1.1 MEDICINAL PLANTS IN DRUG DISCOVERY

1.1.1 History

The use of plants as medicine goes back to early man. Evidences of this early medicinal value association have been found in the grave of a Neanderthal man buried 60,000 years ago after pollen analysis of the numerous plants buried with the corpse. The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. Along with this early medicinal knowledge Pun-tsao, a pharmacopoeia of ancient China which was published around 1600, contained thousands of herbal cures that are attributed to the works of Shen-nung.

In India, ‘Rig-Veda’ the collection of Hindu sacred verses led to a system of health care known as Ayurvedic medicine. One of the useful plants from this body of knowledge is snakeroot, Rauwolfia serpentina, used for centuries for its sedative effects. Today the active components of snakeroot are widely used in Western medicine to treat high blood pressure. In all parts of the world, indigenous people discovered and developed the medicinal uses of native plants, but it is from the herbal medicine of ancient Greece that the foundations of Western medicine were established. Western medicine can be traced back to the Greek physician Hippocrates (460-377 BC), known as the Father of Medicine who believed that a disease had a natural cause and used various herbal remedies in his treatments. Early Roman writing also influenced the development of Western medicine, especially the works of Dioscorides (1st century AD). Although Greek by birth, Dioscorides was a Roman military physician who compiled this information in De Materia Medica, which contained an account of over 600 species of plants with medicinal value. Different therapies followed in different countries which include traditional Chinese medicine (TCM), Japanese medicine (Kampo), Korean traditional medicine, jamu (Indonesia), and Ayurvedic medicine (India), and in Europe, phytotherapy and homeopathy have found medicinal uses.

According to data of the Food and Agriculture Organization (FAO), more than 50000 plant species are being used in the traditional folk medicine throughout the world (Schippmann et al., 2002). The highest percentage of native flora species used for
medication was observed in countries of Southeast Asia, such as India (20%) and China (19%). In the United States and Russia, slightly more than 10% of plant species are used for therapeutic purposes. Studies (1775-1785) of foxglove as a treatment for dropsy (congestive heart failure) by William Withering, was the first in the medical field to scientifically investigate a folk remedy which set the standard for pharmaceutical chemistry. In the nineteenth century a breakthrough in pharmaceutical chemistry came when Friedrich Sertturner isolated morphine from the opium poppy (*Papaver somniferum*) in 1806. After that many such similar developments followed. Quinine from cinchona tree had its origin in the royal households of the South American Incas. In 1860, a German chemist Carl Koler isolated cocaine from Coca (*Erythroxylum coca*), the chemical responsible for the biological activity. He found that cocaine could act as a local anaesthetic in eye surgery. As a local anaesthetic, it revolutionized several surgical and dental procedures. The Jaborandi tree (*Pilocarpus jaborandi*) secretes alkaloid rich oil. Several substances are extracted from this aromatic oil, including the alkaloid pilocarpine, a weapon against the blindness disease, glaucoma. American Indians on the island of Guadeloupe used pineapple (*Ananas comosos*) poultices to reduce inflammation in wounds and other skin injuries, to aid digestion and to cure stomach ache. In 1891, an enzyme that broke down proteins (bromelain) was isolated from the fresh juice of pineapple and was found to break down blood clots. Other pharmaceuticals that have their origin in botanicals include atropine, hyoscine, digoxin, colchicine and emetine. Consequently with increased knowledge of active chemical ingredients, the purely synthetic drugs based on natural products were formulated in the middle of the nineteenth century viz. in 1839, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities and was first synthesized in 1853. This led to the development of aspirin, which is the most widely used synthetic drug today. It is pertinent to note that most of these early discoveries are mainly based on traditional medicines.

Hence, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics. The future of natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be accrued to the patients and the community (Kong Jin-Ming et al., 2003).
1.1.2 Medicines from nature: Natural products and drug discovery

Despite the above mentioned evidence of drugs from medicinal plants, much of the debate is going on the future prospects of medicinal plants for therapeutic use due to different reasons. Sneader (1996) reported that natural products have been the source of most of the active ingredients of medicines and this is widely accepted since olden times even before the advent of high-throughput screening and the post-genomic era. More than 50% of drug substances are natural products or inspired by natural compounds. Newman and Cragg (2007), on the basis of the information presented on sources of new drugs from 1981 to 2007, reported that almost half of the drugs approved since 1994 are based on natural products. On further detailed analysis Newman and Cragg (2007) reported that among 847 low molecular weight medicines introduced into the practice since 1981, 43 agents belong to natural compounds, and 232 agents are derivatives of natural substances. Out of remaining 572 preparations, 262 preparations are originally linked to natural compounds (Newman and Cragg, 2007). Based on very recent information Butler (2008) reported that thirteen natural product related drugs were approved from 2005 to 2007, and, five of these represented the first members of new classes of drugs viz. the peptides exenatide, ziconotide and the small molecules ixabepilone, retapamulin and trabectedin. Ongoing projects also evidenced the utility of natural products in medicine. Over a 100 natural product derived compounds are currently undergoing clinical trials and at least 100 similar projects are in preclinical development. Most of these leads are derived from plants and microbial sources (Butler, 2008).

From the point of view of therapeutic areas, these natural products are useful in 87 % i.e. natural products are useful 48 out of 55 (48/55) therapeutic areas of human including main areas like antibacterial, anticancer, anticoagulant, antiparasitic, and immunosuppressant agents. During 1981 to 2002, there was no introduction of natural products or related drugs for 7 drug categories viz. anesthetic, antianginal, anti-histamine, anxiolytic, chelator and antidote, diuretic, and hypnotic (Newman et al., 2003). More specifically, natural products dominant role is evident in the approximately 60% of anticancer compounds and 75% antiinfective compounds. Of the 90 antiinfective drugs that became commercially available in the United States or were approved worldwide from 1982 to 2002, ~79% traced to a natural product origin (Cragg et al., 2005).
According to World Health Organization (2002) more than 90% of therapeutic classes derive from a natural product prototype and roughly two-thirds to three quarters of the world’s population relies upon medicinal plants for its primary pharmaceutical care.

