Chapter 1

INTRODUCTION
Chapter – 1

Introduction

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1. INTRODUCTION

1.1 ETHNOMEDICINAL PLANTS

The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas, the Bible and those obtained from folklore or traditional practices, has been traced to occurrence of natural products with medicinal properties. It has been confirmed by WHO that herbal medicines, based largely on different species of plants, serve the large needs of millions of people in vast rural areas of developing countries and also in developed world.

India ranks sixth among the 12 mega biodiversity centers of the world, and is home for an unusually large number of endemic species. It supports 15,000 species of flowering plants 5,000 of them exclusively providing shelter to 317 species of mammals [WCMC; 1992] Information on folk medicinal uses of the plants has recently become of renewed interest in the search for the therapeutic agents. India possesses large number tribal communities, amongst those few tribal communities live in Gujarat. Tribal communities are mainly the forest dwellers who have accumulated a rich knowledge on the uses of various forests and forest products over the centuries. Gujarat is home to nearly 7,000 species of plants and animals. Its geographic location is characterized by the mountain ranges of the Aravalies, the Vindhayns, the Satpuras, the Sahayadris and the longest coast line among three Indian states.

A vast knowledge on medicinal plants exists as oral among folklore and primitive societies of India, where a large number of potent medicinal herbs are found growing wild. Based on these considerations, there must be well defined medicinal approach to select natural products which are growing in Dang forest and not been explored much for their therapeutic uses. Many tribal communities dwell in forested belts of Gujarat ranging from Dangs in South to Banaskantha in North. The communities residing in these rich biodiversity areas have rich traditional wisdom of herbal medicines. Since diversity is the core strength of biological situation in Dang forests, the availability and use of medicinal flora is also very diverse and widespread. However, a need was
felt to undertake systemic study encompassing entire state to document human medicines practiced by these communities so that the existing gap in our appreciation of traditional wisdom can be filled and this rich tradition gets a better chance to survive.

Plants belonging to Dilleniaceae family are amongst many plants used by tribal communities of Dang forest and also even in many other forests of India, like Mizoram district, Vindhya region in M.P. Dilleniaceae family comprising of 16 genera and about 400 species which includes trees, shrubs or occasionally vines. The genus Dillenia has 60 species, of which *Dillenia indica*, *Dillenia pentagyna*, *Dillenia alata*, *Dillenia suffruticosa*, *Dillenia papuana*, *Dillenia excelsa*, *Dillenia serrata*, *Dillenia ovata*, *Dillenia philippinensis* etc. which are found to have good medicinal value.

In present investigation we selected two plants belonging to genus Dillenia i.e., *Dillenia indica* Linn. (*D. indica*) and *Dillenia pentagyna* Roxb. (*D. pentagyna*) which widely growing in Dang forest of Gujarat. These plants have been used by tribal and folk communities of India in treatment of different diseases. The leaf, bark, and fruit of these plants are used as traditional medicine is having good therapeutic values. These plants are being used by tribal and folk communities of various regions, fruits of *D. indica* as well as *D. pentagyna* also eaten raw but not much explored in common people [Dubey PC et al., 2009]. Many pharmacological studies has been reported like antioxidant, antibacterial, antidiarrhoeal, antibacterial, antitumour, cytotoxic activities etc. but proper phytochemical investigation needs to be done in order to explore the use of these plants. Although these plants are having great therapeutic value, plants are not much known and lack of information is available.

Herbal medicines which can be used freely by the local community and are well known through long usage by local population in terms of its composition, treatment and dosage are indigenous herbal medicines. If medicines in this category enter in market, they have to meet requirements of safety and efficacy as per national regulations [Kohli K & Jain GK, 2006]. As the folklore medicines are evolved by the individual and ethnic experiences, it needs further investigations in stipulations of diverse branches of medical science to endeavor the issues like that of
standardization, identification, pharmacology etc. [Dhanamani M, 2011]. The isolation, identification of active principles and pharmacological studies of the active phytoconstituents may be considered and studied elaborately to treat effectively for various types of diseases.

Although, a great amount of ethno medicinal research work has been undertaken in various pockets of tribal and rural population scattered throughout the country, more efforts are needed to enhance the utility of these herbs at global level.

“Cure the symptoms, cure the disease.” — Michael Critchton

1.2 STANDARDIZATION AND QUALITY CONTROL OF MEDICINAL PLANTS

“It is a truism that what you really value is what you miss, not what you have” - Jorge Luis Borges.

