Chapter-I

INTRODUCTION
The knowledge of drugs has accumulated over thousands of years as a result of man’s inquisitive nature, so that today we possess many effective means of ensuring health care (Newman et al., 2003). Man, since time immemorial, has been using herbs or plant products as medicine for curing ailments as well as developing immunity or resistance against various diseases. The traditional systems of medicines, Ayurveda, Sidha and Unani are based on the experiences in the use of plant products in the amelioration of common diseases. A good number of herbal remedies have stood the test of time particularly for the treatment of allergic, metabolic and degenerative diseases. The use of plants dates back to almost all civilizations, even primates have been shown to chew leaves when suffering from gastrointestinal disturbances (Cragg 2002). The first records on the use of natural products in medicine were written in cuneiform in Mesopotamia on clay tablets and date back to approximately 2600 BC (Cragg and Newman, 2001). Many pharmaceutical agents have been discovered by screening natural products from plants, animals, marine organisms and microorganisms. The World Health Organization (WHO) estimates that approximately 80% of the world relies on natural sources for primary medical treatment and that the health care systems for the remaining 20% of the population also incorporate natural sources in their medical treatment (Newman et al., 2000). Over twenty new drugs launched in the market between 2000 and 2005 originated from terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates (Chin et al., 2006).

With the advancement in medical sciences, it is becoming clear that many diseases are related to immune system like cancer, AIDS, tuberculosis, diabetes, chronic inflammatory diseases and neurodegenerative disorders and day by day the incidence of these diseases is increasing (Kidd, 2003). Due to inefficacy of modern allopathic medical system to fully cure the chronic diseases and having many side effects, the consumer is shifting to the alternative sources of medical therapy that are reasonably effective and comparatively cost effective. Most acceptable alternative therapy is from the edible plants. The secondary metabolites of plants are often perceived as showing more “drug likeliness and biological friendliness than totally synthetic molecules,” making them good candidates for further drug development (Balunas and Kinghorn, 2005; Jones et al., 2006). However to ensure that preparations
derived from medicinal plants are of acceptable quality, safety and efficacy they should be FDA approved.

Role of free radical-mediated damage in the etiology of many human diseases (like cancer, cardiovascular defects, inflammatory disorders, etc.) is becoming clear. Free radicals are highly unstable and can react with proteins, lipids, carbohydrates and DNA resulting in the destruction of a living cell (Patil et al., 2003). Overproduction of oxidants or diminished levels of antioxidants can lead to an oxidative damage called Oxidative stress which results in the damage of biopolymers including nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates, which is implicated in the pathogenesis of a wide range of diseases. The antioxidant activity in the cancers and immune system of body are generally related to each other. Nutritional antioxidant deficiency may also lead to oxidative stress (Sies and Stahl, 1995; Parker, 1980). Epidemiological studies as well as clinical intervention trials suggest that natural antioxidants may play a pivotal role in preventing or slowing the progression of the diseases associated with oxidative stress, immune response and cancer (Hennekens and Gaziano, 1993; Block et al., 1992).

In the rapidly evolving world of pharmacology, it is a continuing challenge to design a therapy which is both effective and has high specificity for the biology of diseases like cancer/ and immune related-disorders. None of the agents currently under development fulfill this criteria completely (Hoekmann et al., 1999). The impact and the potential for immunologically–derived targets is becoming more and more evident, hence raising an interest in developing immunotherapeutic tools. Thus immunology is one of the most rapidly developing areas of biomedical research and immunotherapy is the natural way of treating the immune dysfunctions. Hence a key point in optimal immunotherapy is to restore/ensure a balanced T and B cell responses and cell-to-cell communication through appropriate cytokines and other signaling molecules (Kidd, 2003). The selectivity and flexibility that is necessary to regulate cell traffic under homeostatic and diseased conditions are provided by the differential distribution and regulated expression of cytokines and their receptors. As a consequence, cytokines are responsible for the development of phenotypes and are, therefore, logical targets for therapeutic immune modulation. Recently many plants or their products have been identified so much that many plant based drugs are available in the market which can
modulate the disease by potentially suppressing or potentiating the immune response. Immunomodulatory drugs, such as thalidomide, lenalidomide (Revlimid, CC-5013) and actimid (CC-4047), have a broad spectrum of activity and have shown remarkable responses in patients with multiple myeloma and related haematological diseases, such as myelodysplastic syndrome. Thalidomide and lenalidomide in combination with dexamethasone have recently been approved by the United States Food and Drug Administration for the treatment of multiple myeloma (Raje et al., 2006). Immunomodulators are considered now as one of the most potent tools in the management of health and disease by modern medicine.

