INTRODUCTION
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Oxidative damage to cells has been implicated in the pathogenesis of a wide variety of clinical disorders (Halliwell, 1987; Droge, 2002) and its broad range of effects in biological systems has drawn attention of many experimental studies. Cellular exposure to exogenously or endogenously generated oxidants cause macromolecular damage including protein oxidation, lipid peroxidation and nucleic acid instability and mutation (Halliwell and Gutteridge, 1990; Janssen et al, 1993; Halliwell, 1998). Oxidative stress is associated with degeneration of cells, tissues, and organs, resulting in diseases such as cancer, cardiovascular failure, cataracts, and Alzheimer's disease, as well as the decline of most measures of physiological performance. Over the past four decades, a great deal of evidence has been gathered which suggests that oxidative damage is the major, although not the only, contributor to cellular degeneration.

Under normal physiological conditions, there appears to be four key sources of Reactive Oxygen Species (ROS): mitochondrial electron transport, peroxisomal fatty acid metabolism, cytochrome P450 reactions and phagocytic cells. The body is equipped with an impressive repertoire of endogenous enzymatic defenses against oxidative damage. These include enzymatic scavengers such as superoxide dismutase, glutathione peroxidase, and catalase; hydrophilic radical scavengers such as ascorbate, urate and glutathione; lipophilic radical scavengers such as tocopherols, flavonoids, carotenoids and ubiquinol; enzymes involved in the regeneration of oxidized forms of the small molecular antioxidants (GSH reductase, dehydroascorbate reductase) or responsible for the maintenance of protein thiols (thioredoxin reductase)
and the cellular machinery that maintains a reducing environment (Glucose-6-phosphate dehydrogenase, which regenerates NADPH).

Several studies have revealed that plants produce potent antioxidants to control the oxidative stress caused by sunbeams and oxygen and represent source of new compounds with antioxidant activity (Scartezzini and Speroni, 2000). Epidemiological, in vivo and in vitro studies have suggested that diets rich in fruit and vegetables may exert protective effects against various stages of the cancer process and Coronary Heart Diseases (CHD) (Block et al, 1992; Hollman et al, 1996; Eastwood, 1999). These effects have been contributed, in part, to bioactive components found in fruits and vegetables that possess antioxidant activities (Block et al, 1992; Hollman et al, 1996; Diplock et al, 1998). The most prominent representatives of these dietary antioxidants include ascorbate, carotenoids, flavonoids, and tocopherols (Diplock et al, 1998; Eastwood, 1999).

Plant and animal products have been the basis of treatment of human diseases since time immemorial. Four major systems of herbal medicines have been recognized which are Asian, European, Indigenous and Neo-western. Out of these, the Asian and European systems go back thousand years, appear in pharmacopia, and with such a tradition of use are better understood than those of indigenous origins that are often only orally or secondarily recorded. The most established types of herbalism are those of Asian origin, particularly from India (Ayurva, Unani and Siddha), China (Wu-Hsing) and Japan (Kampo), and today they still follow the ideas of diagnosis and treatment known for millennia.

Ayurveda is one of the traditional systems of medicine practiced in India and can be traced back to 6000 B.C. (Charak Samhita, 1949). Ayurveda, the traditional Indian health care system (ayus-life, veda-
knowledge, meaning the Science of Life), is the oldest medical system in the world, which exploits the potential of various herbs generally in polyherbal formulations as drugs. Most of the remedies are mixtures of plants, sometimes also containing animal parts and minerals and are formulated to achieve the expected therapeutic goals. There is an ever-increasing interest on research on different plant species to find out their therapeutic applications all over the world.

The medicinal use of plant extracts seems to be a more natural, less expensive way, and involve therapies that are more gentle and largely without side effects. Environmental conditions that induce or favor photo oxidative stress are part of plant's everyday life. Higher plants have a stationary life-style and the potential to adapt to physical, chemical and biological factors to maintain homeostasis. It is critical for plants to readily recognize dangerous and harmful stress factors and to effect suitable responses to them. The accumulated knowledge suggests that plants express new genes and establish new metabolic activities depending on the type and strength of the stress factors (Lamb et al, 1989; Nover et al, 1989; Chandra and Low, 1995). Plants have acquired an essential system to reduce and scavenge active oxygen species, which are naturally generated during photosynthesis and respiration (Asada, 1994). Plants synthesize thousands of metabolites that are used for their growth, development, reproduction, defense against attack by many different kinds of organisms and are able to survive in often harsh and ever changing environments.

