2

Review of Literature

The review of literature pertaining to the present study entitled, **"Evaluation of antioxidant and anticancer potential of *Cassia senna* L. using *in vitro* and *in vivo* methods"** is discussed under the following headings.

2.1. Cancer

2.2. Free radicals

2.3. Oxidative stress and Oxidative damage

2.4. Antioxidant defense system

2.5. Medicinal value of plants

2.6. Phytochemicals

2.7. Medicinal properties of *Cassia species*

2.8. Medicinal properties of *Cassia senna*

2.1. Cancer

Cancer commonly defined as an uncontrolled growth of cells, with loss of differentiation and commonly with metastasis, spread of the cancer to other tissues and organs (Rajwar and Khatri, 2012). Cancer is one among the most serious health problems worldwide, affecting individuals of all sexes, ages and races and it is the group of diseases, characterized by uncontrolled cellular proliferation and invasion from one part to another part, the process referred to as Metastasis. The uncontrolled growth and spreading of cancer cells make them dangerous (Lengauer *et al*., 1998).

2.1.1. Cancer- global scenario

Cancer is a global challenge as this disease remains the second largest cause of death around the world, with some predictions that it will move into the top rank in future. Cancer accounts for one out of every eight deaths annually. Increase in life expectancy and adoption of western diet and lifestyles, owing to tobacco abuse and widespread exposure to carcinogens, are some of the major key factors for increasing the burden of cancer in the developing countries like India (Severi *et al*., 2010). Worldwide about 20 million people per year are diagnosed with cancer and more than 6 million mortalities are recorded and rate of cancer incidence is increased every year (Turgay *et al*., 2005). For patients diagnosed with distinct diseases, the probability of dying of cancer was much higher than the probability of dying of other causes (Edwards *et al*., 2013).

Among all cancers, the three leading cancer killers were different than the three most common forms, (i) Lung cancer is responsible for 17.8 per cent of all cancer deaths. (ii) Stomach cancer is responsible for 10.4 per cent and (iii) Liver cancer is responsible for 8.8 percent of all cancer deaths. Industrial nations with the highest overall cancer rates include: U.S.A, Italy, Australia, Germany, The Netherlands, Canada and France. Developing countries with the lowest cancer were in Northern Africa. Cancer rates could further increase by 50% to 15 million new cases in the year 2020 (Dhanamani *et al,* 2011*)*.

2.1.2 Cancer - Indian scenario

Every year about **8,50,000** new cancer cases being diagnosed in India resulting about **5,80,000** cancer related death every year. India has the highest number of the oral and throat cancer cases in the world. Every third oral cancer patient in the world is from India. In males oral, lungs and stomach cancers was the three most common causes of cancer incidence and death whereas in females cervical, breast and oral cancers were the three main causes of cancer related illnesses and death. Overall cervical cancer was the number one cause of cancer death in India. Average life span was about 58 yrs in India compared to 75 yrs in the developed world (Dhanamani *et al*., 2011).

Table 1

World cancer statistics for most common cancers in 2012

Rank	Cancer	New case diagnosed in 2012(1000s)	Percent of all cancer excluding non melanoma of skin
1	Lung	1,825	13
2	Breast	1,677	11.9
3	Colorectum	1,361	9.7
4	Prostate	1,112	7.9
5	Stomach	952	6.8
6	Liver	782	5.6
7	Bladder	430	3.1
8	Non-hodgkin lymphoma	386	2.7
9	Leukaemia	352	2.5
10	Pancreas	338	2.4
11	Kidney	338	2.4
12	Brain	256	1.8
13	Melanoma of skin	232	1.6
14	Gall bladder	178	1.3
15	Hodgkin lymphoma	66	0.5

Sultana *et al*., (2014)

