The drug design and development is an interdisciplinary process to find new therapeutic agents based on the knowledge of biological targets and interaction of these targets to different bioactive molecules. It requires coordinated efforts among scientists belonging to different disciplines including medicinal chemistry, analytical chemistry, spectroscopy, pharmacology, biochemistry, microbiology and molecular biology. As an interdisciplinary and well organized process with an industrial base, the field is not much older than a century.

Discovery of a new drug is essentially a complex, expensive, resource consuming and time taking process that requires approximately 12 years to reach the clinic, involving more than 1 billion US$ of investments. The field of modern drug design and development originated as theoretical and practical aspects of chemistry have been utilized in form of medicinal chemistry and further supported by the influence of other related disciplines such as pharmacology, biochemistry, pharmacokinetics and analytical chemistry. First of all Paul Ehrlich has postulated the conceptual basis behind the drug discovery. He has demonstrated that differences in the structures of chemoreceptors present on parasites, microorganisms, and cancer cells from those present in host tissues may be exploited in development of therapeutic agents. The selective affinity of some biologically active molecules to these chemoreceptors leads to the search for new targets as well as therapeutic agents specific to these targets. It was the initial step in the field of chemotherapy that achieved its unanticipated height in the course of the 20th century. Current drug discovery technologies in combination with the powerful tools of biotechnology and molecular biology have prompted drug discovery research to focus on target based development of potential therapeutic agents.

The Mother Nature has been continuously serving the mankind as most efficient arsenal, playing an important role in health care and prevention of diseases. Natural products have traditionally been used in form of plant extracts, dry powders, infusions, or other therapeutic preparations to treat several diseases over centuries and continue to play a highly significant role in the modern drug discovery and development process providing a diverse and unique source of medicinally active molecules. Medicinal plants play a major role and constitute the backbone of almost all the traditional systems of medicine. Ayurveda, known as the science of life is one of the oldest system of medicines. This system of using natural resources for betterment of health was originated in India long ago in the pre-Vedic period through day by day experiences and
experimentations with the aim of maintaining health and treatment of various diseases. Several other systems of complementary and alternative medicines including Siddha and Unani are also developed from plant based formulations through experience and interactions with natural resources. The earliest written evidence related to use of plant products as therapeutic agents is available on *Atharvaveda*, one of the four most ancient books of knowledge and culture related to Hindu religion showing the strength of Indian wisdom. As many as 114 different therapeutic formulations have been described for the treatment of different diseases. The therapeutic importance of Indian medicinal plants has been exposed thoroughly in the *Susruta samhita* and *Charaka samhita* during the Vedic period. Indian Materia medica has description of more than 2000 drugs derived from natural resources most of which are originated from different systems of traditional and folk practices. 80% of these drugs are of plant origin whereas the rest are minerals or animal products.

Indian medicinal plants possess enormous therapeutic potential but only a small proportion of it has been explored by mankind leaving the great opportunity to discover novel drugs from natural origin. Numerous therapeutic preparations have been developed by traditional healers and Ayurvedic practitioners for the treatment of various disorders and diseases. Subsequently after the emergence of natural product chemistry thousands of new medicinal plant were identified with immense therapeutic potential. Natural Product chemistry together with analytical chemistry, spectroscopy, pharmacology, biochemistry and other related disciplines demonstrated its value for drug development. It has not only enriched modern medicine with novel bio-active molecules but also provided valuable leads for drug designing. Since the isolation of morphine from *Papaver somniferum* in 1806, extensive efforts are being done to isolate therapeutically active molecules from medicinal plants. Some important examples include emetine, colchicine, atropine, cocaine, ephedrine and quinidine.

World health organization has recently reported that more than 80% of the world population, including millions of people living in the rural areas of developing countries depends primarily on natural products derived medicines for their fundamental health care needs. It is estimated that approximately 250,000 plant species are found all over the world and most of these are still not studied pharmacologically, providing a strong basis to focus on natural resources to fulfill the strong requirement of new drugs with less adverse effects. The immense potential of natural
products for medical application in both the respect of providing novel molecular structures and the range of activities as well as everlasting opportunity to find new drugs has made this field most fascinating. Moreover, the development of target based high throughput screening (HTS) resulted in the need for the generation of large number of libraries of new compounds with diverse structures to satisfy the enormous capacities of this technique. It also prompted the current drug researchers to emphasize on natural products that can continuously provide the diverse array of compounds with biological activities.