Over the past few decades, cancer has remained as the largest cause of mortality worldwide and the number of individuals living with cancer is steadily expanding. Hence, a major portion of the current pharmacological research is involved with the anticancer drug design customized to fit new molecular targets (Xia et al., 2004). The plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities. Traditionally various plants have long been used in the treatment of cancer (Kim et al., 2005; Kintzios, 2006; Indap et al., 2006). In case of cancer over the time frame from around the 1940s to date, of the 175 small molecules, 131 (74.8%), are other than synthetic, with 85 (48.6%), actually being either natural products or directly derived there from. Of the seven antitumor agents approved in 2010, five were derived from natural products (Newman and Cragg, 2012).

More recently, an improved understanding of cancer pathogenesis has given rise to new treatment options, including targeted agents and cancer immunotherapy. Immunotherapy may be effective singly or synergistically with the available chemotherapy. Immunotherapy endeavours to stimulate a host immune response that effectuates long-lived tumour destruction. The discovery of crucial molecular pathways that promote tumour growth and maintenance together with the development of drugs that specifically inhibit these pathways has ushered in a new era of cancer medicine. Analogously, an improved understanding of the mechanisms of protective anti-tumour immunity has translated these concepts into efficacious immunotherapies. A strong foundation has been established to advance the crafting of both preclinical and early stage patient investigations to determine the best ways to integrate targeted agents and immunotherapy (Druker and David, 2003). Plant based substances have been reported
for chemoprotective and immunomodulatory activities and are often employed as supportive or adjuvant therapy to overcome the undesired effects of cytotoxic chemotherapeutic agents and to restore normal health. Plant based immunomodulatory agents exert their antitumor effects by improving host defense mechanisms against the tumors, besides their direct anti-proliferative effect on the tumor cells (Patwardhan et al., 1990).

In addition, the protective effect has been attributed to the fact that such plant foods provide an optimal mix of phytochemicals including natural antioxidants (Alonso et al., 2003). In addition to natural antioxidants, carotenoids, tocoferols and ascorbic acid, most of the anti-oxidative effect of plant based foods is mainly due to presence of non-nutritive phenolic compounds, which have not yet been characterized very well in both fruits and vegetables (Deighton et al., 2000). Phenolic compounds are secondary metabolites, which are associated with flavour and colour characteristics of fruits and vegetables (Kaur and Kapoor, 2002). There are three main classes of dietary phenolics: flavonoids, phenolic acids and polyphenols. Studies to date demonstrated that these dietary phytochemicals can modulate the detoxification enzymes, scavenging of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation, apoptosis and hormone metabolism (Aruoma, 1998). It is estimated that up to 80% of cardiovascular disease, 90% of Type II diabetes, and one third of cancers can be avoided by changing lifestyle, including diet (Ruxton et al., 2006; Serdula et al., 1996). Therefore the importance of such phenolic antioxidants of fruits and vegetables in maintenance of health and in protection from degenerative diseases is of growing interest among scientists, food manufacturers, consumers and health organizations.

Chemoprevention by edible phytochemicals is now considered to be an inexpensive, readily applicable, acceptable and accessible approach to cancer control and management. A wide range of different classes of bioactive molecules have been identified such as organosulphur compounds from garlic, polyphenols in green tea and curcumin in turmeric, resveratrol from grapes, quercetin from onions and apples, pruretin from plums and cherries. Natural products support the treatment of all phases of cancer or interact with several targets simultaneously and often synergistically. These bioactive polyphenols can influence signal transduction factors, inhibit COX-2,
promote cell cycle arrest, increase apoptosis and double multi-drug resistant pumps, curcumin from turmeric has been found to influence sixty such molecular targets in cancer treatment process (Hopking et al., 2006). The National Cancer Institute (NCI-USA) has more than 400 potential agents under investigation and is sponsoring more than 65 Phase I, Phase II and Phase III chemoprevention trials. These involve various substances or their mixtures, many of which are food borne phytochemicals. The much lower risk of colon, prostate and breast cancers in Asians, who generally consume more vegetables, fruits and tea than populations in the West raise the importance of flavonoid components mediated protective effects by acting as natural chemopreventive and chemotherapeutic agents (Middleton et al., 2000). Moreover, when used with chemotherapy agents such as 5-fluorouracil and doxorubicin, antioxidants enhance the cytotoxicity of chemotherapy agents and cause complete remissions, where only partial remission is possible with chemotherapy agents only (Herrmann, 1976). Due to repeated epidemiological studies providing data about correlations between fruit consumption and reduced risk of chronic diseases, fruits have gained an important place in human nutrition (He et al., 2007).

