1.0 INTRODUCTION

The mother earth is a rich reservoir of natural resources particularly medicinal plants. Medicinal plants play a key role in human health care. A large number of medicinal plants and their purified constituents have been shown to have beneficial therapeutic potential (Dahanukar et al., 2000; Agbar et al., 2008; Prusti et al., 2008). Over the past few years however the medicinal plants have required a wide recognition due to an escalating faith in herbal medicine in view of its lesser side effects compared to allopathic medicine in addition, the necessity of meeting the requirements of medicine for an increasing human population. The majority of the world’s population in developing countries still relies on herbal medicines to meet their health needs in cases when synthetic medicine could not relieve patients suffering from illnesses like cancer (Lee et al., 2000; Surendra et al., 2008).

There is increasing interest in the use of herbs for the treatment of human diseases including cancer. Plants contain a wide variety of compounds that may have biological activities including anticancer effect (Kaneshiro et al., 2005; Shoeb, 2006). Plants have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. With the rapid identification of new proteins having significant regulatory effects on tumor cell cycle progression, and their conversion into targets for high throughput screening, molecules isolated from plants and other natural organisms are proving to be an important source of novel inhibitors of the action of these key proteins, and have the potential for development into selective anticancer agents (Cragg and Newman, 2006).

Cancer is a disease of multistep process that entails a progressively more malignant phenotype through the evolution of cellular subsets with cumulative genetic damage (Das, 2004). It is the second most common cause of death. This disease annually affects 9 million people and causes 5 million deaths. It is likely that 300 million new cases of cancer and 200 million deaths from this disease will occur in the next 25 years, with almost two thirds of cases arising in the developing countries (Chaudhuri et al., 2004). Cancer is not a single disease but a wide spectrum of conditions which are biologically
different and even the causation and carcinogenic pathways are different. It can be said that cancer is a generic name for a biologic phenomenon. The only common feature is the uncontrolled proliferation of cells which ultimately ends in death unless successfully treated (Singh et al., 2002).

Cancer is also a disease of cells characterized by a shift in the control mechanism that governs cell proliferation and differentiation (Annapoorani and Hajara Sheereen, 2005). Transplantation of human tumors into Swiss albino mice is an important experimental approach to study the biology of human cancer (Siddiqi et al., 2001; Sivakumar and Alageshaboopathi, 2008; Radha et al., 2008; Adhvaryu et al., 2008). Isolation and identification of some potent antitumor compounds from plants have encouraged scientists to screen different parts of plant species against cancer cell lines (Emami et al., 2005). Daltoms Lymphoma Ascites (DLA) was arisen as a spontaneous carcinoma of the mouse thymus in 1947. Ehrlich Lymphoma Ascites (ELA) was the initial tumors for the Ehrlich solid adenocarcinoma and found by Paul Ehrlich as spontaneous cancer of the mammary gland of mice developed in 1905. The ascetic variant Ehrlich tumor was produced by the intraperitoneal transplantation of Ehrlich solid adenocarcinoma in 1932 (Kuttan et al., 1989).

Recently there has been an increasing interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical induced tissue injury (Pourmorad et al., 2006). Antioxidants play important roles in preventing the diseases induced by reactive oxygen species (Willcox et al., 2004). Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, which results in oxidative stress leading to cellular damage. Thus, antioxidants that can scavenge free radicals play a role in the improvement of diseased conditions which result in oxidative damage to DNA, proteins and other macromolecules and are associated with degenerative pathological events such as aging, asthma and cancer (Klaunig and Kamendulis, 2004; Balaban et al., 2005).

Many human diseases are caused mainly by imbalance between the antioxidants and free radicals. Active principles of medicinal plant play an important role by inducing the enzymic and nonenzymic antioxidants and suppressing the free radicals to maintain them in balance. Antioxidants, either
exogenous or endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders (Buricova and Reblova, 2008; Souri et al., 2008). The natural antioxidants discovered recently are expected to replace synthetic antioxidants that are widely used. Plant extracts such as *Paronychia argentea* L. and garlic with antioxidant activity are traditionally used to strengthen the natural immune defenses. Many studies have been focused on antioxidants as potential anticancer agents (Lau, 2001; Zama et al., 2007).

