Chapter 7

Summary and Conclusions
7. SUMMARY AND CONCLUSIONS

Cancer is one of the most serious clinical problems worldwide and is the second leading cause of death among human population which is next to heart disease. Cancer is multifactorial in its origin with heterogeneous nature and is characterized by uncontrolled growth, invasion that intrudes upon and destroys adjacent tissues, and sometimes metastasis, or spreading into other locations in the body via lymph or blood. 90-95% cancer cases were attributed to environmental factors and 5-10% due to genetic factors. According to global statistics in 2008 approximately 12.7 million cancers occurred and 7.6 million people died of cancer worldwide.

Cancer is originated through a multistep process called carcinogenesis by initiation in which a single cell gets initiated to clonal expansion to form premalignant lesion by mutation of tumor suppressor gene, DNA repair gene and proto-oncogene to oncogene. These initiated cells will have defect in maturation, escapes from senescence and have altered dependence on growth factors and hormones and inhibition of apoptotic cell death. Different genes implicated in cancer are those involved in cell cycle control, apoptosis, DNA repair, aging immortalization, angiogenesis and metastasis. Apoptosis is the essential process for maintaining normal physiology. It is an energy dependent genetically controlled process of cell death by which unnecessary or damaged cells are removed. Evading apoptosis is a common nature of cancer cells (Hanahan and Weinberg, 2000).

Eventhough current treatment modalities are improving in therapeutic efficacy and quality of life; there is only a marginal increase in the survival rate. This is because prognosis is done only at an advanced stage Drug resistance and toxicity is also found to be the major problems in chemotherapeutic treatment (Kummalu et al., 2011). Due to some limitations of these current medical practices, more and more people resort to alternative medicine, which is defined as health care practices used instead of standard ones. Herbal medicine, one type of the alternative medicine, is based on the use of plants
or plant extracts to treat diseases and promote health and has been offered especially for cancer treatment over the last century. This alternative treatment is more widely accepted at the present time. Therefore, medicinal plants have become important and reliable sources for anticancer agents and worldwide efforts are ongoing to find new plants with biological activity (Newman et al., 2003).

Plants are a tremendous source for the discovery of new products of medicinal value for drug development. Today several distinct chemicals derived from plants are important drugs, currently used in one or more countries in the world. Many of the drugs sold today are simple synthetic modifications or copies of the naturally obtained substances (Mulabagal et al., 2004). The search for new plant derived chemicals should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of biodiversity (Phillipson, 1990). Large number of medicinal plants is exploited from natural flora for the commercial production of drugs (Paul et al., 1992). Most of drugs in plants accumulate either in root, stem, or bark, especially in trees after several years of growth. As a result of harvesting these medicinally important parts, these plants are indiscriminately exploited from natural habitats. In order to conserve the natural flora and to meet the increasing demands of plant based drugs in ayurvedic preparations as well as in pharmaceutical industries it is essential to develop an alternate method. In the search for alternatives to production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Ramachandra and Ravishankar, 2002).

Dasamoola is an important combination of ten roots and used in the formulations such as Dasamoolakwatha, Dasamoolarishta, Dasamoolatpalak and Dasamoolaghrta (Ayurvedic Formulary, 1978). The present investigation made an attempt to develop a protocol for the micropropagtion of Solanum xanthocarpum and Tribulus terrestris, two important plants of Dasamoola and their comparison with wild plants. Comparison was studied by cytotoxicity, antioxidant activities and phytochemical screening. The phytochemical analysis was done by HPTLC and HPLC method. Anticancer activities were evaluated by cytotoxicity, antioxidant property, anti-inflammatory activity and antitumor studies. Mechanisms of anticancer activity were analyzed by induction of
apoptosis. For understanding the protective nature of these plants against chemotherapy induced damages, cardioprotection, nephroprotection and gastroprotection were analyzed.

Micropropagation studies were conducted in *S. xanthocarpum* and *T. terrestris*. In *S. xanthocarpum* profuse growth of friable and compact callus (NAA 2mg/L, BA 1.5 mg/L, Table: 3.1.1.3.2) multiple shoot (NAA 2 mg/L, BA 2mg/L, Table: 3.1.1.3.3) and root formation occurs in MS medium supplemented with NAA 3mg/L and BA 2mg/L (Table: 3.1.1.3.4, Fig. 3.1.1.3.1, to2). *In vitro* cultures of *T. terrestris* reveals that, its favorable medium for callus growth occurs in MS medium supplemented with (IAA 3 mg/L, BA2 mg/L, Table: 3.1.2.3.1), multiple shoot (IAA 2 mg/L, BA2 mg/L Table: 3.1.2.3.2) and root (IAA 3 mg/L, BA2 mg/L, Table: 3.1.2.3.3, Fig. 3.1.2.3.1) respectively. Somatic embryogenesis observed in MS medium with (IAA 3 mg/L, BA2 mg/L Table: 3.1.2.3.4, Fig. 3.1.2.3.2). In both cases acclimatized plants in the field appears to be normal and healthy.