Cancer is responsible for about 25% deaths caused due to diseases in the developed countries and is a major public health burden and challenge for world health organization and research organizations. It is considered as an adversary of industrial revolution followed by advanced pattern of socio-cultural life dominated by excessive intake of exogenous chemicals and less physical activities. The number of incidences of different types of cancers is also increasing in developing countries, as the extensive technical advancements in the field of drug development and other areas allowed their populations live longer and make negative lifestyle changes leading to increased risk of cancer. Cancer is a broad group of diseases characterized primarily by uncontrolled cell division leading to increase in the number of malignant cells in a tissue, invasion of adjacent tissues by malignant cells, or spread of malignant cells through lymphatic or circulatory system to regional lymph nodes and distant tissues (metastasis). It develops through multi-step process that initiates with small preneoplastic changes, which may subsequently progress to neoplasia. Under certain conditions, neoplastic cells escape the host's immune surveillance that helps to develop the capacity of growth, invasion and metastasis. Cancer cells behave as independent cells and proliferate continuously without growth regulation, leading to tumor development through multistep process.

Apoptosis is a well organized and evolutionarily conserved intracellular process known as program cell death. It plays a crucial role in maintaining normal tissue homeostasis and also participates in the elimination of infected and damaged cells [1]. It involves the morphological changes including shrinkage of the cell, condensation of chromatin and nuclear fragmentation. The defects in apoptotic pathways are postulated to contribute to tumor initiation, progression or metastasis. Apoptosis is known to be governed by two intricate signaling pathways termed as extracellular and intracellular pathways [2]. Different extracellular and intracellular stimuli
trigger the extracellular or intracellular pathways leading to apoptosis. The major extracellular
triggers include depletion of growth factors, radiation, hypoxia, and loss of cell-matrix
interactions while intracellular triggers include unrepaired DNA damage (caused by exogenous
toxins or due to defective cell-cycle checkpoint), oncogenic mutations resulting in inappropriate
proliferative signals and malfunctioning of telomerase [3] Cellular stress is known to activate 53,
directly promoting the apoptosis mediated by pro-apoptotic factors such as Bax [4].

The extracellular pathway is initiated at the plasma membrane through the activation of cell
surface death receptors such as Fas/CD95, tumor necrosis factor receptor 1 (TNF-R1) and TNF
related apoptosis-inducing ligands (TRAIL) receptors. The binding of cognate trimeric ligand
(Fas ligand) to these receptors induce the association with the adaptor molecule Fas-associated
death domain protein (FADD), which in turn recruits and activates an initiator enzyme caspase-8
(a cell death protease). Activated caspase-8 ultimately triggers the activation of downstream
apoptotic effectors including caspases 3, 6 and 7 leading to programmed cell death. Activation of
caspase-8 also lead to cleavage and activation of pro-apoptotic molecule Bid which induce the
release of cytochrome c from mitochondria [5, 6]. Cytochrome c and Smac/DIABLO may
promote caspase activation in a concerted manner. A serine protease termed as granzyme B, is
known to cleave the pro-apoptotic molecule Bid in to a 15.5-kDa C-terminal fragment and a 6.5-
kDa N-terminal fragment. The C-terminal fragment subsequently translocated to mitochondria
where it interacts with other proapoptotic and antiapoptotic proteins including Bcl-xL, Bax and
Bak, inducing cytochrome c release to the cytosol [7- 9]. TRAIL is postulated to initiates the
apoptosis in cancerous cells through binding to its receptors DR4 or DR5 without involving p53.
Normal cells express decoy receptor, which competitively inhibit the binding of TRAIL to DR4
or DR5.

The intracellular pathway is mediated by mitochondria, and involves the release of proapoptotic
factors in response to growth factors, cytokines and DNA damage. These apoptotic triggers
affect the normal functions of proapoptotic and antiapoptotic factors which subsequently lead to
structural and functional changes in mitochondria such as opening of permeability transition pore
(PTP) resulting in release of cytochrome c from the intramembrane space [10]. Translocation of
pro-apoptotic proteins including Bax, Bac, Bid, Bad and Bim to the mitochondria lead to
permeabilization of mitochondrial outer membrane resulting in cytochrome c release [11-13].
Cytochrome c interacts with apoptotic proteinase-activating factor-1 (Apaf-1) and promotes the activation of caspase-9 ultimately activating the downstream apoptotic effectors including caspases 2, 3, 6 and 10 [14]. Recent studies have established the involvement of caspase mutations in different types of cancer [15, 16].

