



INTRODUCTION

1.0 INTRODUCTION

The human body depends on the continuous holistic intervention between internal and external factors. When this intervention is in a state of equilibrium, man enjoys health, when it fails, either due to internal deficiency or hostile environmental factors, it leads to disharmony and diseases. A vast majority of the diseases are mainly due to the imbalance between the oxidant and antioxidant homeostasis in the body (Haenold *et al.*, 2005). Aerobic organisms require ground state oxygen to live. Oxygen, while indisputably essential for life, can also sometimes participate in the destruction of tissues or lead to functional impairment. Inside the cell, oxygen undergoes reductive unscheduled reactions, leading to the formation of free radicals (Valko *et al.*, 2004).

Generation of free radicals is an integral feature of normal cellular functions in contrast to excessive generation or inadequate removal of free radicals, resulting in destructive and irreversible damage to the cells (Srikumar *et al.*, 2006). The imbalance between the cellular production of free radicals and the ability of cells to defend against them is referred to as oxidative stress (Schaller, 2005). Oxidative stress, arising due to the excessive production of reactive oxygen species, is a challenge faced by all aerobic organisms (Shanker *et al.*, 2005). The generation of reactive radicals in mammalian cells profoundly affects numerous critical cellular functions (Storz, 2005).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are well recognized for playing a dual role as both deleterious and beneficial species (Valko *et al.*, 2007). Generation of ROS and RNS can take place due to endogenous and exogenous factors in many metabolic processes and particularly under pathological conditions (Misiaszek *et al.*, 2004). A certain amount of ROS, RNS and RCS (Reactive Chlorine Species) production, is in fact, necessary, for proper health. For example, it helps the body's immune system to kill the microorganisms. ROS, RNS and RCS are oxidants, i.e, molecules or atoms which can oxidize a substrate and are reduced in this reaction (Polidori, 2003).

A portion of the oxygen may be converted univalently into several intermediates such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\bullet OH$), which subsequently leak out of the electron transport chain (Banerjee *et al.*, 2003). Reactive nitrogen species are also of great biological importance. Nitrogen can form a variety of compounds like nitric oxide (NO), peroxyxynitrite radical ($ONOO\bullet$) and peroxyxynitric acid ($ONOOH$), which cause significant macromolecular destruction. Due to the electron deficient nature of halide ions, haloperoxy radicals are highly reactive and oxidize substances. Free radicals trigger chain reactions that damage different constituents of living organisms by conformational changes in lipids, carbohydrates, proteins, DNA and RNA molecules (Hore, 2004).

It is well known that active free radicals, including oxygen free radicals and non-oxygen free radicals, are byproducts of normal metabolism (Zhao *et al.*, 2004). Increased oxidative stress is manifested by increased lipid peroxidation and other products of oxidation, including increased DNA damage (Vega-Lopez *et al.*, 2004). Protein modifications such as carbonylation, nitration and the formation of lipid peroxidation adducts like 4-hydroxynonenal are products of oxidative damage attributed to ROS (Choksi *et al.*, 2004). Environmental stresses can lead to damage of specific mitochondrial targets through the direct ROS and indirect action of lipid peroxidation (LPO) products (Taylor *et al.*, 2004).

Exposure to stress induces a cluster of physiological and behavioral changes in an effort to maintain the homeostasis of the organisms. Long term exposure to stress, however, has detrimental effects of several cell functions, such as impairment of antioxidant defenses, leading to oxidative damage. Oxidative stress has been recognized as a central feature of many diseases (Torres *et al.*, 2004).

Oxidative processes occurring in living organisms result in the formation of highly reactive free radicals that are known to cause accelerated aging of living cells (Huang and Manton, 2004) and to increase the risk of health impairment including initiation of cancer (Polasek, 2004). In the last few years, there has been

increasing evidence of the correlation between the generation of reactive oxygen species, DNA damage, tumour promotion and carcinogen exposure (Shi *et al.*, 2004). The level of oxygen free radical is found to be high in cancer patients (Valko *et al.*, 2006).

Because of these ill effects, accelerated search for antioxidant principles, which leads to the identification of natural resources and isolation of active antioxidant molecules, is going on. Many herbals and traditional compounds are scientifically validated for use as potential sources of antioxidants (Jagtenberg and Evans, 2005). Antioxidants have the capacity to prevent, delay or ameliorate many disorders (Delanty and Dichter, 2000).

The antioxidants may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing the first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds and chain breaking to prevent continued hydrogen abstraction from substrates (Saha *et al.*, 2004). The antioxidant give up their own electrons to neutralize free radicals and blocks the chain reaction of oxidation. The antioxidants can be derived from the diet. Plant and plant products have been used as a source of medicine for a long time. Human beings rely on traditional medicine for their primary healthcare need for a long time (Ranga *et al.*, 2005).

Herbal extracts have been used for centuries around the world to treat different diseases and there is a long history of the use of plants in many cultures. The supplementation of the natural antioxidant defense system of the body through a balanced diet containing fruits and vegetables could protect the body against various oxidative stresses (Oliveira *et al.*, 2003).

The dietary antioxidants while structurally diverse, have various spin trap abilities; they have one or more reactive groups on their molecular surface that can be easily modified by ROS without significantly altering the overall structure.

The ROS-mediated attacks on the reactive groups, in fact, neutralize the ROS, thereby protecting other sensitive molecules from ROS-mediated damage (Haenold *et al.*, 2005).

