg/disc VII = 3.125 µg/disc S = 10 µg tetracycline/disc C = control

Photo 14 : Determination of MIC of the plant extract on *Pseudomonasaeruginosa*
I = 200 µg/disc II = 100 µg/disc III = 50 µg/disc IV = 25 µg/disc
V = 12.5 µg/disc VI = 6.25 µg/disc VII = 3.125 µg/disc S = 10 µg tetracycline/disc C = control

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CHAPTER 5

SUMMARIZATION AND CONCLUSION

The screening of plants usually involves several approach; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study (Joshi *et al.*, 2011). In the present study ethnobotanical approach and responses of the surveyed respondents were made basis to screen out top twenty potential ethno medicinal plants for phytochemical analysis in near future. The plant species *Hiptage benghalensis* (L.) Kurz scored maximum score and thus selected as candidate plant species for in depth phytochemical analysis in the present study.

Plant extracts and essential oils have been used for thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In vitro* studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many plant extracts has been previously reviewed and classified as strong, medium or weak (Zaika, 1998).

The present study includes extraction, thin layer chromatography, Column chromatography, structural elucidation through melting point IR, NMR, Mass spectrum and elemental analysis.

The root barks of the candidate plant were collected and thoroughly washed and made into powdered form and extracted with methanol using Soxhlet extractor, the extract was then used to test the phytochemical group, the test revealed that

different phytochemical group like alkaloids, tannins and reducing sugars are present in the candidate plant *Hiptage benghalensis* (L.) Kurz

Thin layer chromatographic studies indicates that in the selected solvent system the Dichloromethane : Methanol (9:1) is best suitable solvent for the column chromatography. The isolated compound crystal in Column chromatographic study is pale yellow in colour. The isolated crystal was identified by comparing the melting point, elemental analysis, IR, NMR and Mass spectra spectral datas from the literatures. The compound is identified to be tetrahydropalmatine, (-2,3,9,10-tetramethoxy - 6, 8, 13, 13a - tetrahydro- 5H- isoquinolino [2,1-b]isoquinoline). The molecular formula and molecular weight of the compound are found to be $C_{21}H_{25}NO_4$ and 355.428g/mol respectively.

The antimicrobial activity test was done on four(4) different bacteria-

Micrococcus luteus (MTCC – 106), *Klebsiella pneumonia* (MTCC – 39), *Escherichia coli* (MTCC – 40) and *Pseudomonas aeruginosa* (MTCC – 424) by disc diffusion method. The result showed that the plant has antimicrobial activity and it can be concluded that the methanolic extract of the selected plant may be due to the presence of alkaloid.

Intensive use of antibiotics often resulted in the development of resistant strains (Sydney *et al.*, 1980), these create a problem in treatment of infectious diseases, furthermore antibiotics sometimes associated with side effects (Cunha, 2001) whereas there are some advantages of using antimicrobial compounds of medicinal plants such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Vermani and Garg, 2002). Because of this, the search for new antibiotics continues unabated. These findings support the traditional knowledge of local users and it is a

preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources.

Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings. In conclusion, the results of the present study support the folkloric usage of the studied plant and suggests that some of the plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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2. National seminar on Economic and Educational Security With Reference to North East India(3rd – 4th March, 2010) at I & PR Conference Hall, Aizawl.