Multidisciplinary scientific investigations are making best efforts for the treatment of cancer, but the reliable, sure-shot treatment is yet to be achieved. Identification of antiproliferative molecules with selective cytotoxicity is the fundamental step for the development of anticancer agents. Recent advances in cancer treatment however attributed to the identification of unique biochemical pathways in cancerous cells which could be targeted selectively by drug molecules. Moreover the new findings in molecular biology as well as the unexpected achievements in the field of proteomics and genomics have offered a number of novel drug targets, leading to changes in conventional patterns of anticancer drug discovery in the direction of molecular target based therapeutics. This changing scenario led to drastic revolution in the field of anticancer drug development.

An ideal anticancer drug would restore normal growth regulation and cell cycle control to cancer cells through restoring aberrant molecular signaling pathways and inducing apoptosis in these cells. It should selectively target different components of physiological and biochemical pathways related to different stages of cancer development without affecting the normal cells [17]. The discovery of new compounds with novel mechanisms of action, contribute to improved and highly effective methods for cancer treatment.

A newer dimension in the anticancer drug research is the increasing awareness about natural product based chemotherapy. Several studies have demonstrated that different plant-based foods such as onion, grapes, garlic, ginger, soybean, turmeric, cabbage, cauliflower, broccoli and tomato can offer significant anticancer potential. Natural products have provided four important categories of antitumor agents: taxanes, camptothecins, bisindole alkaloids also known as vinca alkaloid and epipodophyllotoxins. Microorganisms have also provided several potent anticancer drugs in form of anticancer antibiotics such as doxorubicin, actinomycin and mitomycin C.

A natural product is a chemical compound or substance produced by a plant, animal or microorganism and usually has a pharmacological or biological activity which can be utilized in pharmaceutical drug discovery and drug design [18-20]. Chemically natural products are
secondary metabolites, specifically produced by a particular group of organisms and have been postulated to play an important role in self defense against predators as well as in interspecies interactions [21, 22]. Their role is exceptionally pronounced in the field of anticancer drug research. 73% of the 155 small molecules introduced during 1940 to 2007 were of non synthetic origin. Roughly 50% of the new chemical entities introduced during that period were either natural products or derived from natural products through structural modifications. Due to enormous advancement in the field of medicinal chemistry, design of natural product or natural product-mimetic scaffolds can be achieved readily in one-step with the help of multicomponent reactions [23]. Usually, natural products are isolated only in minute amounts and thus subsequent techniques are required to scale-up of the biologically active molecules.

Semisynthesis is a process of chemical synthesis in which a naturally occurring compound is used as starting materials. It has given a newer dimension to medicinal chemistry [24]. It involves structural modifications in different functional groups present in natural products. It is applied to synthesize structurally complex novel compounds, which cannot be produced by total synthesis. It is also used to improve the pharmacological properties of natural compounds such as efficiency, water solubility or stability in the human body. Important examples of semisynthetic drugs include LSD (derived from ergotamine), docetaxel (derived from paclitaxel), flavopiridol (derived from rohitukine), and artemether (derived from artemisinin).

Together with cancer, metabolic diseases are also considered as leading causes of death all over the world. It is well established that metabolic disorders are directly associated with increased risk for the development of cancer in a population with a rapidly increasing incidence of these disorders [25]. Several recent epidemiological studies have shown the direct link between the metabolic syndrome-associated disorders to an increased risk for initiation and progression of different types of cancer as well as mortality from them. In addition, hyperglycemia is shown to play a significant role as independent risk factor for overall cancer incidence.

Overweight and obesity caused due to sedentary life style followed by other factors are shown to be associated with increased risk of cardiovascular disease and non insulin dependent diabetes mellitus [26]. Lipid lowering drugs Statins are shown to be associated with reduced risk of esophageal, colorectal, gastric, hepatocellular and prostate cancer as these may produce energy depletion to rapidly dividing cancer cells. Moreover, several recent studies established the association between obesity and cancer-related mortality. Different metabolic changes caused
due to obesity, and altered adipocyte function increase the risk of cancer. Obesity as well as increased body mass index is shown to be associated with an increased incidence of colorectal, endometrial, thyroid, renal, gallbladder, pancreatic and ovarian cancers [27-28]. Consequently, many natural compounds which are known to possess anti-adipogenic potential such as genistein, kampferol and epigallocatechin have also found to be effective as anti-cancer agents [29-31]. Multiple therapeutic potential of a compound provides additional benefit to patents suffering with a multifactorial disease including, Cancer, diabetes, and other metabolic disorders. Similarly, targeting a molecule with central roles in different signaling pathways may provide opportunity to find a novel biological activity in already existing drugs.