As many synthetic antioxidants have been shown to have one or the other side effects (Musk et al, 1994; Nocentini et al, 2001), there has been upsurge in the therapeutic potential of medicinal plants as antioxidants in reducing free radical induced tissue injury (Siddique et al 2000; Engelhart et al, 2002; Koleva et al, 2002). Numerous plant products have been shown to have antioxidant activity (Auroma and
Cuppelt, 1997; De Groot and Rauen, 1998; Scartezzini and Speroni, 2000; Koleva et al, 2002). In addition to antioxidant vitamins (Vitamin A, E and C), flavonoids and other polyphenolic compounds of plant origin have also been reported as scavengers of free radicals and inhibitors of lipid peroxidation (Hanasaki et al, 1994; Formica and Regelson, 1995; Tapiero et al, 2002).

Herbal products have been used for the relief of the gastrointestinal system, urogenital system, diabetes and for improving the cardiovascular and immune system. For example, reserpine, which is widely used for the treatment of high blood pressure, was originally extracted from the plant Rauwolfia serpentina while digitalis, used as a heart stimulant, was derived from the foxglove plant (Digitalis purpurea).

A number of herbal products are known to facilitate enhancement of the immune system. These include Echinacea, garlic, cat’s claw, astragalus, licorice and other herbs. Flavonoid and carotenoid rich herbs may be expected to enhance the immune system (Kuhnau, 1976; Tyler, 1994). These plants have been found to promote the activity of lymphocytes, increase phagocytosis and induce interferon production. A number of commonly used herbs have been identified as possessing cancer-protecting properties. These herbs include members of the Allium species (garlic, onions, chives); members of the Labiatae (mint) family (basil, mint, oregano, rosemary, sage, sweet savory, thyme); members of the Zingiberaceae family (turmeric, ginger); licorice root, green tea, flax; members of the umbelliferae (carrot) family (anise, caraway, celery, chervil, cilantro, coriander, cumin, dill, fennel, parsley); and tarragon. These beneficial substances act as antioxidants, stimulate the immune system, inhibit the formation of DNA adducts, inhibit hormonal actions and metabolic pathways associated with the development of cancer, hepatic diseases, inflammation, CHD, AIDS, Parkinson’s and
Huntington's disease (Block et al, 1992; Howard and Kritchevsky, 1997; Floyd, 1999; Engelhart et al, 2002).

During the last decade, seabuckthorn has attracted a lot of global attention. Seabuckthorn (Hippophae rhamnoides L., Elaegnaceae) is a thorny nitrogen fixing deciduous shrub, native to Europe and Asia (Rousi, 1971) and currently being cultivated in various parts of the world for its nutritional and medicinal values (Beveridge et al, 1999; Xu et al, 1994). In India, it grows very widely in Himalayan states of Himachal Pradesh, Ladakh regions in Jammu-Kashmir, Uttaranchal, Sikkim and Arunachal Pradesh. Indian Himalayas is believed to possess tremendously rich source of seabuckthorn.

The fruits and leaves of seabuckthorn (SBT) are very rich in vitamins and many other useful compounds, which have found many applications in pharmaceutical industries. It has been also used extensively as a medicinal plant in Tibetan and Mongolian traditional medicines (Lu, 1992). All parts of the plant are considered to be a good source of a large number of bioactive substances. The ripe fruit has been reported to be a rich source of vitamin C, E and carotenoids, which are potent antioxidants (Chen et al, 1990; Zheng et al, 1990; Kallio et al, 2002). The leaves are a rich source of flavonoids, which comprises of leucoanthocyanidins, quercetin, isorhamnetin, epicatechin and flavonols (Novruzov, 2001). Oil of seabuckthorn fruit (3-5%) is very precious material for the development of many medicines and creams for skin problems. Consequently seabuckthorn fruit and leaves are being used for the production of medicines for curing tumors, immunity related problems, cardiovascular diseases, lung disorders, skin disorders, memory loss and wound healing. These medicinal effects of SBT may be due to high content of antioxidant and immunomodulatory substances present in the plant.
For studying antioxidant and immunomodulatory properties of any substance many experimental models have been used. Animal models are the most commonly used for such studies and rats form a good model for evaluating the bioactivity of any drug or extract. However, the complexity of biological interactions that occur in the whole animal often make the study very difficult. For this reason, most detailed research these days is done on isolated tissues or cells \textit{in-vitro}.