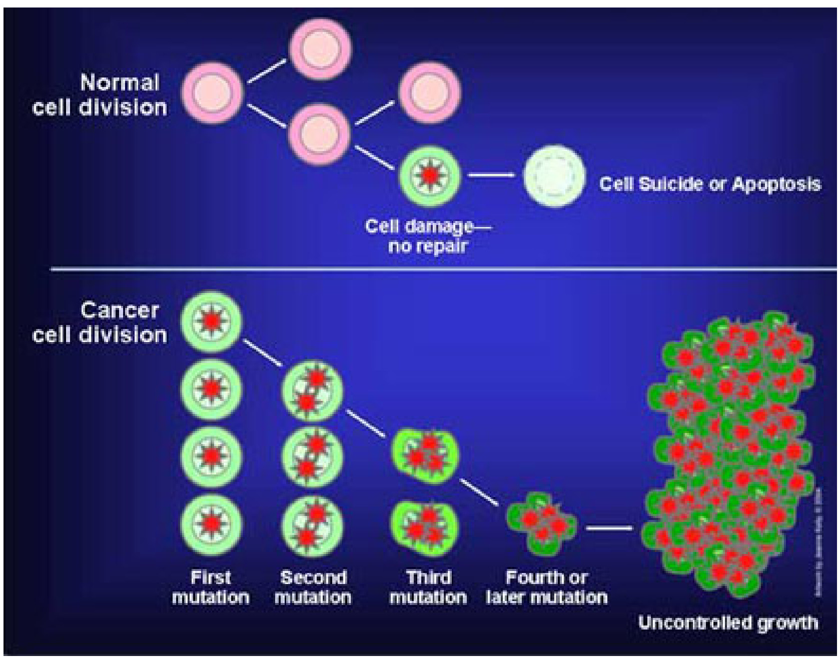
2.1.3. Properties and stages of cancer

Cancer cells are defined by two heritable properties:

1. Reproduce in defiance of the normal restraints on cell division and
2. Invade and colonize territories normally reserved for other cells.

As long as the neoplastic cells remain clustered together in a single mass, however, the tumor is said to be benign. A tumor is considered as cancer only if it is malignant, that is, when its cells have acquired the ability to invade surrounding tissue. Invasiveness usually implies an ability to enter the blood stream or lymphatic vessels and form secondary tumors called metastases, at other sites in the body. Tumors induce the formation of new blood vessels that invade the tumor to nourish it, a process called angiogenesis. New blood vessels nourish the growing tumor, allowing it to increase in size and thus increase the probability that additional harmful mutations will occur (Alberts *et al*., 2002).

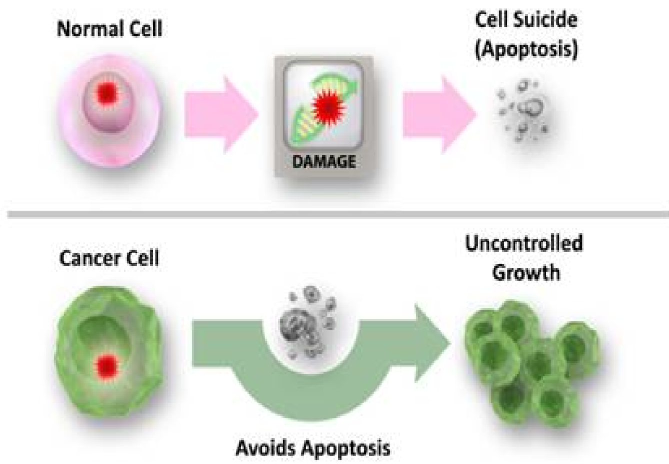
Figure 1 : Loss of normal growth control in cancer cells



Cancer progrssion

Carcinogenesis occurs and develops in three steps. Initiation, where the normal cells in the organ turn carcinogenic. Promotion, in this stage the surrounding cells of the newly developed carcinogenic cell also become tumor cells. Progression, this is the most critical stage where the cancer cells break from the parent organ, mixes into the circulatory system, flows and settles somewhere else in the body and causes tumor in that particular organ (Farber, 1984).

Figure 2 : Lack of apoptosis in cancer cells



Types of cancer cells

Cancer cells are classified according to the tissue and cell type from which they arise.

- ✓ Leukemias and Myelomas-Tumors of the leukocytic system.
- ✓ Erythroblastomas-Tumors of the erythroblastic lineage.
- ✓ Lymphomas-Tumors of the lymphocytic lineage.
- ✓ Neuroblastoma- Tumors of the neuroblastic origin.
- ✓ Fibroma- Tumors of the fibroblastic origin.
- ✓ Carcinomas-Cancers arising from epithelial cells.
- ✓ Sarcomas-Cancers arising from connective tissue or muscle cells.

In parallel with set of names for malignant tumors, there is a related set of names for benign tumors.

- ✓ Adenoma- A benign epithelial tumor with a glandular organization.
- ✓ Adenocarcinoma- The corresponding type of malignant tumor of adenoma
- ✓ Chondroma- A benign tumors of cartilage
- ✓ Chondrosarcoma- A malignant tumor of cartilage

About 90% of human cancers are carcinomas, perhaps because most of the cell proliferation in the body occurs in epithelia or because epithelial tissues are most frequently exposed to the various forms of physical and chemical damage that favour the development of cancer (Becker and Deamer, 1991).

Morphology of cancer cells

- larger than normal cell
- show nuclear and cellular polymorphism
- hyperchromatism, altered nuclear: cytoplasmic ratio, abundant mitosis.
- Often grow over one another and form multilayers.
- Diminished adhesion.