Elaborating above saying, Quality of Herbal medicines especially ethno medicinal and folklore medicines are extremely important for evaluation of therapeutic potential of plants. If we focus on development of quality parameters, herbal plants may serve the purpose of being most valuable source for disease management. A variety of reasons have been cited for the need of scientific evaluation, quality control and standardization. There is no uniform or standard procedure for maintaining inventory of herbal plants and the knowledge about their medicinal properties. [Govindarajan R & Vijayakumar M, 2006].

Throughout human history people relied on natural products and plants. But due to greater awareness to traditional knowledge of herbal medicinal plants, the allopathic medicines in many ways are replaced by the herbal traditional drugs. Thus, to promote this diversion, a number of research centers have been established, e.g. Medicinal Plant Association and Product Council, National Institute of Medicinal Herbalists, American Herbal Product Associations, Council for Scientific and Industrial Research, National Institute of Science Communication and Information
Sources, etc. The WHO has also estimated that 80% of population uses some or other form of herbal medicines in day to day preparations. [Kohli K & Amin S, 2006]

Over the years, physicians, pharmacists and patients have come to rely on plant drugs found in urban markets as well as from forests which may be adulterated. Therefore, the need for quality control and quality assurance is widely recognized to develop standard for subsequent use of medicinal plants. No pharmacopoeia in the world is comprehensive to cover all medicinal herbs and give concrete methodology for routine quality control [Ahuja PS et al., 2006]. Special emphasis is required to be paid to the establishment of quality standards of raw material generate comparative data with the materials actually available in Indian market. It can be achieved by applying well defined strategy and advance tools of modern science. Therefore, identity of plant species, time of harvest, drying, storage and packing are some important steps for producing quality herbal medicine having consistency and desirable therapeutic effects. [Tirpathi YB, 2006; WHO, 1998; WHO, 1999; WHO, 2001; WHO, 2003]

1.2.1 Qualitative and Quantitative aspects of herbal drugs

Herbal medicines are defined (as per WHO) on the basis of assessment of quality. The quality assessment includes pharmacopoeial assessment like official pharmacopoeias. Authentication of medicinal plants, foreign matters, organoleptic evaluation, microscopy, physicochemical parameters, microorganisms, chromatographic profiling as well as market components.

For standardization purpose three attributes are desirable:

- Authenticity/Identity (Right identity of the plants).
- Purity (Absence of adulterants or Substituent's).
- Quality/Assays (Chemical and Biological profiles).

Authenticity corresponds to the quality of raw material, whether it is of right identity, correct morphology, microscopy and chemical analysis. The purity refers absence adulterants present in plant material, which evaluated by developing pharmacognostical characteristics, physicochemical, assays treated to chemical and

Presence of foreign matters, pesticides and heavy metals in the finished products is also another issue, which must be targeted carefully. Steps must be taken as per WHO guidelines for keeping raw materials, devoid of these components. There is need to generate data-base on micro-organisms pesticides and heavy metal residue of these medicinal plants in order to meet WHO requirements and also to generate safety evaluation data. [Tirpathi YB, 2006]

1.2.2 Chemical background of herbal drugs

Herbal products are available as either raw materials or extracts of portions of the plants. Extraction involves boiling or percolating the herbs in water, alcohol or other solvents to release biologically active constituents of the plant [Govindarajan R & Vijayakumar M, 2006]. All plants produce chemical compounds as part of their normal metabolic activities. These can be split into two broad categories—primary metabolites and secondary metabolites. Secondary metabolites, which are much more specialized substances with wide variety of functions. These secondary metabolites produces therapeutic actions in humans, which can be refined to produce drugs [Chirangini P et al., 2006]. Both raw herb and extract contain complicated mixtures of organic chemicals, which may include flavonoids, glycosides, alkaloids, sterols, triterpenoids, tannins etc. [Rotblatt and ziment, 2002].

Determination of such metabolites chemical evaluation is necessary step for quality control. Chemical evaluation comprises of different chemical tests and chemical assays, the isolation, purification and identification of active constituents. [Govindarajan R & Vijayakumar M, 2006]. To ascertain the quantitative aspects of any medicinal plants, active constituents are to be considered. The quantitative estimation of herbal plants is carried out with novel techniques like TLC, HPTLC, HPLC etc. [Kohli K & Amin S, 2006].