Some of the prominent commercial plant-derived medicinal compounds include colchicine, betulinic acid, camptothecin, topotecan (Hycamtin®), CPT-11 (Irinotecan, Camptosar®), 9-aminocamptothecin, delta-9-tetrahydrocannabinol (Dronabinol, Marinol®), β-lapachone, lapachol, podophyllotoxin, etoposide, podophyllinic acid, vinblastine (Velban®), vincristine (Leurocristine, Oncovin®), vindesine (Eldisine®, Fildisin®), vinorelbine (Navelbine®), docetaxel (Taxotere®), paclitaxel (Taxol®), tubocurarine, pilocarpine, scopolamine (Patwardhan et al., 2004).

Many such examples motivate us for discovering new drugs with chemical diversity from natural resources based on Ayurvedic principles and ethnopharmacology.

1.1.3 Traditional Wisdom – Ayurveda

As mentioned in earlier sections traditional medicine has been having a big impact on human health all over the world and Ayurveda remains one of the most ancient medical systems widely practiced in the Indian subcontinent and has a sound philosophical, and experimental basis. Ayurveda (Ayur: Life; Veda: Science, means science of life in Sanskrit) aims at holistic management of health and disease. Atharvaveda (around 1200 BC), Charak Samhita and Sushrut Samhita (1000–500 BC) are main Ayurvedic classics, which describe over 700 plants along with their classification, pharmacological and therapeutic properties with a commentary from modern medicine and scientific viewpoint, gives some glimpses of ancient wisdom (Valianthan, 2003). Nearly 5800 clinical signs and symptoms are available in Ayurvedic texts. More than 1200 species of plants, nearly 100 minerals and over 100 animal products comprise the Ayurvedic pharmacopoeia. Thousands of single drugs, multiple combinations and processed formulations are described in Ayurvedic literature along with details of drug actions. Indian healthcare consists of medical pluralism and Ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions (Waxler-Morrison, 1988).

This wisdom helped to create Ayurvedic database by CSIR (www.tkdl.res.in). Exhaustive information is available in Ayurvedic literature that can be converted into a large database giving information of various foods, herbs, and medicines with their
taste, actions and utility in different disorders (Moringstar, 1990). It can be used for bioprospecting to identify new sources of medicine and to provide information about likely effects ranging from primary taste to its post-digestive effects. Information about safety, efficacy along with possible indications and contraindications is also provided. Valuable information of therapeutic potential and selective benefits to people with different constitutions can be obtained from this database. This will greatly facilitate intentional, focused and safe natural products drug discovery and development.

1.1.3.1 Approaches for drug discovery by utilizing Ayurvedic concepts

- A recent example of this is an innovative method developed to provide quantitative representations of various Ayurvedic concepts of medicinal plants, including, Prakruti, Rasa, Guna, Virya, Vipak etc., has been developed by the Indian Institute of Chemical Technology, Hyderabad. This patented technology has been registered as Herboprint and essentially gives a three dimensional HPLC fingerprint of these plants with Ayurvedic property profile (Vijay Kumar, 2002).

- Patwardhan (2003) coined the term Ayurgenomics, based on the information available in Ayurvedic texts and understanding of the human genome, which helped in understanding scientific basis of individual variation. Ayurgenomics includes person as a whole concept which describes the basis of individual variations and it has clear similarities with the pharmacogenomics that is expected to become the basis of designer medicine. Medical practice has become more predictive, individual and customized. For years physicians have noted these individual differences, but had no way to predict them. Pharmacogenetics is the study of the hereditary basis for differences in response of populations to a drug. The same dose of a drug will result in elevated plasma concentrations for some patients and low concentrations for others. Some patients respond well to the drugs, while others do not. A drug might show adverse effects in some patients, but not in others. Populations and enzyme polymorphisms are known. Importance of such individual variations in health and disease is an important basic principle of Ayurveda and was underlined by Charaka some 4000 years ago as follows: ‘Every individual is different from another and hence should be considered as a different entity. As many variations are there in the Universe, all are seen in Human being. Understanding
the possible relationship between Prakruti and genome is important. Functionally, this involves creation of three organized databases that are capable of intelligently communicating with each other to give a customized prescription. These are human constitution (genotype), disease constitution (phenotype) and drug constitution (Patwardhan, 2003).

Long before these budding concepts have been applied for drug discovery from Ayurveda, considerable amount of research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on Ayurvedic medicinal plants (Patwardhan et al., 2004). Numerous molecules have come out of Ayurvedic experimental base, including Rauwolfia alkaloids for hypertension, psoralens from Psoralia corylifolia for vitiligo, Holarrhena alkaloids in amoebiasis, guggulsterons as hypolipidemic agents, Mucuna pruriens for Parkinson’s disease, piperidines as bioavailability enhancers, bacosides for mental retention capacity, picrosides for hepatic protection, phyllanthins as antivirals, curcuminoids for inflammation, withanolides and many other steroidal lactones and their glycosides as immunomodulators (Patwardhan, 2000). Keeping this success in mind, efforts are underway to establish evidence based therapeutic and safety practice of Ayurvedic medicine. Development of standardized herbal formulations is underway as an initiative of the Council for Scientific and Industrial Research (CSIR) New Millennium Indian Technology Leadership Initiative (NMITLI). Randomized controlled clinical trials for rheumatoid and osteoarthritis, hepatoprotectives, hypolipidemic agents, asthma, Parkinson’s disease and many other disorders have reasonably established clinical efficacy. These excellent evidence-based researches and approaches have now resulted in wider acceptance of Ayurvedic medicines (Chopra, et al., 2007). Thus the Ayurvedic knowledge database allows drug researchers to start from a well-tested and safe botanical material. The Ayurvedic texts are valuable and the basis of usefulness of traditional medicine is in its use for a number of years and therefore its clinical existence comes as a presumption (Patwardhan and Hooper, 1992). However, for bringing more objectivity and also to confirm traditional claims, systematic clinical trials are necessary. In Ayurvedic medicine research, clinical experiences, observations or available data becomes a starting point. In conventional drug research, it comes at the end. Thus, the drug discovery based on Ayurveda follows a ‘reverse pharmacology’ path (Vaidya et al., 2001).
Ayurvedic knowledge and experimental database can provide new functional leads to reduce time, money and toxicity – the three main hurdles in drug development.

