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Introduction

Cancer is the most deadly disease that causes the serious health problems, physical disabilities, mortalities and morbidities around the world. It is the second leading cause of death all over the world (Sultana *et al*., 2014). More than six million deaths each year occur in the world is due to cancer. In the world wide, about 12.7 million cancers were diagnosed and 7.6 million deaths were reported in 2008 (Jemal *et al*., 2011). In 2012, there were an estimated 14.1 million cases around the world, of these 7.4 million cases were men and 6.4 million women. This number is expected to increase to 24 million by 2035 (Ferlay, 2012).

Cancer is a multifactorial disease characterized by uncontrolled growth and spread of abnormal cells (Baskar *et al.,* 2012) and it is a hyper proliferative disorder that causes transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis (Ranganathan and Jebaraj, 2014). It is one of the most life-threatening diseases in which deregulating proliferation of abnormal cells invades and disrupts surrounding tissues (Gennari *et al*., 2007).

Cancer constitutes serious public health problems in both developed and developing countries. The limited success of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in treatment of cancer indicates that there is an imperative need of alternative strategies in cancer management (Dai and Mumper, 2010).

Chemoprevention, which consists of the use of synthetic or natural agents (alone or in combination) to block the development of cancer in human beings, is an extremely promising strategy for cancer prevention. The control of cell proliferation is fundamental in maintaining cellular homeostasis and loss of this mechanism is a principle hallmark of cancer cells. Synthetic chemotherapeutic agents are used to stop the growth and eliminate cancer cells even at distant sites from the origin of primary tumor. However, synthetic agents do not distinguish between a cancer and normal cell, and

eliminate not only the fast-growing cancer cells but also other fast-growing cells in the body, including hair and blood cells (Tousson *et al*., 2014a).

Discovery and development of anticancer agents are the key foci of several pharmaceutical companies as well as nonprofit government and non-government organizations (Narang and Desai, 2009). It is necessary to develop new anticancer agents with antitumor and antimetastatic activities but without adverse reactions such as gastrointestinal toxicity, myelotoxicity and immune suppression caused by cancer chemotherapeutic drugs (Kimura, 2005).

A huge reservoir of bioactive compounds exists in many species of plants and only a small percentage of which have been examined and continued to be an important source of anticancer agents. Worldwide efforts are ongoing to identify new anticancer compounds from plants. With the current decline in the number of new molecular entities from the pharmaceutical industry, novel anticancer agents are being sought from traditional medicines (Dhanamani *et al*., 2011).

Free radicals are highly active, unstable compounds due to the presence of unpaired electron in their outer shell, which are produced as a result of cellular metabolism, lipid peroxidation, cellular injury and oxidative stress. These free radicals are also responsible for the aetiology of more number of chronic and degenerative diseases (Murugan *et al*., 2013). Free radicals are toxic byproducts of natural cell metabolism and are responsible for causing a wide number of health problems (Sirappuselvi and Chitra, 2012).

Several diseases like cancer, arthritis, Parkinson's disease and heart diseases are caused due to oxidative stress induced by reactive oxygen(ROS) and nitrogen species(RNS) (Tabaraki and Nateghi, 2011). ROS which contain oxygen, are chemically reactive compounds due to the presence of unpaired electrons and may be present in the human body during environmental stress and can be very harmful. This may result in significant damage to cell structures. Most of the damaging effects of oxygen are due to ROS, which include superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$), hydroperoxyl radicals ($HOO^{\bullet}$), peroxyl ($ROO^{\bullet}$) and non-free radical species such as hydrogen peroxide (H_2O_2), ozone (O_3), and singlet oxygen. ROS can lead to more

diseases such as atherosclerosis, coronary heart diseases, aging and cancer (Ercan *et al*., 2013). Reactive nitrogen species(RNS) such as nitric oxide (NO·), nitrogen dioxide (NO_2), peroxynitrate (ONOO−), nitrous acid (HNO_2) and dinitrogen trioxide (N_2O_3) are generated spontaneously in cells through metabolism (Pacher *et al*., 2008).

Oxidative stress is caused by an imbalance in the production of reactive oxygen and the biological ability to detoxify the reactive intermediates. Pro-oxidant, as a chemical substance induces oxidative stress, either by generating reactive oxygen species (ROS) or by inhibiting antioxidant systems. The oxidative stress induced by these substances (ROS) can induce injury to the cells and tissues which is known as oxidative damage (Alzoghaibi, 2013). The oxidative stress happens by the overlapping of pro-oxidizing compounds in relation to the antioxidants. This imbalance generates the oxidation of biomolecules with later loss of biological function consequently causing oxidative damages in cells and tissues. The chronic effect of these processes result in relevant implications on the aetiology of chronic diseases, for instance, atherosclerosis, diabetes, obesity and cancer (Vidigal *et al*., 2012).