Dietary factors that act as antioxidants to decrease membrane lipid peroxidation and protein carbonyl formation may contribute a protective effect against cancer, atherosclerosis and age related diseases (Luqman and Rizvi, 2006). Reports indicate that there is an inverse relation between the dietary intake of antioxidant rich foods and incidence of human diseases. Antioxidants in their natural formulations are more active than their isolated form (Srikumar *et al.*, 2006).

Phytochemicals are naturally occurring non-nutritive chemicals in plants. The phytochemicals work alone or in combination with vitamins and other nutrients in food to prevent, halt or lessen diseases (organicashitaba.com/phytochemicals.html). Non-nutritive polyphenols, such as flavonoids are also present in significant amounts in fruits and vegetables and have been shown to possess strong antioxidant properties in chemical systems (Duthie *et al.*, 2000).

Better understanding of structure-activity relationship of these phytochemicals, synergistic mode of action and their relative importance in different mechanisms may provide deeper insight in finding out better and safer therapeutics. The concept of synergy is central to the holistic approach. However, the natural/holistic approach attempts to solve the problem by taking these in their entirety, with all their interlinkages and their complexity (Tiwari, 2001).

Even as we commence the new century with its existing prospect of gene therapy, herbal medicine remains one of the common forms of therapy available to much of the world's population (Kuruvilla, 2002). Medicinal and aromatic plants play an important role in the healthcare of people around the world, especially in developing countries. The Indian systems of medicine, Ayurveda, Siddha and

Unani entirely, and Homeopathy to some extent, depend on plant materials or their derivatives for treating human ailments (Rao *et al.*, 2004).

According to a World Health Organization estimate, about 80% of the world population relies on the traditional system of medicine for primary healthcare, where plants form the dominant component over other natural resources (Mukherjee and Wahila, 2006). There has been a revival of interest in herbal products (botanicals) at a global level and the conventional medicine is now beginning to accept the use of botanicals, once they are scientifically validated (Gilani and Rahman, 2005). The demand for medicinal plants is increasing in both developing and developed countries due to the growing recognition of natural products being non-toxic, having no side effects and easily available at affordable costs (Taylor *et al.*, 2004).

Naturally derived or originated compounds still play a major role as drugs and as lead structures for the development of synthetic molecules. About 50 percent of the drugs introduced to the market during the last 20 years are derived directly or indirectly from small biogenic molecules (Vuorela *et al.*, 2004). A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals. Some of the phytochemicals are pharmacologically active and can exert a therapeutic action on the body (Miliauskas *et al.*, 2004).

Many plants show antioxidant properties, which have protective effect on human carcinogenesis (Park and Pezzuto, 2002). Cancer chemoprevention has traditionally been defined as a dietary or therapeutic approach for the prevention, delay or reversal of carcinogenesis. Some natural products may be administered as dietary supplements (Mehta and Pezzuto, 2002). Similarly, many ethnobotanicals and components of vegetable diets with free radical scavenging activity have a profound effect on diabetes (Sabu and Kuttan, 2002), Alzheimer's disease (Howes *et al.*, 2003), ulcers (Borelli and Izzo, 2000) and even cancer

(Mantle *et al.*, 2000). Herbal derived substances remain the basis for a large proportion of the commercial medications used today for the treatment of heart diseases, high blood pressure, pain, asthma and other illnesses. Today, a great number of modern drugs are still derived from natural sources, and 25 percent of the prescriptions contain one or more active ingredients. Therefore, the herbs are considered as a potential source of drugs for all clinical purposes (Aithal, 2005).

The plants and plant products are part of the vegetarian diet and a number of them exhibit medicinal properties (Naik *et al.*, 2003). Some of the leafy vegetables are treated as weeds in different parts of the world and as indigenous / traditional vegetables in other. Such is the case of the *Solanum nigrum* species related to the blacknightshade. *Solanum nigrum* and related species are classified as an under-utilized and neglected species. They are, however, also widely used as leafy herbs and vegetables, as source of fruits and for various medicinal purposes. Therefore, the human consumption of their leaves and fruits as food is widespread, particularly in Africa and South East Asia (Edmonds and Chewya, 1997). It is commonly used in traditional medicine as a remedy for various diseases (Jainu and Shyamala Devi, 2004).

The present study was undertaken to evaluate the antioxidant and anticancer properties of the leaves of *Solanum nigrum* (L) complex, one bearing the black berries and the other bearing the red berries. According to a recent taxonomic study, the red berry bearing plants are identified as *Solanum nigrum* L. subsp.*punicerum* (Kirschl), also known as *Solanum villosum* var.*punicerum* (Kirschl) (Reemakumari, 2006). The plants were authenticated by the Botanical Survey of India (BSI), Coimbatore, Tamilnadu, India.

The objectives of the present investigation were as follows:

- Analyzing the antioxidant contents of the leaves -
 - ◆ Enzymic, non-enzymic and mineral antioxidants
- Assessing the antioxidant and anticancer properties using various *in vitro* systems
 - ◆ Scavenging of free- and non-radical oxidants
 - ◆ Antioxidant activity against biological molecules
 - ◆ Effect on antioxidant contents of oxidative stress induced goat liver slices
 - ◆ Cytotoxic effect on cancer cell lines
- Confirming the antioxidant and anticancer effects *in vivo*
 - ◆ Analysing the antioxidant effect against oxidative stress induced in rats
 - ◆ Evaluating the anticancer activity against Dalton's Lymphoma Ascites tumour induced in mice
- Identifying the active components in the leaves using various techniques like TLC, FT-IR, NMR and GC-MS.

Thus, the present study was designed to validate the antioxidant and anticancer effects of these plants in a systematic manner to provide information for preventing and treating diseases, which may in turn raise the economic importance of these plants.

A brief review of the salient points of the literature relevant to this study is presented in the next chapter.