### 1.1.4 Ethnopharmacology

One of the approaches of utilization of the above mentioned Ayurvedic principles is ethnobotanical and ethnopharmacological studies which involves field explorations of indigenous medical knowledge and biodiversity that can serve as an innovative and powerful discovery engines for newer, safer and affordable medicines (Soejarto et al., 2005; Patwardhan, 2005). This resulted in cultural acceptability and importance of traditional medicine, along with perceptions of affordability, safety and efficacy which played a role in stimulating scientific research and validation of traditional medicines and dramatic increase in use of herbal medicines in developing countries (WHO, 2002; Vandebroek et al., 2004; Vicente et al., 2007).

There are a few examples to support this. Gerard’s *Herball*, first published in 1597, yielded 16 currently prescribed drugs (Cox, 1998). There are ethnomedical reports on about 14,300 species of plants in NAPRALERT (about 5.2% of all plant species), and 58% of these species have never been examined biologically or chemically (Cordell and Quinn-Beattie, 2005). Yet, of those plant-derived products currently available as prescription products, 74% are used in a manner which equals their ethnomedical use (Fabricant and Farnsworth, 2001).

Ethnopharmacology investigations involve traditional healers, botanists, anthropologists, chemists and pharmacologists. But some groups of researchers played major role than physicians in ethnopharmacological investigations. Historical data shows that discovery of several important modern drugs of herbal origin owe to the medical knowledge and clinical expertise of physicians but rising cost of modern drug development is attributed to lack of classical ethnopharmacological approach. Physicians can play multiple role in the ethnopharmacological studies to facilitate drug discovery as well as to rescue authentic traditional knowledge of use of medicinal plants. These include: (1) Ethnopharmacological field work which involves interviewing healers, interpreting traditional terminologies into their modern counterparts, examining patients consuming herbal remedies and identifying the disease for which an herbal remedy is used; (2) Interpretation of signs and symptoms mentioned in ancient texts and suggesting proper use of old traditional remedies in the
light of modern medicine; (3) Clinical studies on herbs and their interaction with modern medicines; (4) Advising pharmacologists to carryout laboratory studies on herbs observed during field studies; (5) Work in collaboration with local healers to strengthen traditional systems of medicine in a community. In conclusion, physician’s involvement in ethnopharmacological studies will lead to more reliable information on traditional use of medicinal plants both from the field and the ancient texts, more focused and cheaper natural product based drug discovery, as well as bridge the gap between traditional and modern medicine.

1.1.5 Chemical diversity

The plants evolved as chemical factories capturing energy from the Sun for the production of a large variety of compounds that are needed not only for the construction and functioning of the plant but also for plant defense against adverse environmental factors and for strengthening the competitiveness of a given species in the plant community (Raskin et al., 2002). The chemical defense is almost the only effective instrument in the struggle of plants against pathogenic organisms and multiple herbivorous animals. For the effective defense against pathogens, plants have developed a complicated system comprising structurally different chemicals with different mechanisms of action. These biologically relevant properties of natural products are likely to continue to be sources of new commercially viable drug leads. The large proportion of natural products in drug discovery stemmed from the diverse structures and the intricate carbon skeletons of natural products. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more drug-likeness and biological friendliness than totally synthetic molecules (Koehn and Carter, 2005) making them good candidates for further drug development (Balunas and Kinghorn, 2005; Drahl et al., 2005).

Along with this biological relevance, the chemical novelty associated with natural products is higher than that of any other source. Synthetic chemistry has less than 40% of the chemical scaffolds that are reported in natural products database, Dictionary of Natural Products, Chapman & Hall.. This is particularly important when searching for lead molecules against newly discovered targets for which there are no known small-molecule leads. Feher and Schmidt (2003) reported that approximately 5750 different natural product skeleta, from the perspective of interactions with enzymes and receptors, represent substantially greater chemical diversity space and is more
reflective of the chemical diversity space of drugs, compared with the known range of combinatorial compounds. These facts proved that natural products have an edge over combinatorial libraries. Comparative analysis of structural diversity in natural product mixtures and combinatorial libraries suggests that nature still has an edge over synthetic chemistry, despite the fact that combinatorial libraries use more nitrogen, phosphorus, sulfur, and halogens. Natural products generally have higher molecular weight and exhibit a different distribution of heteroatoms. They comprise structural elements that are under-represented by synthetic compounds and contain significantly more rings and chiral centers. Besides their sterically complex structures natural products are distinguished by a characteristic combination of pharmacophoric groups which differs strongly from those of drugs and synthetics. (Henkel et al., 1999).