For preliminary screening of an extract for any bioactivity, \textit{in-vitro} approaches offer several advantages over conventional \textit{in-vivo} methods. Cells are easy to isolate and there is no need for sacrificing animals. The effect of the extract directly on the cell can be studied using cell culture. Further, the effect of extract on various functions at the cellular level can be studied \textit{in-vitro}. They are cost effective, rapid and reliable. Once the dose and the activity has been evaluated using \textit{in-vitro} approaches, the results can be validated by doing \textit{in-vivo} experiments.

Although many cell lines such as hepatic cells, kidney cells and neural cells are in routine use for evaluating the antioxidant activity of extract, immune cells offer an advantage over the other cells. Immune cells offer an excellent model for evaluating antioxidant and immunomodulatory properties of herbal extracts. Immune cells like lymphocytes and macrophages are the first line of defense against invading organisms and are sensitive to changes in oxidant-antioxidant balance because of higher percentage of polyunsaturated fatty acid (PUFA) in their membrane (Meydani \textit{et al}, 1995). Immune cells are also exposed to changes in this balance because of the high number of reactive oxygen species (ROS) produced as part of their normal physiological function. Membrane related signaling and gene expression, which are sensitive to oxidative stress, are critical in maintaining normal
function of immune cells and their ability to defend against the wide range of foreign antigens they are exposed to.

The site of free radical formation is very important in determining the antioxidant activity. The hydrophilic free radicals such as hydroxyl radical and superoxide radical are generated in the cytoplasm in the cell. Antioxidant such as vitamin C effectively scavenges these aqueous free radicals but cannot scavenge radicals within the membranes. Lipophilic free radicals such as peroxyl radical, peroxynitrite are generated within the membranes. Antioxidants like vitamin E and vitamin A, are responsible for scavenging such lipophilic radicals but are ineffective against aqueous free radicals. Hence, the antioxidant activity of seabuckthorn was evaluated using two types of oxidants, chromium (VI) and sodium nitroprusside (SNP). Importance of chromium (VI) as environmental toxicant is largely due to impact on the body to produce cellular toxicity. Growing evidence suggests that reactive oxygen species (ROS) play a major role in chromium (VI) induced cell injuries (Klein et al, 1991; Snow, 1991; Ercal et al, 2001). Chromium (VI) compounds also induce tumors, nephrotoxicity and hepatotoxicity in experimental animals. Chromium (VI) reduction to various intermediates via hydrogen peroxide in vitro has been shown to produce hydroxyl radicals by fenton reaction (Shi and Dalal, 1990; Florez and Perez, 1999; Bagchi et al, 2002). Since hydroxyl radicals are hydrophilic in nature, the damage produced by Cr (VI) is mainly in the cytoplasm.

Sodium nitroprusside (SNP) has been used by many investigators to study the nitric oxide mediated cellular injury. NO at lower concentrations has been shown to be part of the oxidative war chest of the immune system and demonstrated to have anti-tumor and antimicrobial response. However, at higher levels NO reacts with superoxide to form peroxynitrite and have been implicated in various inflammatory and degenerative diseases (Beckman et al, 1990). This can directly
oxidize low-density lipoproteins resulting in irreversible damage to the cell membrane (Bernabe et al, 2001), hence producing a lipophilic attack in the cells. Since seabuckthorn is rich in various antioxidants, these two oxidants Chromium (VI) and SNP were selected to evaluate the efficacy of seabuckthorn against both hydrophilic and lipophilic oxidative damage.

In the present investigation the antioxidant, immunomodulatory and adaptogenic potentials of seabuckthorn (SBT) were evaluated using in-vitro and in-vivo experimentation.