Preliminary screening for chemical profile by High Performance Thin Layer Chromatography (HPTLC Fig. 3.2.3.1.1 Fig. 3.2.3.2.1) reveals that both *in vitro* and *ex vitro* plants have same type of peaks in chromatogram revealing its similarity. But in their quantification by High Performance Liquid Chromatography (HPLC Fig. 3.2.3.1.2 Fig. 3.2.3.2.2) while using diosgenin as standard it was observed that in *S. xanthocarpum in vitro* plants have more diosgenin content than *ex vitro* plants (Table: 3.2.3.1.1). In the case of *T. terrestris* its *in vitro* plants have very low content of quercetin even though the explant used in this study was fruit wall (Table: 3.2.3.2.1).

*In vitro* antioxidant activity of *Solanum xanthocarpum* and *Tribulus terrestris* were assessed by 2, 2Diphenyl-2-Picrylhydrazyl (DPPH), 2, 2'-Azino-Bis(3-ethylbenzthiazoline-6-Sulphonic acid) (ABTS), Super oxide, Hydroxyl, Nitric oxide radical scavenging activity, inhibition of Lipid peroxidation and measuring Ferric reducing antioxidant power (FRAP). The results are compared with antioxidant activity of *in vitro* cultured plants (Table 4.1.3.1, Table: 4.1.3.1 and Fig. 4.1.3.1. to Fig. 4.1.3.6). Our observation suggests that wild plants have more potential in antioxidant activities.

The efficiency of *Solanum xanthocarpum* and *Tribulus terrestris* in preventing inflammation were carried out by using carrageenan/ dextran induced acute and formalin induced chronic inflammatory models of mice paw edema. Results were compared with
standard reference drug diclofenac. Oral administration of drug decreased the paw edema in a dose dependent manner. Results indicated that both extract have potent anti-inflammatory activity (Table: 4.2.3.1, Fig. 4.2.3.1 to 3). S. xanthocarpum 250 mg/kg body weight and T. terrestris 100 mg/kg have inhibitory activity almost nearer to diclofenac.

Short-term cytotoxic activity of the Solanum xanthocarpum and Tribulus terrestris extract was assayed by determining the percentage viability of DLA and EAC cells using the try pan blue dye exclusion technique, and it is compared with the in vitro cultured plants. In vitro cultured plants showed less cytotoxicity than ex vitro (Table: 4.3.3.1, Fig. 4.3.3.1 to 2).

The anti-tumor activity of Solanum xanthocarpum and Tribulus terrestris were performed using DLA induced solid and EAC induced ascites tumor models. The results were compared with standard reference drug cyclophosphamide. Administration of drug continuously ten days after the induction of tumor results in increase in life span, and reducing the increase in body weight of ascites tumor bearing animals. In solid tumor model significant reduction in tumor volume was observed. The results were significant (p<0.01) in lower and higher concentration of both plant extract. S. xanthocarpum 250 mg/kg body weight and T. terrestris 100 mg/kg showed more potentiality than standard drug (Table: 4.4.3.1, 4.4.3.1 to 3).

Mechanisms of anticancer activities were determined by inducing apoptosis in MCF 7 human breast cancer cell lines. Nuclear condensation analysis was carried out by using hoechst 33342 dye. Results indicates that both extracts were capable of inducing 100% of nuclear condensation.(Fig.5.3.1) Cell cycle analysis were conducted by Fluorescence activated cell sorter using propidium iodide. In cell cycle analysis after 24 h treatment with the extract in G0 phase DNA fragmentation is observed. There is no significant variation in S and G1 phase, but in G2 phase of cell cycle significant inhibition is observed when compared to control (Fig Fig.5.3.2 and 3). The steroid content of S. xanthocarpum and flavonoid content of T. terrestris may be the factor accountable for cell cycle arrest and induction of apoptosis in these plants.