Although natural product libraries are competing with synthetic chemistry for drug discovery, natural product scaffolds are being used as cores of compound libraries made by combinatorial techniques. There are several examples of libraries based on alkaloids, polyketides, terpenoids (Boldi, 2004) and flavonoids (Yao et al., 2007). It is generally believed that the complexity of plant-produced secondary metabolites and the vast number of natural products will constitute a resource beyond the capacity of current synthetic chemistry for a long time (Koch et al., 2005)

1.1.6 Standardization

Earlier for the traditional medicinal plants which were being marketed all over the world as phytotherapeuticals, the quality control was typically very poor or non-existent. This is because there was absence of clear and harmonious regulations regarding quality control and marketing of herbal drugs. The issues of safety and efficacy were being both understated and neglected for traditional medicines. Consumers need to be assured of the authenticity, safety, efficacy, and shelf-life of any herbal preparation. Keeping this in mind many government organizations and authorities like World Health Organization (WHO, 2000), European Agency for the Evaluation of Medicinal Products and European Scientific Cooperation of Phytomedicine (Anonymous1, 2001), US Agency for Health Care Policy and Research (Anonymous2, 2000), European Pharmacopoeia Commission, Department of Indian Systems of Medicine have started creating new mechanisms to induce and regulate quality control and standardization of botanical medicine. WHO aimed to harmonize the terms being used, to summarize the issues for developing research methodologies,
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to improve the quality and value of research in traditional medicine, and to provide appropriate evaluation methods to facilitate the regulation and registration of traditional medicines. For Ayurvedic medicine and other traditional medicines, newer guidelines of standardization are stated.

On a batch-to-batch basis there must be botanical, chemical, and biological standardization of products, and collateral studies which would establish the safety, efficacy and shelf-life of the product. A botanical drug or a preparation thereof is now regarded as one active substance in its entirety, whether or not the constituents with therapeutic activity are known. In such cases, the concept of active markers in the process of standardization needs a flexible approach in favour of the complex nature of these materials. This will be a major step in the development of new generation standardized botanical medicines.

1.1.6.1 New analytical techniques for standardization

- Multicomponent analytical systems like HPLC/ESMS/NMR have a significant impact in the area of routine chemical standardization.
- Multi-component botanical formulations can be standardized with newer techniques such as DNA fingerprinting, high performance thin layer chromatography (HPTLC), liquid chromatography–mass spectroscopy.
- Real time PCR analysis on a microchip become a standard procedure for the authentication of plant materials (Carles et al., 2001; Zhang et al., 2005).
- Quick, cheap, accurate, and clinically relevant biological systems, mostly micro array-based, demonstrate the level of biological activity for each batch of marketable product (Prasad et al., 2005).

With these techniques inhouse monographs need to be evolved and critically followed.

1.1.7 Problems related to natural product drug discovery and strategies to overcome these problems

As mentioned in earlier sections natural products have historically provided many novel drugs, and they are expected to play pivotal role in the drug discovery strategy of pharmaceutical companies in future. Pharmaceutical industry has consistently voided aspects of natural product research indicating several reasons of variable cogency (Cordell and Colvard, 2007). However, most big pharma companies have terminated or
significantly scaled down their Natural Product operations in the last 10 years (Butler, 2004; Cordell, 2002; Strohl, 2000; Harvey, 1999).

This fall in drug discovery from natural products reflects in some of the recent reviews. Analysis of database on natural products by Harvey, 2008 revealed that in 2008 about 108 natural product drug discovery projects are under different developmental stages as compared to 312 projects in 2001. This indicates a drop of about 30% in natural-product-based development projects between 2001 and 2008 (Harvey, 2008).

![Figure 1.1: FDA drug approvals. New molecular entities and biologic license applications approved by the US FDA by year (Cordell and Colvard, 2005).](image)

Harvey (2007) reported a downslope in FDA drug approvals from 40 in 1996 to 20 in 2006 (Figure 1.1), and that is responsible for decline in natural product-based drug discovery in pharmaceutical industry.

**1.1.7.1 Problems**

The reason behind this fall in natural product drug discovery in pharmaceutical industry has been analyzed by some experts and concluded that bioprospecting from plants and other organisms is losing to many issues.

*Social and ethical issues*

At least 25 % of all modern drugs originally came from rainforests. Still it is not sure, whether these drugs will make major impact on future drug discovery due to a few social and ethical issues. Firstly, as only less than 1 % of the world’s tropical forest plants have been tested for pharmaceutical properties, there is fear of widespread destruction of these ecosystems and it threatens to eliminate thousands of species that have never been scientifically investigated for medical
potential. Secondly, the drugs that were being developed were not for global population, but for a privileged few people in the developed and developing world. Most of them were first discovered and used by indigenous people (Cordell and Colvard, 2007).

*High-throughput drug discovery*

Since 1990s there was arrival of high-throughput drug discovery. This high-throughput drug discovery relies on combinatorial chemistry (Adang and Hermkens, 2001; Schreiber, 2000) and computational drug design (Clark and Pickett, 2000). The basic premise was that combinatorial chemistry would generate libraries consisting of millions of compounds, which would be screened by high throughput screening (HTS) and produce drug leads by sheer weight of numbers. The leads would be delivered in quicker time and in greater numbers for all therapeutic areas as compared to traditional drug discovery methods, and as a consequence, most of the big pharma companies quickly changed their drug discovery strategies to include a significant proportion of combinatorial chemistry (Balkenhohl et al., 1996; Lee and Breitenbucher, 2003). On the contrary, the availability of compounds from their biological source, the chemical complexity and stereochemistry, compound stability, unreliability in assay systems and the most named reason among others: natural product research is very cost intensive makes natural products unfit for high-throughput drug discovery (Mishra et al., 2008). Hence there is a decline in drug discovery from natural products. However, during the period 1981–2002 there has not been a single *de novo* combinatorial compound approved as a drug (Maureen, 2003). Thus, high-throughput drug discovery created obstacle in natural product drug discovery.