Many medicinal plants, herbal drugs and fruits have been studied for their immunopharmacological activities. Total extracts, polar and non-polar extracts or formulations, prepared from medicinal plants and fruits have been mentioned in Ayurveda. A number of fruits used in the traditional medical system of remedies in India, have been shown to possess immunomodulatory activity acting at the different levels of the immune system (Puri et al., 2000). Researchers continue to explore the benefits of “superfruits”, a unique group nutrient-rich fruits that contain natural compounds shown to have potential disease-fighting properties. Few fruits fall in this category and emerging science shows that sour cherries (*Prunus cerasus* L.) are one among them. Sour cherry is a medicinal fruit claimed to possess number of therapeutic uses including anti-inflammatory as well as antioxidant activities (Saric et al., 2009).

*Prunus cerasus* L. or sour cherry is a species of genus *Prunus* in the sub-genus *Cerasus* (cherries) belonging to *Rosacea* family, native to much of Europe and Southwest of Asia. In India, cherries are grown mainly in Jammu and Kashmir and Himachal Pradesh. It is closely related to wild cherry or sweet cherry but has a fruit that is more acidic. Sour cherry tree have been used widely for its fruit, eaten fresh and also used in
cooking. Both the fermented fruit and the crushed pits are used in making European liqueur kirsch. In particular, red cherry fruits are used in a traditional herbal remedy for various diseases such as heart failure, beriberi, dropsy, mastitis. The stalk from sour cherry has been used medicinally as an astringent, detoxificant and relaxant. The bark of sour cherry is astringent, bitter and an infusion of this bark has been used in the treatment of fevers, coughs and colds. The seed (pit) is nervine and an edible drying oil obtained from these seeds is also used in cosmetics. A green dye can be obtained from the leaves and can be used as a natural colouring agent (Ponnumswamy et al., 2011).

Convincing phytochemical studies affirmed that sour cherries are one of the few known food sources that are a rich source of powerful antioxidants including flavonoids, polyphenols and anthocyanins and thus they can serve as a good source of bio-functional phytochemicals in our diet. Sour cherries contain a wide array of a unique package of antioxidants and other phytonutrients (including melatonin, kaempherol, chlorogenic acid, p-coumaric acid, gallic acid, perillyl alcohol and ellagic acid) (Wang et al., 1999). A nutritional research study found that sour cherries ranked 14 in the top 50 foods for highest antioxidant content per serving (Halvorsen et al., 2006).

The role of *P. cerasus* to modulate a variety of biological responses has been constantly reported. It has found extensive use in many indigenous nutraceutical preparations for its medicinal values and different pharmacological actions such as, anti-arthritis, cardioprotective, ant-inflammatory, anticancer, anti-diabetic retinopathy, neurological disorders, weight management, anti-aging and muscle recovery. However it may be mentioned here that there are various varieties of sour cherries grown in different parts of the world reported in literature. Although most of these varieties have been studied for chemical profile and varied pharmacologic activities, *P. cerasus* cultivar grown in Kashmir region of India still remains unexplored. Hence the present study was designed to study the unexplored *P. cerasus* fruit of Kashmir with reference to its chemical signature and immunotherapeutic as well as anticancer activities.
This thesis embodies the results to seek answers to the proposed aims and objectives as:

- To screen various extracts prepared from different parts of *Prunus cerasus* fruit for immunological activities under in vitro conditions and select the most active extract.
- Isolation of the major compounds from the selected bioactive (immunomodulator) extract of *Prunus cerasus*.
- In vitro immunomodulatory characterization of isolated compounds from selected bioactive extract of *Prunus cerasus*.
- In vitro or In vivo evaluation of therapeutic potential of selected bioactive extract.
- Attempts to evaluate therapeutic potential of isolated most bioactive compound (depending upon the quantity of the compound isolated).