So far, during the development, processing and circulation of herbal plants, there is no comprehensive and integrated quality control measure to reflect the variations of
Herbal products, and to effectively control the quality in the whole process. The research and establishment of fingerprints contributed much to solving the problem. The fingerprint analysis have been internationally accepted as one of the efficient methods to control the quality of herbal medicines [Liang YZ et al., 2004]. Scientific evidence can be developed for the medicinal plants by developing quality parameters. Further, the combination of qualitative fingerprinting and quantitative multicomponent analysis is a novel and rational method to address the key issues of quality control of herbal medicines. To facilitate such an approach, the procedures various techniques are recommended for herbal drug research.

1.3 PHYTOCHEMICAL TECHNIQUES IN HERBAL DRUG RESEARCH

Scientific validation of existing and new herbal drugs is extremely significant before they put to pharmacological and clinical applications. Standardized drugs provide authentic and reliable range of active principals. For developing method for evaluation of such active principles various techniques needs to be applied such as chromatography, advanced extraction procedures, spectroscopic techniques etc [Sabulal B & George V, 2006]. Fingerprinting of herbal medicines is utilized on the authenticity and quality control of HMs and the total producing process of herbal preparation. The combination of qualitative fingerprinting and quantitative multicomponent analysis is a novel and rational method to address the key issues of quality control of herbal medicine [Yongyu Z et al, 2011]. These techniques are broadly employed for successful isolation and characterization of phytochemicals described as follows:

Various conventional extraction methods like maceration, infusion, digestion, decoction, percolation, hot continuous extraction (Soxhlet), aqueous alcoholic extraction by fermentation, counter-current extraction and ultrasound extraction (sonication) are used for isolation of pharmacologically active compounds from plant sources. Standardization of extraction procedures contributes significantly to the final quality of the herbal drug. Recently besides it, so many methods are used for extraction purpose such as; Sonication-assisted extraction (Ultrasound assisted extraction (UAE), Solvent extraction, Counter Current Extraction (CCE), Accelerated Solvent Extraction (ASE), Phytonics Process, Solid Phase Micro-extraction (SPME)
etc. are applied for extraction of active constituents [Handa SS et al., 2008; Pai R et al., 2011; Nattaponga S et al., 2008; Chang CW, et al., 1999].

1.3.1 Ultrasound Extraction (Sonication)
The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases but its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules [Fermeglia et al., 2008].

1.3.2 Solid Phase Extraction (SPE)
Solid phase extraction (SPE) is applied for isolation of analytes from a liquid matrix and purified herbal extracts. This technique has many advantages such as: high recoveries of the analyte, concentration of analyte, highly purified extracts, ability to simultaneously extract analytes of wide polarity range, ease of automation, compatibility with instrumental analysis and reduction in organic solvent in comparison with more traditional sample preparation techniques [Choudhary N & Sekhon BS, 2011].

1.3.3 Accelerated Solvent Extraction
Accelerated solvent extraction (ASE), also referred as pressurized fluid extraction (PFE) and pressurized liquid extraction (PLE), is a liquid solvent extraction technique that uses aqueous and organic extraction solvents at elevated temperatures and pressures. Accelerated solvent extraction is performed by using the same solvents as in the traditional approaches, but at higher temperatures than is possible in these techniques. This increase in temperature improves the kinetics of the process, resulting in more efficient extraction (faster and using less solvent) compared with traditional approaches. The solvents are used under pressure so that their liquid state is maintained under heated conditions. Compared with conventional extraction times ranging from 4 to 48 h in length, ASE extractions are normally performed in 12 to 20 minutes. ASE method has been developed for a class of compounds, that same method can be applied successfully to a variety of matrix types without adjusting the
1.3.4 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is the most preferable process for the extraction of the bioactive chemical from the medicinal and aromatic plants. SFE has emerged as a highly promising technology for production of herbal medicines and nutraceuticals with high potency of active ingredients. SFE techniques have been found useful in isolating the desired phytoconstituents from the herbal extracts [Choudhary N & Sekhon BS, 2011].