Biochemical changes of cancer cells

- Increased synthesis of DNA and RNA
- Show increased rate of glycolysis
- Show alterations of permeability and membrane charge.
- Changes in composition of glycoproteins and glycosphingolipids on cell surfaces.
- Appearance of new antigens and loss of certain antigens (Chatterjea and Shinde, 2005).

2.1.4. Chemotherapy

Chemotherapy is one of the main modalities available for cancer treatment but the current synthetic chemotherapeutic drugs currently available are reported to exhibit toxicity towards normal tissues and possess undesirable side effects. Most cancer chemotherapy regimens make use of highly cytotoxic drugs that target proliferating cell populations. The non-discriminatory use of these drugs leads to severe side effects in normal cells with the high proliferative index, leading to drug resistance and limiting the effective dose of anticancer drug that can be administered. Overcoming these side-effects with anticancer agents from plants may provide a strategy for overcoming the adverse effects of chemotherapy as they are less toxic. Development of naturally derived anticancer drugs, therefore, is crucial, and isolation of novel compounds has become an important part of cancer research (Cardellina *et al*., 1999).

Synthetic chemotherapeutic drugs for cancer such as 5-fluorouracil derivatives, cisplatin and mitomycin have been used extensively for the treatment of certain types of cancer. However, with these treatments, severe gastrointestinal toxicity with diarrhea, mucosis and hematological toxicity with leucopenia and immune suppression appear to be dose-limiting factors (Kimura, 2005). The inhibition of tumor cell growth without side effects is recognized as an important target for cancer therapy (Koppikar *et al.,* 2010).

Since ancient times, natural products, herbs and spices have been used for preventing several diseases, including cancer. Till date large number of natural products have been screened for their anticancer potential through various experimental models. This has resulted in the discovery of 30 effective anticancer drugs (Ramnath *et al*., 2002). Around the world, tremendous resources are being invested in prevention, diagnosis and treatment of cancer (Narang and Desai, 2009).

2.2. Free radicals

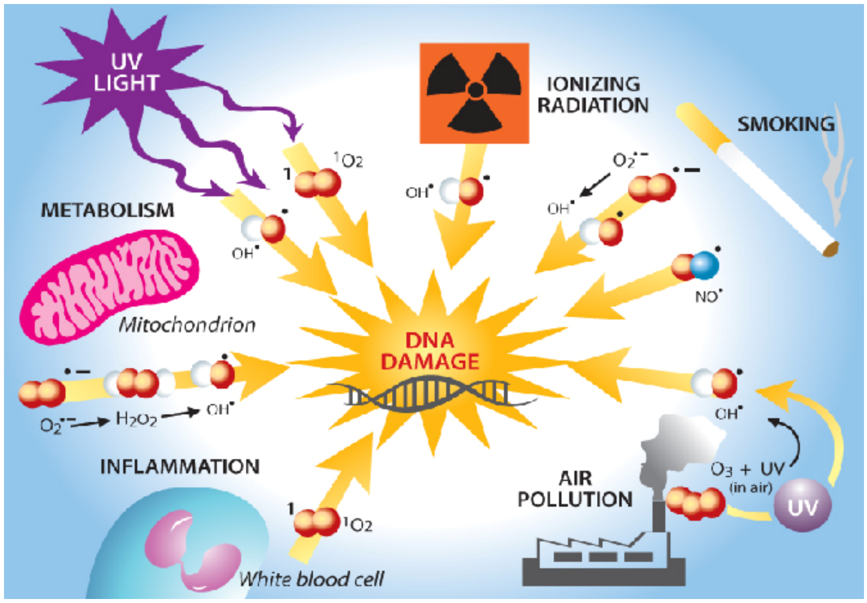
Free radicals are highly reactive molecules produced during mitochondrial aerobic metabolism (Li *et al*., 2013). A free radical has one or more unpaired electrons in its outer orbital. Such unpaired electrons make these species very unstable and therefore quite reactive with other molecules. They try to pair their electron(s) and generate a more stable compound (Shekhawat *et al*., 2010).

Free radicals tend to be reactive and participate in chain reactions, in which a single free radical initiation event can be propagated to damage multiple molecules. Studies of the chain reaction in purified chemical systems show that a single initiation event can be propagated to damage 200- 400 lipid molecules, before two radicals react to eliminate the unpaired electrons and terminate the reaction sequence (Jones *et al*., 2008).

Formation and effects of free radicals

Living cells may generate free radicals as a result of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases such as cancer, diabetes, aging, and other degenerative diseases in humans (Shrivastava *et al.,* 2013). They are known to be the underlying cause of oxidative stress which is grossly implicated in the pathogenesis of various diseases such as cancer, diabetes, cardiovascular diseases, aging and metabolic syndrome (Raghuveer and Tandon, 2009).