Gastric ulcer was induced in fasted animals by oral ingestion of ethanol. Administration of, S. xanthocarpum and T. terrestris extracts prior to ethanol ingestion protected the stomach of the rat from ulcer formation, exhibited statistically significant
gastro-protective effect. The ulcer index values, expressed as a percentage of total stomach surface area affected by the ulcer, were lowered from $4.71 \pm 0.66$ to $1.68 \pm 0.23$ in *S. xanthocarpum* and $2.01 \pm 0.16$ in *T. terrestris*. At the concentration under study, *S. xanthocarpum* crude drug (250 mg/kg) was more effective than the reference compound ranitidine 50 mg/kg (Table: 6.1.3.1, Fig.6.1.3.3). In biochemical analysis ethanol induced groups showed significant increase in MDA levels and decrease in antioxidant enzymes like SOD, catalase, GPx and reduced Glutathione levels in gastric mucosa. The extract treated groups showed significant increase in the antioxidant status and reduced glutathione, whereas lipid peroxidation was found to be decreased (Table: 6.1.3.2,. Fig. 6.1.3.1 and 2). Histopathological studies confirmed the results of the in vivo test (Fig. 6.1.3.4) The result of present investigation suggests the significant gastro-protective effect of *S. xanthocarpum* and *T. terrestris*. The drug that possesses both anti-ulcer and anti-inflammatory activities is of great therapeutic importance because most of the anti-inflammatory drugs used in modern medicine are ulcerogenic (Surender, 1999).

In cisplatin induced nephrotoxicity there was elevated levels of serum creatinine and urea, a measure of kidney damage were reduced by the treatment with the extract. The status of major antioxidant enzymes, antioxidants and lipid peroxidation was studied in kidney tissue samples of all the groups. Cisplatin treated group showed significantly decreased level of antioxidant enzymes with higher extent of lipid peroxidation. But the treatment with extracts showed all the parameters tended towards normal levels in a dose dependent manner. Hence, the present study indicated that the extract of *S. xanthocarpum* and *T. terrestris* showed an ameliorative effect on the cisplatin induced nephrotoxicity (Table: 6.2.3.1, Fig. 6.2.3.1 to 4).

The protective role of *Solanum xanthocarpum* and *Tribulus terrestris* against the myocarditotoxicity, nephrotoxicity and hepatotoxicity of doxorubicin was investigated in rats. Doxorubicin-induced elevations of serum creatine phosphokinase and lactate dehydrogenase activity a measure of myocardiac damage, creatinine and urea indicators of kidney function and serum glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and alkaline phosphatase activity, reflecting hepatic damage, were reduced by treatment with the extract in a dose dependent manner. Doxorubicin produced significant increase in malondialdehyde levels indicating tissue lipid peroxidation and
potentially inhibiting the activity of antioxidant reduced glutathione and antioxidant enzymes catalase, super oxide dismutase and glutathione peroxidase. In the present investigation result showed that in heart, kidney and liver the treatment with extract significantly restored the activity of these enzymes and reduction in MDA level suggesting the antioxidant potential of these extracts in ameliorating the cardiotoxicity, nephrotoxicity and hepatotoxicity induced by doxorubicin. Histo-pathological evidence also supports the biochemical results (Table: 6.3.3.1 to 3, Fig.6.3.3.1 to 11).

*S. xanthocarpum* and *T. terrestris*, two high value medicinal plants and ingredients of Dasamoola were used in this study. Phytochemical studies of *S. xanthocarpum* showed the presence of steroids and glycoalkaloids and *T. terrestris* have flavonoids. Present investigation revealed their antioxidant potential, anticancer activities and protective nature against chemotherapeutic drugs.

The following conclusions could be drawn from this investigation.

- Clonal multiplication of *S. xanthocarpum* through micropropagation can be achieved from nodal explants.
- In vitro shoot regeneration of *T. terrestris* from mesocarp.
- Somatic embryogenesis from *T. terrestris* might offer a potential source for production of important pharmaceuticals.
- Diosgenin content of regenerated *S. xanthocarpum* through tissue culture was higher than that of wild plants.
- *S. xanthocarpum* and *T. terrestris* have potent cytotoxicity against DLA and EAC.
- *S. xanthocarpum* and *T. terrestris* possess anti-oxidant potential
- *S. xanthocarpum* and *T. terrestris* showed anti-inflammatory activity.
- *S. xanthocarpum* and *T. terrestris* exhibited significant reduction of solid tumors and increase in life span of ascites tumor bearing animals.
- *S. xanthocarpum* and *T. terrestris* can induce nuclear condensation in MCF 7 human breast cancer cells
- *S. xanthocarpum* and *T. terrestris* can induce cell cycle arrest at G2 phase of cell cycle condensation in MCF 7 human breast cancer cells.
- Mechanism of anti-cancer activity of *S. xanthocarpum* and *T. terrestris* may be due to the induction of apoptosis.
- *S. xanthocarpum* and *T. terrestris* have protective activity against doxorubicin induced cardiotoxicity, nephrotoxicity and hepatotoxicity.
- *S. xanthocarpum* and *T. terrestris* can ameliorate the renal damage induced by cisplatin.
- *S. xanthocarpum* and *T. terrestris* have cytoprotective role against ethanol induced gastric ulcer.
- *S. xanthocarpum* and *T. terrestris* can be used as chemotherapeutic drug against cancer in future.