There are restrictions on the use of synthetic antioxidants, as they are suspected to be carcinogenic. Therefore development of a potential natural antioxidant molecule is gaining importance in the recent years as it plays an important role in preventing or delaying the onset of certain pathological consequences such as hepatotoxicity, heart diseases and cancer (Ashwini and Krishnamoorthy, 2011).

Many synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are very effective but they possess certain health risks and toxic properties to human health. Recent results show that plant polyphenolics and their various metabolites may scavenge oxygen and nitrogen free radicals, which controls oxidative stress and oxidative damage to blood lipids and proteins (He *et al*., 2010).

Antioxidants are compounds that can protect or delay the oxidation of oxidizable products by scavenging free radicals and reducing oxidative stress (Alzoghaibi, 2013)**.** Antioxidant components are microconstituents that inhibit lipid oxidation by inhibiting the initiation or propagation of oxidizing chain reactions, and are also involved in scavenging of free radicals. Clinical approaches of antioxidants increased

multifold during the recent time for the management and therapeutic implication of neurodegenerative disorders, aging, and chronic degenerative diseases (Irshad *et al*., 2012). Studies have shown that many antioxidant compounds possess anti-inflammatory, antitumor, antimutagenic and anti-carcinogenic activities (Sala *et al*., 2002).

Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage (Mattson and Cheng, 2006).

Several studies have indicated that antioxidants could quench free radicals or suppress the generation of free radicals by interrupting oxidation chain reactions (Kerr *et al*., 1996). Antioxidants that trap free radicals and lipid peroxides may delay the onset of lipid peroxidation, inhibiting the production of free radicals and suppressing damages induced by enzymes that can degrade connective tissues (Halliwell *et al*., 1995).

Antioxidants neutralize dangerous free radicals and stop the chain reactions that spoils the living cells through the reduction of free radical intermediates and inhibition of other oxidation reactions. Hence the plants and animals regulate complex systems of multiple nonenzymic antioxidants, such as glutathione, Vitamin C and Vitamin E along with some enzymic antioxidants like catalase, superoxide dismutase and various peroxidises (Joseph *et al.,* 2010). Several of the investigated herbs contain substantial amounts of free radical scavengers and can serve as a potential source of natural antioxidants for medicinal and commercial uses (Kratchanova *et al*., 2010).

Medicinal plants are an essential part of the traditional health care systems. There are more than 8,000 plant species in South Asia with known medicinal uses. Thus, historically it is evident that South Asia is home to many rich traditional systems of medicine. In Indian medicine systems, Ayurveda, Siddha and Unani entirely and Homeopathy partially depend either on plant materials or their derivatives for treating human ailments (Mandal and Rath, 2015)**.**

Medicinal plants have always been the principal source of medicine in India. Today about 80% of the world's population relies mainly on plants and plant extracts for healthcare. In recent years, researches on medicinal plants are useful for developing new

products and medicines to treat diseases. Medicines derived from plants are potentially safer and more reliable compared to synthetically produced drugs. The medicinal plant wealth is our national heritage and it seems to be the first and foremost line of defence for the treatment of many diseases (Ripunjoy, 2013).

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine (Danish *et al*., 2011).

The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of human medicine. Plants have great potential uses, especially as traditional medicine and pharmacopoeial drugs. A large proportion of the world's population depends on traditional medicine because of the scarcity and high costs of orthodox medicine (Hudaib *et al.*, 2008). Medicinal plants possess unlimited and untapped wealth of chemical compounds with high drug potential which make these plants useful as sources of biomedicines (Deshpande and Bhalsing, 2013).

India is a rich home to rare medicinal plants of high medicinal importance with antioxidant activity (Dubey *et al*., 2015). Indian medicinal plants have a great history for their utility as remedy for treatment of variety of ailments. The impact of medicinal plants and their isolated pure compounds is in variety of therapeutic areas but the major contribution is in the field of anti-infective and anticancer drug discovery (Newman and Cragg, 2007).

Plants are the effective source of anticancer agents and over 60% anticancer agents are derived from plants (Crag and Newman 2005). The National Cancer Institute has screened around 114,000 extracts for anticancer activity. Still there are number of plants with anticancer potential have not yet been fully investigated (Shoeb, 2006).