Figure 3 : Formation of free radicals



Types of free radicals

Free radicals are classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS are oxygen centered free radicals, which include radicals such as superoxide anion, hydroxyl radical and alkoxyl and non-radicals, hydrogen peroxide and singlet oxygen. RNS are nitrogen centered radicals, which include nitric oxide and peroxynirite (El-Bahr, 2013).

Reactive oxygen species (ROS)

Superoxide ($O_2^{\cdot -}$)

The incomplete reduction of oxygen during oxygen transfer reactions in the electron transport chain in mitochondria leads to the generation of singlet oxygen and superoxide anion (Gutteridge and Halliwell, 2010). Superoxide anion is the primary free radical that is produced inside the biological system during oxidative damage (Chan *et al*., 2011).

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is a toxic ROS that induces damage to various biological structural molecules (Petrov and Breusegem, 2012). Hydrogen peroxide is generated from superoxide, which is catalyzed by the enzyme superoxide dismutase. Plants generate hydrogen peroxide in response to abiotic stress such as temperature, UV light from various sources. It plays a critical role in the metabolism of plants and the accumulation of H_2O_2 inside the cells causes toxic effects (Mazid *et al*., 2012).

Hydroxyl ($OH^\bullet$)

Hydroxyl radical is the most unstable and damage inducing free radical with average life of milliseconds and cannot be detected directly under *in vivo* conditions. The hydroxyl radical is very reactive in nature and can cause damage to biomolecules DNA, RNA, proteins and lipids in both the nucleus and mitochondria (Tang *et al*., 2012). Hydroxyl radical can readily react with deoxy ribose and bases present in the DNA that causes deterioration of DNA, which results in aging (Sanders *et al*., 2011).

Singlet oxygen ($^1O_2$)

Singlet oxygen is one of the ROS generated inside the biological system and it operates through mechanisms that are different from that of other ROS (Bohm *et al*., 2012). Singlet oxygen is generated in the aerobic organisms and the excess production of this leads to the oxidation of lipids, DNA and RNA (Agnes- Lima *et al*., 2012).

Reactive nitrogen species

Nitric oxide and Peroxynitrite

Nitric oxide is an important signalling molecule, which plays a critical role in mediating cellular activities and damage during various disease conditions. The nitricoxide induces toxic effect on cells, which is mainly through another free radical peroxynitrite, which is generated by the reaction between nitric oxide and superoxide. Peroxynitrite is capable of inducing direct oxidative damage to the biomolecules including lipids, DNA and proteins. Peroxynitrite is also involved in the pathogenesis of various cardiovascular and neurodegenerative disorders (Pacher *et al*., 2008).

Table 2 : Types of Free radicals and their action

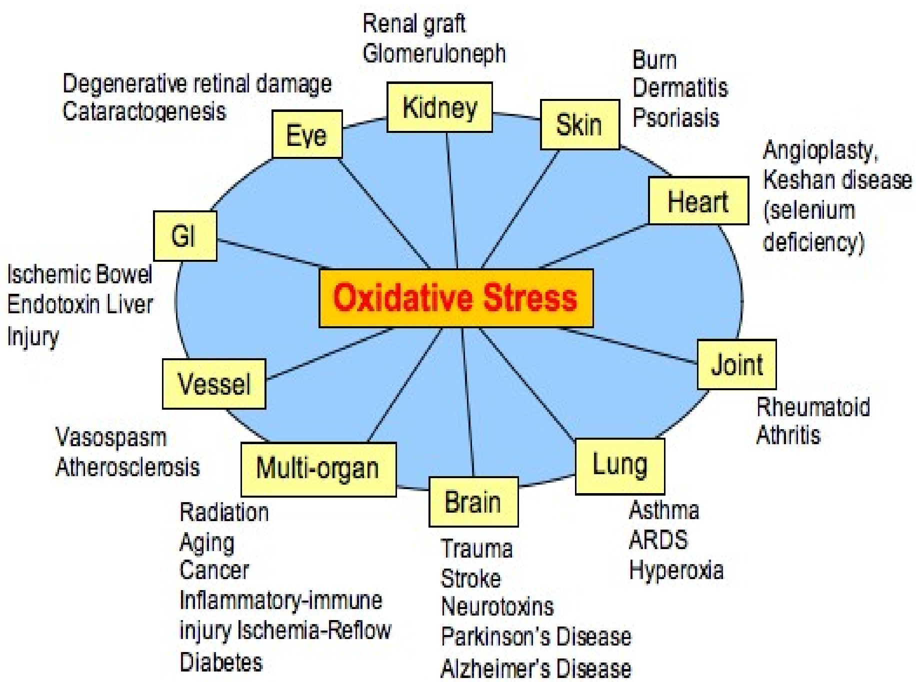
Species	Chemical stucture	Description	Occurrence	Action
Superoxide radical	$O_2^{\cdot -}$	Most potential radical in the induction of cellular damage	Almost all aerobic cells	Majority of reactions as a reducing agent
Hydroxyl radical	$OH^{\bullet}$	$O_2^{\cdot -}$ Acid conjugate, highly reactive	Formed through water radiolysis	DNA, proteins, carbohydrates and lipids
Hydroperoxyl radical	$HO_2^{\bullet}$	Protonated form of the $O_2^{\cdot -}$	From hydrogen peroxide	Biological membranes
Hydrogen peroxide	H_2O_2	It's not a free radical, because did not submit electrons paire in the last layer	Reactions for the production of $OH^{\bullet}$	Proteins and lipids
Singlet oxygen	$^1O_2$	Excited form of molecular oxygen. It's not a free radical, because did not submit electrons paire in the last layer	Generated by phagocytes, luminous induction and catalyzed reactions by peroxidises	DNA changes