A supercritical fluid can diffuse through solids like a gas, and dissolve materials like a liquid. The basic principle of Supercritical fluid extraction is that the solubility of a given compound (solute) in a solvent varies with both temperature and pressure. At ambient conditions (25°C and 1 bar) the solubility of a solute in a gas is usually related directly to the vapor pressure of the solute and is generally negligible. Unlike other processes, the extraction process leaves no solvent residue behind if CO₂ alone is used as a solvent. Moreover, the CO₂ is non-toxic, nonflammable, odorless, tasteless, inert, and inexpensive. CO₂ has a low surface tension and viscosity, and high diffusivity which make it attractive as a supercritical solvent. Solubility increases with increasing density (that is with increasing pressure). Rapid expansion of supercritical solutions leads to precipitation of a finely divided solid. This is a key feature of flow reactors. SFC is considered “green” because the carbon dioxide has been recycled and it replaces more obnoxious fluids. In many countries, stricter regulations regarding the use of organic solvents (i.e. hexane) to address safety, health and environmental issues is forcing the industry to search for alternative processes [Simandi B & Sawinsky J, 2013; York O et al., 2004].

1.3.5 Microwave Assisted Extraction (MAE)

MAE technology includes the extraction of high-value compounds from natural sources including phytonutrients, nutraceuticals and functional food ingredients and pharmaceutical actives from biomass. MAE finds utility in production of cost effective herbal extracts and helpful in extraction of various metabolites. [Choudhary N & Sekhon BS, 2011]
Microwave radiation interacts with dipoles of polar and polarizable materials. The coupled forces of electric and magnetic components change direction rapidly (2450 MHz). Polar molecules try to orient in the changing field direction and hence get heated. In non-polar solvents without polarizable groups, the heating is poor (dielectric absorption only because of atomic and electronic polarizations). This thermal effect is practically instantaneous at the molecular level but limited to a small area and depth near the surface of the material. The rest of the material is heated by conduction. Thus, large particles or agglomerates of small particles cannot be heated uniformly, which may be a major drawback of microwave heating. It may be possible to use high power sources to increase the depth of penetration. However, microwave radiation exhibits an exponential decay once inside a microwave-absorbing solid [Handa SS et al., 2008]

Compared to conventional solvent extraction methods, advantages of this technology include: improved product, purity of crude extracts, stability of marker compounds and use of minimal toxic solvents, reduced processing costs, increased recovery and purity of marker compounds, very fast extraction rates, reduced energy and solvent usage. [Choudhary N & Sekhon BS, 2011]

1.3.6 Spectroscopic techniques
Fingerprinting and marker compounds are used for identification and standardization of botanical drugs. Chemical and chromatographic techniques may be used to aid in identification of an herbal material or extract. Chromatographic techniques such as HPLC, TLC, GC and capillary electrophoresis and spectroscopic methods such as IR, NMR, and UV also used for fingerprinting. Marker compounds may be used to identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes and obtain stability profiles. [Sabulal B & George V, 2006]

1.3.7 High Performance Thin Layer Chromatography (HPTLC)
TLC is the preliminary fingerprint method for herbal analysis. With this technique, authentication of various species is possible, as well as the evaluation of stability and consistency of their preparations from different manufactures. HPTLC technique is widely employed in pharmaceutical industry in process development, identification
and detection of adulterants in herbal product and helps in identification of pesticide content, mycotoxins and in quality control of herbs and health foods. High Performance Thin Layer Chromatography (HPTLC) is a sophisticated and automated form of TLC. Main Difference between HPTLC and TLC is particle size and pore size of sorbents. Thin Layer Chromatography (TLC) is a solid-liquid technique in which the two phases are a solid (stationary phase) and a liquid (moving phase). HPTLC is a sensitive, fast, simple and inexpensive analytical technique. TLC involves spotting the sample to be analysed near one end of a sheet of glass, aluminium or plastic that is coated with a thin layer of an adsorbent. The sheet is placed on end in a chamber containing a shallow layer of solvent. As the solvent rises by capillary action up through the adsorbent, differential partitioning occurs between the components of the mixture dissolved in the solvent the stationary adsorbent phase [Anyakora, Chimezie, et al., 2008; Ganesan M et al., 2012; Rao UB, 2009].

Chromatographic methods are required for method development, which are rapid, fit for purpose, inexpensive, and reliable. Steps for method development includes selection of stationary phase, selection of mobile phase, selection of visualization technique and optimization of TLC separations [Chimezie A et al., 2008; Saravanan J et al., 2010; Rao UB, 2009]. Method Validation includes Specificity, Linearity, Range, Accuracy, Precision, Repeatability, Reproducibility, Robustness and System Suitability Testing. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose [ICH, 2006]