*Natural product discovery is time consuming*

Major pharmaceutical companies were not interested in the evaluation of plant extracts. The reasons are quite simple. Firstly, when an extract shows activity in a bioassay, the active principle must be isolated and characterized. This is expensive and may take a long time, depending on the availability of appropriate amount of extract or plant material, the time for bioassay, and the ease of unambiguously determining the structure (Corley and Durley, 1994). By
this time, the synthetic “hits” will have moved to the next stage of decision-making and the natural product is left behind.

*Lack of reproducibility*

The lack of reproducibility of activity for more than 40% of plant extracts (Cordell, 2000) is one of the major obstacles in using plants in pharmaceutical discovery, despite the great diversity of compounds they synthesize. The activities detected in screens often do not repeat when plants are resampled and extracted. Moreover, the biochemical profiles of plants harvested at different times and locations vary greatly. This, in turn, creates a major difficulty for the prioritization, characterization, and isolation of active compounds. Complex plant extracts complicate the determination of potency and novelty of the active ingredient, which is often present in trace amounts and obscured by pigments and polyphenols that interfere with many screens.

*Pharmacokinetic issues*

Traditional pharmacokinetics methods cannot lead to discovery of the pharmacokinetics properties of plant products, due to lack of knowledge on their active components. Minimal effective dose and minimal toxic dose of certain plant products are completely derived from clinical experiences or ancient books (Ko, 2004; Siow et al., 2005). In addition, traditional pharmacokinetics methods using animal models in drug discovery and development may not be suitable for plant products, although they have been clinically used for thousands of years. Species difference cannot be excluded by these methods. Therefore, a human-derived evaluation system is urgently needed in the development of plant products, by which quantitative and accurate evaluation of pharmacokinetics properties of plant products can be achieved.

*Natural products screening*

There are about 250,000 species of plants in the world and around 10% only have been tested for some type of biological activity or the other (Verpoorte, 1998). Even fewer of these have gone through extensive HTS programmes. This is partly because of the difficulties perceived in using complex plant extracts in HTS. Biochemical assays are too sensitive to screen complex extract mixtures. Natural extracts contain chemically reactive compounds that are
inappropriate for biochemical screens because they tend to modify target proteins covalently, inducing false-positive results in the assay (Rishton et al., 1997).

**Quantity of pure chemical substances**

Concern over the availability of enough quantity of a chemical entity required for development and market needs of natural products, has been the one most limiting factor for the pharmaceutical industry’s interest in natural products. Market demand can reach a scale of hundreds to thousands of kilograms per annum. Total synthesis will not economically provide the complex natural product to meet this market demand.

### 1.1.7.2 Solutions and strategies

After carefully examining the reasons behind the decline in natural product drug discovery, some strategies and solutions had been put forward by experts for drug discovery from natural products. We need to adopt and implement these strategies to make most from the natural resources.

**Collaborative work**

In natural product sciences there is a need to create alliances, both locally and globally. This topic was discussed previously in various formats by Cordell (1990, 1993a, 1995a, 2000a). There is a great need for government research funding sources in Asia, Europe, and South America, as well as international funding agencies, to develop programs which can bring together academic and industrial researchers to address their national issues in a focused manner. Many of these alliances are operating formally or informally at the present time between academic institutions and industries. Development of selected libraries of natural product extracts and compounds and the screening of corporate libraries against inaccessible bioassays are the areas for collaboration (Short, 2002; Borman, 1997, 2001). Merlion Pharmaceuticals in Singapore is one of the examples which has a unique and very large collection of natural product samples available for evaluation against corporate bioassays.

**Utilization of botanical resources**
Cordell mentioned on several occasions, that there is a tremendous waste of manpower and resources in bringing dried plant materials back to the laboratory for biological evaluation and to establish a library of plant extracts (Cordell, 1990, 1995a, 2000a, 2002). Marine drug discovery groups used strategy of in-field biological evaluation of materials more efficiently. There is a need to develop simple genomic-based tests for plant extracts so that when activity is observed, collection can take place of the same plant population. Consequently, it will be only those plants which show activity that will be collected, dried, and brought to a laboratory for further chemical and biological evaluation. Such studies would also require in-field access to large database systems, such as NAPRALERT, in real time to assess prior knowledge.

Access to biodiversity

In Rio de Janeiro, the Earth Summit (3–14 June 1992) was held where introduction of the United Nations Convention on Biological Diversity (CBD) (Baker, 1998) took place in which concern regarding loss of biodiversity was highlighted. The CBD recognized that countries have sovereign rights over the biological resources within their boundaries and sets out conditions for the preservation and sustainable use of biodiversity. Biodiversity-rich countries have to facilitate access to the biological resources, but access must be in accordance with appropriate legislation, involving prior informed consent. The source country should be involved in R&D projects relating to its biodiversity, benefit from technology transfer and share any commercial benefits resulting from the use of its biodiversity. Since 1992, over 180 countries have ratified. Since the introduction of the CBD, there has been little progress in accessing plant material from more diverse geographical areas. These include the collection programme of the National Cancer Institute (Frederick, MD, USA) (Suffness et al., 1995), Bioresources Development and Conservation Programme (Silver Spring, MD, USA) in parts of Africa (Iwu, 1996) and the Indian Council for Scientific and Industrial Research’s Coordinated Programme on Bioactive Molecules from Natural Product Sources New Delhi, India. They focus on the exploration of plants that have a history of use in systems of traditional medicine.
New techniques

Technology investments at Bristol–Myers Squibb have played a crucial role in the progress of drug discovery significantly and more successfully (Houston et al., 2008). Following are some of the techniques applied for the natural products.

- Introduction of microarray analysis helped to learn about the effects of traditional medicinal plants on the human genome and the difference in effectiveness between the pure active compounds and plant extracts became clearer.
- Cordell and others had developed a partial approach to this latter issue using a dereplication protocol (Cordell, 2003), involving a HPLC/electrospray mass spectral/bioassay/database system. This process eliminated 50% of cytotoxic extracts from the fractionation process because they would yield a known active.
- Advanced separation techniques such as SEP Box coupled with LC–MS and newer techniques like Super-critical fluid (SFC) extraction play an important role in systematic studies of natural compounds.