2.3 Oxidative stress and Oxidative damage

Oxidative stress, a key player in several diseases such as cancer, diabetes mellitus, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases, results from an imbalance between formation and neutralization of prooxidants pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation (Sun *et al*., 2012).

Overproduction of free radicals or lowering of antioxidant defences results in oxidative stress. Oxidative stress is excess formation and/or incomplete removal of highly reactive molecules such as ROS, including superoxide radical, hydroxyl radical as well as nonradical species such as hydrogen peroxide. Free radicals are implicated in the etiology of several degenerative diseases such as stroke, coronary artery diseases, rheumatoid arthritis, diabetes and cancer. These radicals are capable of damaging essential biomolecules such as proteins, DNA and lipids (Rekha *et al.,* 2012). Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress (Zengin *et al.,* 2011).

Figure 4 : Oxidative Stress



http://www.oxidativestresssource.org/img/oxidative_stressoval.jpg

2.4. Antioxidant defense system

Development of a potential 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).

As antioxidants have been reported to prevent oxidative stress and damage caused by free radicals, they can interfere with the oxidation process by reacting with free radicals, chelating agents, catalytic metals and also by acting as oxygen scavengers. As a result, a recent upsurge of interest has been made in the therapeutic potentials of plants as antioxidants in reducing free radical induced tissue injury. Although several synthetic antioxidants, such as ascorbic acid, butylated hydroxyanisole and butylated hydroxytoluene, are commercially available, they are quite unsafe and their toxicity is a problem of concerns (Vinay *et al*., 2010).

Plants have developed a complex anti-oxidative defence system to combat heavy metal induced oxidative damage caused by ROS. This is repaired by several antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and glutathione reductase (Li *et al*., 2013). Plants also have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants such as ascorbic acid, α-tocopherol, flavonoids and carotenoids which are capable of attenuating ROS- induced oxidative damage (Kasote *et al*., 2015).

Huaman body has several enzymatic and nonenzymatic antioxidant mechanisms to combat oxidative stress. The non enzymatic antioxidants are either produced naturally in the body or supplied through foods and supplements. Antioxidants derived from plants are presumed to be safe since they are natural origin and have capability to counteract the damaging effect of ROS (Mishra *et al*., 2011).

Enzymic antioxidants

The enzymic antioxidant system includes superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase and polyphenol oxidase.

Superoxide dismutase (SOD)

Superoxide dismutase is the primary defence chain breaking antioxidant which catalyses the conversion of highly reactive superoxide anion radical produced during electron transfer reactions in mitochondria to less reactive hydrogen peroxide and prevents lipid peroxidation (Sankaran *et al*., 2010; Li *et al*., 2013).

Catalase (CAT)

Catalase is the second major defensive antioxidant enzyme next to superoxide dismutase. It catalyses the conversion of hydrogen peroxide to water and molecular oxygen (Goyal *et al*., 2012; Goyal and Basak, 2012). Catalase imparts oxidative stress tolerance and adaptive response in cells against free radical mediated damage (Sankaran *et al*., 2010).

Glutathione peroxidase (GPx)

The glutathione peroxidase enzyme catalyses the removal of lipid peroxides from the biological system and protects the biological membranes (Margis *et al*., 2008). Four isoenzymes of GPx are identified in mammals (GPx1, 2, 3 and 4). All these isoenzymes contains selenocysteine in the active site, which is responsible for its catalytic action. GPx 1, 2 and 3 reduce H2O2 and peroxides from free fatty acids and GPx 4 reduces peroxides of phospholipids and cholesterol (Toppo *et al*., 2009).