With reference to above facts, the present study has been designed to identify anticancer molecules which possess activity against metabolic syndrome as well.

The majority of anticancer drugs nonspecifically act on cancer cells as well as rapidly dividing normal cells including bone marrow cells, germinal epithelium cells, hair follicles and mucosal cells of digestive tract. These effects results in common side effects such as anemia, nausea, hair loss and ulcer in gastrointestinal system. Gastric ulcer is considered as a major side effect related to most of the anticancer drugs and thus an anticancer drug possessing gastroprotective potential may provide better option for chemotherapy. A therapeutic agent showing dual activities could be proved a good anticancer agent without adverse effect.

Parkinson’s disease is a common progressive neurodegenerative disease characterized by death of nerve cells. It affects brainstem extrapyramidal neurons of middle-age individuals affecting approximately 2% of the population. An estimated 4 million individuals above 50 had Parkinson’s disease in 2005. Moreover, recent studies established the strong relation between the Parkinson’s disease and cancer [5, 6]. These studies explained that the association between melanoma and Parkinson’s disease was present during and after the appearance of Parkinson’s disease.

Keeping these interrelated observations in consideration we have evaluated the natural product based extracts and pure compounds for above mentioned activities and the results of our study showed that the plants selected for the study have prominent anticancer as well as other activities that can be pursued for further studies to develop the therapeutic agents for the treatment of cancer and other metabolic disorders. The CSIR-Central Drug Research Institute, Lucknow is actively involved in the development of new drugs. As a part of drug development from natural
resources it is running an integrated program of screening the biological activity of indigenous plants and marine organisms. Chemists and biologists are exploring the chemical constituents and new pharmacological potential along with their mechanism of action, the active compounds are modified for optimization of their activity through chemical transformation. Based on these innovative activities, the thesis entitled “Evaluation of Anti-Carcinogenic Potential of Bioactive Compounds Derived from Indian Medicinal Plants” describes our endeavors leading to the identification of new Anti-cancer, Anti-dyslipidemic, Anti-Parkinsonian and Anti-ulcer agents. The present study has been accomplished with following objective

1. Extraction and Bio activity guided fractionation of medicinal plants selected for the study
2. Isolation and characterization of pure compounds from active extract/ fractions.
3. Semisynthetic modification of lead molecules.
4. Evaluation of biological activities of isolated compounds and their semisynthetic analogs
5. Determination of mechanism of actions of most active compounds

The present thesis covers the result of these studies and has been divided into four chapters.

Chapter 1: Development of chemotherapeutic agents from Natural Products: Review

This chapter covers the history of drug development from natural resources, anticancer compounds isolated from different natural resources, brief view of pathophysiology of cancer and mechanism of action of currently used anticancer agents.

Chapter 2: Phytochemical investigation of *Dysoxylum binectariferum* (stem bark)

This chapter includes description of phytochemical and pharmacological studies of the stem bark of *Dysoxylum binectariferum*. Description of different compounds isolated from different parts of the plant together with their pharmacological activities has been included. Further it includes isolation and characterization of four known compounds DB-1 to DB-4 based on the spectral data and literature review. The chapter also includes the semi-synthetic derivatization of lead molecule rohitukine to synthesize novel derivatives as well as evaluation of these molecules for their Anti-cancer potential and antiadipogenic activity.

Chapter 3: Phytochemical investigation of *Xylocarpus molluccensis* (fruits)

This chapter deals with the phytochemical and pharmacological studies of the fruits of *Xylocarpus molluccensis*. Phytochemical investigation resulted in isolation and characterization
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of six known compounds XM-1 to XM-6 on the basis of spectral data and literature review. It also includes the evaluation of these molecules for therapeutic effect against Parkinson’s disease.

Chapter 4: Phytochemical investigation of *Rheum emodi* rhizomes

This chapter includes description of plant, phytochemical investigation and pharmacological properties of the rhizomes of *Rheum emodi*. Bioactivity guided fractionation of ethanolic extract resulted in isolation and characterization of four known compounds RE-1 to RE-4 on the basis of spectral data and literature review. This chapter also includes evaluation of ethanolic extract and its active constituents as Anti-dyslipidemic and antiulcer agents.

References