Most chemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their synthetic derivatives (Solowey *et al*., 2014). Plant based medication has definitely found a role in cancer healing (chemotherapy), and the mechanism of interaction between many phytochemicals and cancer cells has been studied extensively. In particular, there is growing interest in the pharmacological estimation of various plants used in Indian traditional system of medicine. There are more than 2,70,000 higher plants existing on this planet. But only a small portion has been surveyed phytochemically. So, it is anticipated that plants can provide potential bioactive compounds for the development of new 'leads' to combat cancer (Shoeb, 2006).

Phytochemicals are naturally occurring chemicals present in medicinal plants, leaves, vegetables and fruits that have their own defence mechanisms and protection against various diseases (Wadood *et al*., 2013). Phytochemicals may either be used as chemotherapeutic or chemo preventive agents with chemoprevention referring to the use of agents to inhibit, reverse or retard tumorigenesis. In this sense chemo preventive phytochemicals are applicable to cancer therapy, since molecular mechanisms may be common to both chemoprevention and cancer therapy (Sarkar and Li, 2006).

Several phytochemical constituents such as flavonoids, polysaccharides, anthracene derivatives, alkaloids and tannins have therapeutic properties toward diabetes, dys-lipidemia, conjunctivitis, renal and liver disorders, ulcer, asthma,skin disease and cancer (Juan-Badaturuge *et al*., 2011).

The phytochemicals possess antioxidant activity and protect body against free radical damage (Gupta *et al*., 2011). Active phytochemicals derived from plants are found to be alkaloids, flavonoids, polyphenols, terpenoids and polysaccharides (Govind, 2011). The reason for using medicinal plants lies in the fact that they contain chemical substances of therapeutic value namely secondary metabolites that have definite physiological action in the human body. The most important among these bioactive compounds of plant are alkaloids, flavonoids, tannins and phenolics (Nisa *et al.,* 2010).

Many natural products discovered from medicinal plants are secondary metabolites such as terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins, alkaloids, which exhibit significant antioxidant and other activities and have

played an important role in the treatment of cancer (Kaur *et al*., 2011). Medicinal plants have thus become a focal point to improve the present and future health needs against cancer. This is because secondary metabolites present in medicinal plants could maintain the health and cure various diseases including cancer with less harmful effects (Harun-ur-Rashid *et al*., 2002). The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bio-activities (Kaviraj *et al*., 1993).

Plants belonging to the genus, *Cassia* are considered as leguminous plants that are useful for their medicinal value and edible quality. Due to their medicinal, agricultural and economic value, *Cassia* species have more important attention worldwide (Pant *et al.,* 2014a). This large genus is widely distributed in several parts of the world, including India, Mauritius, China, East Africa, South Africa, America, Mexico and Brazil (Mazumder *et al*., 2008).

Several *Cassia* species are of high medicinal significance and commercial since they are used in traditional medicines (Danish *et al*., 2011). *Cassia* species are reported to have various pharmacological activities such as antitumor (Gupta *et al*., 2000), antioxidant (Danish *et al*., 2011; Siddhuraju *et al*., 2002), hepatoprotective (Kalantari *et al*., 2011; Mondal *et al*., 2012) and hypoglycemic activity (Nirmala *et al*., 2008; Pari and Latha, 2002).

The antitumor and antimetastatic actions of stilbene derivatives isolated from *Cassia* species were examined and were reported to inhibit tumor-induced neovascularization in *in vitro* and *in vivo* models (Kimura, 2005). Some important members of *Cassia* genus namely *Cassia fistula*, *Cassia siamea*, *Cassia tora*, *Cassia senna* and *Cassia auriculata* are rich in bioactive molecules which are responsible for their antioxidant (Yen *et al*., 1998; Yen and Chung, 1999), antimicrobial and antidiabetic activities (Pari and Latha, 2002).

Although many scientific works have been carried out on the various species of *Cassia* belonging to the family Fabaceae/Leguminoseae with respect to their biological activities, not much work has been done on the anticancer activiy of *C. senna.*

With this background, the present study entitled "Evaluation of antioxidant and anticancer potential of *Cassia senna* L. using *in vitro* and *in vivo* methods" was designed with the following objectives:

- To determine the antioxidant potential and the phytochemical constituents of leaf and pod of *C. senna*
- To screen the effective extract of *C. senna* by radical scavenging effect, chromatographic analysis and *in vitro* cytotoxic activity.
- To evaluate the *in vivo* antitumour activity of the selected extract of *C. senna* on cancer induced mice
- To characterize the active principles of the selected extract by spectral analysis.

A brief review of the vast literature available, relevant to the present study, is presented in the next chapter.