Glutathione S-transferase (GST)

Glutathione S-transferases (GST's) are regulatory antioxidant enzymes involved in various cellular responses. They directly interact with the cellular enzymes and proteins which are involved in the protection against ROS and RNS. This enzyme is also involved in the regulation of cell proliferation, differentiation and apoptosis (Townsend, 2008).

Polyphenol oxidase (PPO)

Polyphenol oxidase is an oxidative enzyme which is otherwise called as catecholase, tyrosinase and o-diphenol oxidase (Masum *et al*., 2010). It catalyses the oxygen dependent oxidation of mono and o-diquinones, highly reactive intermediates, the secondary reactions of which are believed to be responsible for the oxidative browning that occurs as a consequence of plant senescence, wounding and pathogen infection. Functions of polyphenol oxidase have been proposed, including roles in the phenylpropanoid pathway, the Mehler reaction, electron cycling, oxygen regulation, flower petal coloration and plant defense (Thipyapong *et al*., 2007).

Non-enzymic antioxidants

The major non-enzymic antioxidants include vitamins C, E, and reduced glutathione.

Vitamin C

Vitamin C is a water soluble antioxidant molecule that can readily cross the biological membranes. It directly removes the superoxide and hydroxyl radical from the biological system. It also has the ability to regenerate oxidized vitamin E (Silva and Goncalves, 2010).

Vitamin E

Tocopherol is a lipid soluble antioxidant vitamin which is believed to be the source of membrane antioxidants. It prevents lipid peroxidation and increases membrane stability. Among the four isomers of tocopherol (α, β, γ, δ) present in plants, α-tocopherol exhibits maximum antioxidant activity (Sarvajeet and Narendra, 2010).

Reduced glutathione

Glutathione is a tripeptide containing three amino acids namely L-glutamate, L-cysteine and L-glycine and is involved in various biological functions including protection against ROS and RNS (Vivancos *et al*., 2010; Lushchak, 2012). The antioxidant activity of GSH is mainly due to the presence of thiol group and it exerts its action directly by reacting with ROS and RNS or as a cofactor for various enzyme catalyzed reactions (Foster *et al*., 2009; Cooper *et al*., 2011).

2.5 Medicinal value of plants

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Medicinal plants have been used for centuries as remedies for human and animal diseases as they contain phytochemicals of therapeutic values (Verma *et al.,* 2013). Herbal remedies have been used throughout the world over 4000 years ago for treatment of various diseases due to the presence of beneficial chemical elements in them. In essence, medicinal potentials of plants lie in their phytochemical constituents that produce a definite pharmacological action when applied to the human body (Wadood *et al*., 2013).

Modern techniques and pharmacological screening procedures have resulted in new plant drugs which usually find their way into modern medicines. Nowadays maximum number of plants are being screened for their possible pharmacological value. The plant kingdom still holds many plant species containing substance of medicinal value which have yet to be discovered (Tambe *et al.,* 2013). Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design (Vijayalakshmi and Ravindran 2012).

The high cost of drugs and the inability of many developing countries to avail modern drugs have forced communities to look for products in the form of medicinal plants that are proved to be effective, safe, inexpensive and culturally acceptable (Adefa and Abraha, 2011). Pharmaceutical and scientific communities have recently received the attention of the medicinal plants and various publications have documented the therapeutic worth of natural compounds to validate the claims of their biological activity (Das *et al*., 2010).

India is also known to harbour a rich diversity of higher plant species (about 17000 species) of which 7500 are known as medicinal plants and out of these species 1000 plant species have been used in the traditional system of medicines like Ayurveda, Unani and Siddha (Reddy *et al*., 2012). There has been an increasing interest worldwide on therapeutic value of natural products. The nature provides the mankind, vast therapeutic flora with a wide variety of medicinal potential (Singh *et al.,* 2011).

In the ancient India, medicinal plants were used to prevent various critical diseases. The plant kingdom is an important source of herbal drugs. Even in recent years, there has been an increasing awareness about the importance of medicinal plants. Generally, herbal drugs are easily available, safe, less expensive, efficient and rarely have side effects. According to World Health Organization, medicinal plants would be the best source to obtain variety of drugs (Yadav and Agrawala., 2011).

Plants have a long history of use in the treatment of cancer. The approach for minimizing unwanted toxicity is to employ newer natural products that may act by different and distinct mechanism(s) and/or precipitate less serious adverse effects. A number of plant or other natural product extracts have been studied for their anticancer activity leading to the development of several clinically useful anticancer agents (Rocha *et al*., 2001).

Natural therapies, such as the use of plant-derived products in the treatment of cancer, may reduce adverse and toxic side effects. However, many plants exist that have shown very promising anticancer activities *in vitro* and *in vivo* but their active anticancer principle have yet to be evaluated. Combined efforts of botanist, pharmacologist and chemists are required to find new lead anticancer constituent to fight disease (Sultana *et al*., 2014).