Making available samples for screening

Following approaches can be used for making samples available for screening

- The problem of complexity of plant extracts for screening could be avoided by making these plant extracts more ‘assay friendly’. Treatment to remove tannins and other protein-precipitating components are routinely used for the above purpose (Rishton, 1997).
- Combinatorial chemistry can be used for natural products, such as alkaloids, steroids, diterpenes, and lignans.
- There are also attempts to create collections of isolated plant chemicals by large-scale purification before any biological testing (e.g. by Analyticon, Berlin, Germany and Molecular Nature, Aberystwyth, UK).
- Academia, research establishments, small companies and professional providers of natural products such as Dutch SPECS and BioSPECS BV, Interbioscreen in Moscow, bioLeads GmbH, Germany, AnalytiCon
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Discovery and Aventis Pharma, Hans-Knoell-Institut in Jena, Germany, Asta Medica, Aventis Crop Science, Boehringer Ingelheim Pharma, E Merck, Hoffmann-LaRoche, Oncotest, Schering and AnalytiCon Discovery have been collecting natural compounds (Abel et al., 2002).

New screening strategies

As described in the earlier sections microarray based assays demonstrate the level of biological activity for each batch (Prasad et al., 2005). Yi Wang et al., developed data mining method for identifying active components of complex extract of Panax ginseng (Wang et al., 2008).

For natural product drug discovery to continue successfully, new and innovative approaches are required. By applying these new approaches in a systematic manner to natural product drug discovery, it might be possible to increase the current efficiency in identifying and developing new drugs from natural products.

1.2. MALARIA AND ITS TREATMENT

1.2.1 Malaria Milestones

400 Hippocrates’ description of malaria. Charaka and Sushrutha gave vivid descriptions of malaria and even associated it with the bites of mosquitoes
1640 A.D.Huan del Vego – Cinchona bark for malaria treatment
1696 Morton first detailed picture of Malaria
1717 Lanicsi linked malaria to bad air in swamps and thus originates the name malaria
1816 Gize-Extraction of quinine from cinchona bark
1820 Pelletier and Caventou – extraction of pure quinine alkaloids
1880 Laveran identifies malarial parasite under microscope
1895 Golgi-Identification of P. vivax & P. malariae
1889-90 Sakharov, Marchiafava, Celli-identification of P. falciparum
1897 Ronald Ross—Demonstration on malarial oocysts in gut of female anopheles mosquito
1934 Chloroquine synthesized by Germans.
1939 Paul Miller – Insecticidal properties of DDT
1950 Elderfield – Synthesis of primaquine
1967  WHO – emphasis on control of malaria rather than global eradication of the disease
1990’s  Synthesis of quinine analogue mefloquine. Artemesinins obtained from Quinghaosu introduced for resistant malaria
1994  Sequencing of *P. falciparum* Genome begun
1999  WHO Recommends – Combined therapy to delay resistance development to anti-malarials including Artemesinin
2000  Chloroquine resistance gene identified as PfeRTK767
2002  Sequence finished and published

1.2.2 Cause and clinical manifestation

Malaria is caused by the *Plasmodium* parasite that requires two hosts to complete its life cycle. The vertebrate hosts include birds, reptiles, rodents, primates and humans, while the invertebrate host is normally the Anopheles mosquito. Human malaria may also be transmitted by blood transfusion, and contaminated syringes (Phillips, 1983). There are five species of *Plasmodium* known to infect humans - *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. The most prevalent of these is *P. falciparum* as a result of its virulence and drug resistance. The pathogenicity of this parasite is as a result of its rapid rate of asexual reproduction in the host and its ability to sequester in small blood vessels (Winstanley, 2000). None of the other three species characteristically causes severe disease. However, all the three species can cause anaemia, low birth weight, splenomegaly and nephrosis (with *P. malariae*). *P. vivax* and *P. ovale* have hepatic ‘hypnozoites’ which, if not killed using primaquine or tafenoquine, can cause relapse, usually up to 40 weeks after the primary attack. *P. malariae* lacks hypnozoites, but can persist in the blood for many years if inadequately treated.

1.2.3 Epidemiology

The burden of *falciparum* malaria is carried mainly by tropical Africa, where most people become infected during childhood, and most morbidity and mortality are seen in children under the age of five years. Most children gradually develop partial immunity, and this protects them from severe disease (Snow et al., 1997). However, a small proportion (but a numerically large group) develops severe malaria, and this kills about a million people annually. Falciparum malaria is less common in South America, the Indian subcontinent, Southeast Asia and China than in tropical Africa. As a result of this low transmission, partial immunity does not develop as readily, and all age groups can be affected by severe disease. *P. vivax* is encountered in temperate regions and
throughout the tropics and is relatively uncommon in tropical Africa. *P. ovale* is found principally in tropical Africa, whereas *P. malariae* has a widespread distribution throughout the tropics and subtropics.