1.3.8 High Pressure Liquid Chromatography (HPLC)
Preparative and analytical HPLC are widely used in pharmaceutical industry for isolation and purification of herbal compounds. There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high pressure HPLC (pressure >20 bar). The important parameters to be considered are resolution, sensitivity and fast analysis time in analytical HPLC whereas both the degree of solute purity as well as the amount of compound that can be produced per unit time i.e. throughput or recovery in preparative HPLC [Chimezie A et al., 2008; Saravanan J et al., 2010; Rao Udaykumar B, 2009]

High performance liquid chromatography (HPLC) is an important tool for the analysis
of pharmaceutical drugs, for drug monitoring and for quality assurance. HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector that provides a characteristic retention time for the analyte. The detector may also provide other characteristic information (i.e. UV/Vis spectroscopic data for analyte if so equipped). Analyte retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent(s) used, and the flow rate of the mobile phase. It is a form of liquid chromatography but smaller column size, smaller beads inside column and higher pressures. The hyphenated technique is developed from the coupling of a separation technique and an on-line spectroscopic detection technology. The remarkable improvements in hyphenated analytical methods like LC-IR, LC-MS, LC-NMR over the last two decades have significantly broadened their applications in the analysis of chemicals, biomaterials, especially natural products. 45, 50, 51, 52, 53

1.3.9 Other recent advances in techniques for herbal drug research

Other recent advances in method includes chromatography like Gas chromatography (GC) and GC-MS and related techniques, electro-migration techniques (e.g. electrophoresis, electro-chromatography), ultra-performance (UPLC) have increased the analysis efficiency, so that shorter analytical time and better separation have been achieved (Zheng X.T. et al., 2008; Chen J.H. et al., 2008) hyphenated and other multi-dimensional techniques, sample preparation etc. Chemical fingerprints obtained by chromatographic, spectroscopic, thermogravimetric analysis, capillary electrophoresis and polarography techniques have become the most potent tools for quality control of traditional herbal medicines. Moreover, all herbal products manufacturers must follow WHO guidelines for quality control. Further, the combination of qualitative fingerprinting and quantitative multicomponent analysis is a novel and rational method to address the key issues of quality control of herbal medicines. The advancement of analytical techniques will serve as a rapid and specific tool in the herbal research, thereby, allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf life of herbal drugs. The applications of high-technology oriented advanced hyphenated techniques will serve as a rapid and unambiguous tool in the herbal research, thereby, benefiting the entire pharmaceutical industry [Choudhary N & Sekhon BS 2011].
1.4 PHARMACOLOGICAL SCREENING OF HERBAL DRUGS

Ethno medicinal herbs are used by tribal communities and only oral information regarding therapeutic uses of plants available. If plants are collected from forest region, checking purity as well as safety of plants is a crucial step. The safety and efficacy data are available for few herbs, their extracts and active ingredients and the preparation containing them. Notwithstanding the potential pharmacological benefits of medicinal plants in general, herbal pharmacopeia are largely lacking in providing detailed information regarding therapeutic uses of plants.

There are many pharmacological screening tests available. In the random selection program in the US, plants are randomly selected, extracted, and the extracts are evaluated for its therapeutic potential. An extension of this procedure is to isolate metabolites or “active compounds” from the plant that had shown most promising activity and subject them to pharmacological tests. In another approach, plants containing specific types or classes of chemical compounds, for example alkaloids, are tested. Simple tests such as colour reactions are carried out on various parts of the plant in the field, and assays are carried out in the laboratories [Farnsworth NR et al., 1977]. In terms of cost–benefit ratio, these “shotgun” approaches are considered to be very unsatisfactory.

Another method involves random collection of plants and subjection of their extracts to several broad screening methods and pharmacological tests. The success of this method depends on the number of samples assayed, adequate funding, and appropriate predictable bioassay protocols. Broad-based empirical screening, which is time consuming and expensive, can detect novel activities but is not suited for screening large numbers of samples [Farnsworth NR et al,1977; Bandaranayake WM, 2006; Wagner AH et al., 1977; Aswal BS, 1977].

The proofs of pharmacology activity that are available at present are mostly based on empirical experience. The scientific proof then becomes the most important thing, in order to eliminate the concern of using medicinal plants as drugs for alternative treatment [Adotey JP et al., 2012]. The main problem facing the use of traditional medicines is the proof requirement that the active components contained in medicinal
plants are useful, safe, and effective. This is required to assure the medical field and the public regarding the use of medicinal plants as drug alternatives.

FIGURE 1.1 Authentication and standardization of herbal raw material (Handa SS, 1997)
1.5 REFERENCES


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