2.6 Phytochemicals

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 (Nisa *et al.,* 2010). The phytochemical is a natural biologically active substance found in plants to work as a defence system against disease. Primary phytochemical compounds are common sugars, amino acids, proteins and chlorophyll while secondary compounds are of alkaloids, terpenoids, flavonoids, tannins and phenolic compounds (Charu *et al*., 2012). These secondary metabolites possess some therapeutic properties therefore, some plants are also classified as Herbs (Lincoln and Eduardo, 2006).

Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well (Pandey *et al*., 2013). Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Numerous research groups have also reported such studies throughout the world (Raphae, 2012).

These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, antiinflammatory, anticarcinogenic, antimalarial, anticholinergic and antileprosy activities (Negi *et al*., 2011).

To identify and develop anticancer agents which cause minimal harm to cells while effectively killing cancer cells, a number of naturally occurring phytochemicals in medicinal plants and food have been investigated. The epigenetic mode of action of various phytochemicals and their potential role as safe and clinically useful chemopreventive stratagies have been studied (Thakur *et al*., 2014). Some herbal anticancer drugs derived from medicinal plants like *Catharanthus roseus, Camptotheca acuminate, Podophyllum* species and *Taxus* species are wildly used because of their well defined mechanism of action as anticancer drug (Rajwar and Khatri, 2012).

The most important bioactive constituents of plants are alkaloids, flavonoids, phenolic compounds and tannins (Doss, 2009).

Phenols and flavonoids

Polyphenols(phenolics and flavonoids) are the largest group of phytochemicals widely distributed in plants. Studies have shown that the plant polyphenols possess significant antioxidant properties and helps in the prevention of various oxidative stress associated diseases such as cancer, Parkinson's disease, cardiovascular disease and osteoporosis (Dai and Mumper, 2010). Polyphenols act synergistically with antioxidant vitamins and enzymes as a defence against oxidative stress. They also act as metal chelators. For example, polyphenols chelate Fe2+ ions, thereby inhibiting the hydroxyl radical induced oxidation mediated through Fenton reaction (Perron and Brumaghim, 2009).

Alkaloids

The significance of medicinal plants is directly linked to the wide range of chemical compounds synthesised by the various biochemical pathways. One major category of such compounds is alkaloids. Although they vary greatly in their chemical structures, alkaloids have several common characteristics: they possess nitrogen and are alkaline(basic) groups, but have nonbasic forms such as quaternary compounds and N-oxides. The alkaloid extracts obtained from medicinal plant species have multiplicity of host-mediated biological activities, including antimalarial, antimicrobial, antihyperglycemic, anti-inflammatory and pharmacological effects (Ameyaw and Duker-Eshun, 2009).

2.7. Medicinal properties of *Cassia species*

Table 3 : Taxonomical classification of the Genus *Cassia*

Taxonomical classification of the Genus *Cassia* : Scientific Classification	
Kingdom	Plantae
Sub-Division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub-Class	Rosidae
Order	Fables
Family	Fabaceae/Leguminoceae
Sub-Family	Caesalpiniceae
Genus	*Cassia*

Cassia species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values (Singh *et al.,* 2013). All *Cassia* species are rich source of secondary metabolites and have been used in Chinese and Ayurvedic preparations. A review of phytochemistry of some medicinally important *Cassia* species has been presented, considering the fact that there are about 580 species of this genus scattered all around the world (Deshpande and Bhalsing, 2013). The clinical research on *Cassia* species indicated it as a source of effective liver tonic, antibiotic, antiinflammatory and antifungal agents (Farswan *et al*., 2009).

Plants belonging to *Cassia* species are used extensively in various parts of the world against a wide range of ailments, the synergistic action of its metabolite being probably responsible for the plants beneficial effects (Verma *et al*., 2013). *Cassia* species are well known for their medicinal and therapeutic importance. The drug has been known to Arabs for many centuries and it is believed that it was introduced into Indian and European medicine through them. One of the preparations of *Cassia* species is known as Constivac(Lupin Herbal) a bowel regulator, relieves constipation. It is also one of the ingredients of the preparations known as Pilex, Purian (Himalaya Drug Company) for piles and detoxifier, respectively (Danish *et al*., 2011). Species of *Cassia* are rich sources of polyphenols, anthraquinone derivatives (Ayo, 2010), flavonoids and polysaccharides.

C. tora is one of the well-known anthraquinone containing plant and has been used in Chinese and Ayurvedic medicine. The whole plant as well as specific part such as roots, leaves and seeds have been used and claimed against different diseases by rural and traditional practioners of Satpura region of Madhaya Pradesh. This plant has great contribution in modern system of herbal medicine for new drug development (Jain and Patil, 2010).