### 1.2.4. Plasmodium life cycle

The *Plasmodium* life cycle requires both *Anopheles* mosquito and human host. When the female mosquito takes her blood meal, she injects an anticoagulant with the saliva to ensure an even-flow of blood. Sporozoites from the salivary gland are then injected into the capillary bed of the skin and enter the bloodstream. Sporozoites move to the liver within about half an hour and enter the hepatocytes. Once in the hepatocyte *P. falciparum* and *P. malariae* sporozoites immediately enter schizogony whereas *P. ovale* and *P. vivax* sporozoites either enter schizogony or develop into dormant hypnozoites (Phillips, 1983). Preerythrocytic schizogony takes between 5 to 15 days depending on the species. The role of this stage is the production of merozoites. Merozoites leave the liver and enter the bloodstream, and within minutes invade red blood cells, where they grow and divide. In the red blood cell the parasite requires nutrients for growth. Hemoglobin is degraded by a cysteine protease (Salas et al. 1995) and aspartyl protease (Oaks et al., 1991) resulting in amino acids which the parasite uses to synthesize proteins for the developing merozoites. Inside the red blood cells, merozoites differentiate into rings and trophozoites. These trophozoites produce and insert proteins into the erythrocyte membrane responsible for cytoadherence (Oaks et al., 1991). Trophozoites that mature into schizonts release merozoites which then invade other erythrocytes. Every 48-72 hours the erythrocytes rupture, releasing parasites along with waste products and toxins into the blood stream. At this stage, clinical symptoms such as fever and chills arise. The patient feels weak and shows signs of fatigue and episodes of high fever and shivering.

This asexual cycle could be repeated several times, raising the parasitaemia level (Phillips, 1983). Some trophozoites develop into the sexual forms, macrogametocytes and microgametocytes. These are the forms that are ingested by the mosquito when she takes the blood meal. The gametocytes once in the midgut of the mosquito, lose the erythrocyte membrane and undergo gametogenesis (Sinden, 1984). The microgametocyte undergoes three nuclear divisions producing eight microgametes but the macrogametocyte only forms one macrogamete. Once mature, fertilization occurs forming a zygote, and after 18 to 24 hours the zygote develops into a motile ookinete.
The ookinete elongates and penetrates the midgut wall to lie under the membrane and above the basal lamina. There it differentiates into an oocyst and grows over the next 7-12 days. The hemolymph provides the growing oocyst with nutrients. Sporogony occurs within the oocyst, producing many sporozoites. When the oocyst ruptures, the sporozoites migrate to the salivary gland where they generally remain viable for the life of the mosquito (Beier, 1998), ready to move on to another victim when the mosquito takes a blood meal.

1.2.5 Unique aspects of antimalarial drug discovery (Rosenthal, 2003)

As burden of malaria prevalence is more in developing countries, there is a major need of widespread treatment of malaria in developing countries.

- Besides this, as most of the antimalarial drugs are natural products or derivatives of natural products, there is resource limitation for antimalarial drugs.
- A new antimalarial drug should be effective with single-daily dosing, and that curative regimens should be short, ideally 1-3 days in length.
- Antimalarial drug development should be economic and very inexpensive so that they could be available to populations in need in developing countries.
- Since malaria prevalence is primarily in poor countries, investment in antimalarial drug discovery and development has been small. Thus, drug discovery directed against malaria is particularly reliant upon shortcuts that may obviate excess cost.
- Though research centers and academics have been investing time and money in antimalarial drug discovery, most of the companies are no more interested in antimalarial drug discovery.

Overall antimalarial drug discovery remains neglected.

1.2.6 The Prospects

Although millions are at risk, malaria is economically unattractive to the pharmaceutical industry. The scientific community has the responsibility of pointing out this to the public and to the governments. It is the responsibility of national governments to make antimalarial drug discovery favorable to industrial research and
development. By keeping this in mind a number of such approaches, to improve the success rate of drug development, had been adopted in antimalarial drug discovery. These include the re-design of existing drugs, novel use of older drugs, development of drugs from natural products and rational targeting of novel parasite-specific targets as identified by an improved understanding of parasite biology. All of these strategies had proved useful in developing important drugs (Table 1.1) and have the potential to produce newer drugs and it is hoped that in the near future a new arsenal of drugs will be available to stem the tide of antimalarial drug resistance.

Table 1.1 Approaches for antimalarial drug discovery (Rosenthal, 2003)

<table>
<thead>
<tr>
<th>Approach</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>To Optimize therapy with existing agents</td>
<td>Amodiaquine/sulfadoxine/pyrimethamine</td>
</tr>
<tr>
<td></td>
<td>Amodiaquine/artesunate</td>
</tr>
<tr>
<td></td>
<td>Artesunate/sulfadoxine/pyrimethamine</td>
</tr>
<tr>
<td></td>
<td>Artesunate/mefloquine</td>
</tr>
<tr>
<td></td>
<td>Artemether/lumefantrine</td>
</tr>
<tr>
<td></td>
<td>Chlorproguanil/dapsone</td>
</tr>
<tr>
<td></td>
<td>Chlorproguanil/dapsone/artesunate</td>
</tr>
<tr>
<td></td>
<td>Atovaquone/proguanil</td>
</tr>
<tr>
<td>Develop analogs of existing agents</td>
<td>New aminquinolines</td>
</tr>
<tr>
<td></td>
<td>New endoperoxides</td>
</tr>
<tr>
<td></td>
<td>New folate antagonists</td>
</tr>
<tr>
<td>Natural products</td>
<td>New natural products</td>
</tr>
<tr>
<td>Compounds active against other diseases</td>
<td>Folate antagonists</td>
</tr>
<tr>
<td></td>
<td>Antibiotics</td>
</tr>
<tr>
<td></td>
<td>Atovaquone</td>
</tr>
<tr>
<td></td>
<td>Iron chelators</td>
</tr>
<tr>
<td>Drug resistance reversers</td>
<td>Verapamil, desipramine, trifluoperazine</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine</td>
</tr>
</tbody>
</table>

1.3 GENESIS OF THE PROJECT

Malaria is the most important parasitic disease in the world, responsible for 500 million new cases and 2 to 3 million deaths every year (WHO, 2003) and the number of clinical attacks due to *Plasmodium falciparum* seems to be 50% higher than WHO estimates (Snow et al., 1997). This situation occurred due to progressive spread of resistance to almost every drug (Table 1.2). Interestingly, this loss of effectiveness of the newer antimalarial drugs has also occurred at an alarming rate.
Resistance to artemisinin derivatives and artemisinin based combination therapy (ACT) was also reported (Luxemburger et al., 1998; Gogtay et al., 2000; Sahr et al., 2001; Meshnick, 2002; Duffy and Sibley, 2005). To avoid resistance problem combination therapy associating long and short acting compounds with different modes of action was adopted. It offers efficient but expensive treatments. Also vaccines with sufficient efficacy will not be available in the near future (Greenwood et al., 2005). Hence, there is a clear need for a low cost, efficient, curative (and possibly preventive) malaria treatment which does not induce resistance.