Cassia spectabilis has been commonly used in traditional medicine for many years. Information in the biomedical literature has indicated the presence of a variety of medicinally-important chemical constituents in *C. spectabilis*. Pharmacological studies by various groups of investigators have shown that *C. spectabilis* possesses significant biological activity such as antibacterial, antibiofilm, antifungal and antioxidant properties. Beside this, toxicity studies of this plant have revealed no toxic effect on mice (Jothy *et al.,* 2012).

C. fistula popularly called "Indian Laburnum" has been extensively used in Ayurvedic system of medicine for various ailments. The flower, pod and root are prescribed as a tonic, astringent, febrifuge and strong purgative and useful in fever, heart diseases, joint pain, migraine and blood dysentery (Kiritikar and Basu, 2006). *Cassia nigricans* is used in ulcers, gastro intestinal disorders, antioedema activities, diarrhoea and skin diseases due to its high therapeutic value (Jacob *et al.,* 2002).

C. auriculata commonly known as Tanners senna is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine (Kirtikar and Basu, 2006) and conjunctivitis (Pari and Latha, 2002). This plant has been widely used in traditional system of medicine as a cure for rheumatism. The various parts of this plant were reported to exert a beneficial effect to alleviate the symptoms of diabetes (Surana *et al.,* 2008).

Cassia occidentalis is an Ayurvedic plant with huge medicinal importance (Raghunathan and Mitra, 1999) It is used for fever, menstrual problems, tuberculosis, as diuretic for anaemia, liver complaints and as a tonic for general weakness and illness (Kirthikar and Basu, 2006). The leaves, seeds and roots are useful in vitiated conditions of vata, kapha, leprosy, erysipelas, pruritus, wounds and ulcers, cough, strangury, bronchitis, asthma, pharyngodynia, constipation fever, hydrophobia and inflammation, diabetes, elephantiasis, ring worm and flatulence respectively (Prajapati *et al*., 2003).

Cassia sophera is an important drug of Islamic System of Medicine (Unani Medicine). It has been used for its efficacy in respiratory disorders. In ethno botanical literature it is effective in the treatment of pityriasis, psoriasis, diabetes and convulsions of children (Agarwal and Paridhavi, 2005).

Sixty-four accessions from 20 medicinally important species of *Cassia* including *Cassia senna* were collected and analyzed for these four DNA barcoding markers. PCR amplification was 100% successful for all four markers and the nucleotide sequences for 20 species of *Cassia* were submitted to the database of Genbank at the NCBI with the accession numbers (Purushothaman *et al*., 2014)

2.8. Medicinal properties of *Cassia senna*

Cassia senna (Common Names-Senna, Indian senna, English – sanay, Hindi- sana ka pat) belonging to the family Fabaceae. The Fabeceae, also called Leguminosae or bean and pea family, is the third largest family of angiosperms around 250species. This plant is widely found in India, Pakistan, Srilanka, Tropical Africa, Egypt, Sudan and Bangladesh. It is a small shrub grows uo to 70cm height with erect or spreading branches. Leaves are paripinnate, flowers are racemes and yellow, fruits are flat legumes greenish brown or dark. Seeds are dark brown and smooth. The plant leaves are pinnate which has the hydroxyanthracene derivatives and are present as glycosides (sennosides) mainly, respectively glycones are called sennidies. Hydroxyanthracene (Diathrones) are not natively present compounds but formed enzymatically in the plant during drying at low temperature. They acts as prodrugs, and pass through the upper part of the gastrointestinal tract nearly unaltered to reach the caecum and colon, where they are transformed to the rheineanthrae, the active the metabolite. Mode of action via rheinanthrone formation is well accepted and understood. The systemic availability of this compound is less than 5% get it potentially activates pesristalis of the colon, resulting in laxative effects.

C. senna leaves have been investigated for the presence of secondary metabolites and evaluated for the biological activities of the crude extracts with special emphasis to the antimicrobial, cytotoxic and thrombolytic activities and also in brine shrimp(*Artemia salina*) lethality bio-assay, the significant cytotoxicity of methanol extract of *C. senna* has been reported at very low concentration (Hossain *et al.,* 2012). The ethanolic extract of *C. senna* leaves was found to suppress prostate tumor growth in experimental animals induced with prostate carcinogenesis using Testosterone and N-methyl N-nitrosourea (Kumar *et al*., 2010).

Although many reports are being avalilable on the various species of *Cassia* belonging to the family Fabaceae with respect to their biological activities, studies on antioxidant and anticancer activity of *C. senna* are very scarce and hence the present study is focused on exploring the antioxidant and anticancer potential of *C. senna* using *in vitro* and *in vivo* methods.