Table 1.2 Reports of resistance to common antimalarial drugs (Wongsrichanalai et al., 2002)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Introduced</th>
<th>First Reported Resistance</th>
<th>Effectiveness (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>1632</td>
<td>1910</td>
<td>278</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>1945</td>
<td>1957</td>
<td>12</td>
</tr>
<tr>
<td>Proguanil</td>
<td>1948</td>
<td>1949</td>
<td>1</td>
</tr>
<tr>
<td>Sulfadoxine-pyrimethamine</td>
<td>1967</td>
<td>1967</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>1977</td>
<td>1982</td>
<td>5</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>1996</td>
<td>1996</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Plants have been an integral part of life in many indigenous communities including India. The evaluation of traditional medicines that were employed for the treatment of malaria represents a potential source for the discovery of lead molecules for development into potential antimalarial drugs (Phillipson and Wright, 1991; Kayser et al., 2003). Leaman et al. (1995) showed that plants widely used as antimalarials by traditional healers are significantly more active in vitro against *P. falciparum* than plants that are not widely used, or not used at all, for the treatment of malaria. The molecular diversity and efficacy of antiparasitic plants, extracts, and herbal preparations have been intensively discussed in a few reviews (Schwikkard & Van-Heerden, 2002; Willcox & Bodeker, 2004; Wright, 2005). Antimalarial properties of *Cinchona* bark, known for more than 300 years and recent development of artemisinin derivatives as essential antimalarial drugs reveals that majority of antimalarial drugs used historically have been derived from medicinal plants or are structures modeled on lead compounds from plants (Klayman, 1985).

WHO (2002) report states that although there is a widespread use of traditional herbal remedies in the management of malaria, scientific understanding of these plants is largely unexplored. In Indian Systems of Medicine several plants have been mentioned
for the treatment of malarial fever (Vishmajwara, in Ayurveda) (Sastri, 2002), that are proved to have good potency. To support this hypothesis there are scientifically proved evidences, e.g. Triphaladiyogam, Trichatupanchadravya, Panchakolaghrutam, Vardhamana pippali, are some of the preparations used for the treatment of fever and malaria in Ayurveda. Ellagic acid and piperine are two of the major phytochemicals present in these preparations and these two compounds have been shown to have antimalarial activity (Tekwani and Lary, 2005; Staines et al., 2005). We thought it is worthwhile to undertake work on systematically collecting information on antimalarial plants based on traditional claims for Vishamajwara in Ayurveda (Satya Narayana Sastri, 2002) and further evaluation of the efficacy of the extracts, fractions and compound/s from selected plants for antimalarial activity.

1.4 OBJECTIVES OF RESEARCH WORK

• To screen selected medicinal plants for antiplasmodial activity using in vitro/in vivo models.
• Activity guided fractionation leading to a possible isolation of active compound/s and their pharmacological evaluation for antiplasmodial activity.
• To study the possible mechanism of action of the isolated compound/s.
• To evaluate synergy between different isolated phytochemicals.

1.5 STRUCTURE OF THE THESIS

Chapter 1

This chapter describes the role of natural products in the drug discovery, need for the antimalarial drug discovery, rational behind antimalarial drug discovery from traditional medicine and objectives of the proposed research work.

Chapter 2

Detailed protocol for continuous in vitro cultivation of malarial parasite Plasmodium falciparum is described in this chapter.

Chapter 3

Criteria for the selection of medicinal plants, their collection, authentication and preliminary antiplasmodial screening in two different in vitro models is explained in this chapter.
Chapter 4
Review of the available literature on ethnomedical uses, chemistry, pharmacology and toxicity of the selected plants *Adhatoda zeylanica* leaf and *Embelia ribes* fruit is illustrated in this chapter.

Chapter 5
This chapter describes the fractionation and activity guided isolation of the *Plasmodium falciparum* lactate dehydrogenase (PfLDH) inhibiting agent/s from the leaf of *Adhatoda zeylanica* and fruit of *Embelia ribes*. Structural elucidation of the active principle with PfLDH inhibition properties through the usual spectroscopic techniques including IR, Mass, $^1$H and $^{13}$C-NMR.

Chapter 6
Various experiments carried out to establish the probable mechanism of action of the isolated compounds, vasicine, vasicinone and embelin as antimalarial agents is portrayed in this chapter. These experiments include hemozoin formation inhibition, drug-heme interaction, GSH dependent heme degradation, protein kinase inhibition, plasmepsin inhibition, histidine rich protein-2 inhibition.

Chapter 7
Antiplasmodial synergy evaluation among vasicine, vasicinone, embelin and standard drugs is revealed in this chapter.

Chapter 8
This chapter describes the TLC fingerprint profile of the methanolic extract, co-chromatography with vasicine and vasicinone standards in *Adhatoda zeylanica* and embelin standard in *Embelia ribes* and their quantification by TLC densitometric method using HPTLC.

Chapter 9
This chapter summarizes the outcome of the entire work and conclusions drawn from the results.
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(NCNPDDG) and International Cooperative Biodiversity Group (ICBG) programme. *International Journal of Pharmacognosy* 33, 5–16