The antitumor effect of the ethanol extracts of *Pisonia aculeate* Linn. (Senthilkumar et al., 2008) was found to retard the development of solid and Ehrlich ascites carcinoma tumor and increase the lifespan of tumor bearing mice. Antimutagenic and antioxidative activities are exhibited by the protein of regular green tea and Se-enriched green tea (Yu et al., 2007). Proteins also have excellent potential as antioxidant additives in foods because they can inhibit lipid oxidation through multiple pathways including inactivation of reactive oxygen species, scavenging free radicals, chelation of prooxidative transition metals, reduction of hydroperoxides, and alteration of the physical properties of food systems (Elias et al., 2008). Proteins and various plant or herbal extracts have been reported to be radical scavengers and inhibitors of lipid peroxidation (Akter et al., 2008). There is an increasing interest in the natural antioxidants contained in medicinal plants, which are important for the prevention of oxidative damage (Morales et al., 2008).

The most important components of immune system in the initial stages of the defense is the phagocytic cells which include neutrophils, eosinophils, macrophages and monocytes that recognize foreign substances and invading microorganisms. These cells engulf and destroy the foreign substances with their intracellular killing mechanisms by innate immune response (Ranjith et al., 2008). The improved defense response under normal circumstances is due to the marginalization of the above phagocytic cells particularly neutrophil which denotes the increase in per cent neutrophil adhesion (Block and Mead, 2003).

Immunomodulation is a process that can alter the immune system of an organism by interfering with its functions (Shivaprasad et al., 2006). The immune system may be the last line of defense against cancer development. According to
the most recent point of view about cancer immunology, the key issue is whether recognition of tumor antigens by the immune system leads to activation of tolerance (Pardoll, 2003). The immune system not only plays a crucial role in preventing tumor growth, but also it can suppress cancer progression (Shankaran et al., 2001). Thus, the immune system is a double-edged sword in terms of preventing tumor growth (Yang et al., 2004).

There are many plants, which are having immunostimulatory whereas other has immunosuppressant activity (Oladunmoye, 2007). Apart from being specifically stimulatory or suppressive action, certain agents have been shown to possess activity to normalize or modulate pathophysiological processes and are hence called immunomodulatory agents (Bafna and Mishra, 2005). The plant products have long been used as immunomodulators by the traditional healers. Scientific literature is continuously reporting plant drugs having immunomodulatory activity (Tan and Vanitha, 2004; Gauniyal et al., 2005). Immunomodulation using plant extracts such as Eclipta alba, Centella asiatica, T. chebula, T. bellerica and E. officinalis can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defense mechanism has to be activated under the condition of impaired immune response (Jayathirtha and Mishra, 2004; Srikumar et al., 2006; Meera, et al., 2008).

Immunomodulators are compounds capable of interacting with the immune system to up regulate or down regulate specific aspects of the host response (Tzianabos, 2000). These immunomodulators can influence innate and cell mediated immunity through interactions with T cells, monocytes, macrophages and polymorphonuclear lymphocytes. This activity depends on a number of factors, including dose, route of administration and timing of administration of the compound in question. It may also depend on the mechanism of action or the route or site of activity. The ability to modulate the immune response in an appropriate way can enhance the host’s immune response to certain infections (Manosroi et al., 2004).

The active principle of plants acting as antioxidants and immunomodulators can reduce the toxicity of specific chemotherapeutic agents used in the treatment of cancer. The immunomodulators have a biphasic effect on the immune response either by stimulating or inhibiting the immune
response and other host defense mechanisms. Immunomodulators not only have immunorestorating function but also can be used to produce an immunocompetent state so as to ward off many diseases. Therefore, great attention is given for the plant materials that can stimulate immune system. Plant extracts with antioxidant activity are traditionally used to strengthen the natural immune defenses. Immunomodulatory agents of plant origin enhance the immune responsiveness of an organism against a pathogen by nonspecifically activating the immune system (Razdan and Roy, 2008).

The plants selected for the present research work were *Cynodon dactylon* (*C. dactylon*) and *Terminalia catappa* (*T. catappa*). *C. dactylon* (Poaceae) commonly known as arugampul, is a perennial grass, forming thick mats by means of stolons and rhizomes. It is an important medicinal plant which is used for treatment of various ailments in Ayurvedic system of medicine. It is bitter, sharp, hot taste, good odour, laxative, brain and heart tonic, emetic, expectorant, carminative and useful against pains, inflammation and tooth ache (Mahesh and Brahatheeswaran, 2007). *C. dactylon* is traditionally used as an agent to control diabetes in India (Brahmvarchas, 2003; Singh *et al.*, 2007). Increasing evidence indicated that *C. dactylon* extract had significant application in dropsy and secondary syphilis (Kesari *et al.*, 2006).

*Terminalia catappa* (Combretaceae) also known as badam, is a large deciduous tree well known for its therapeutic values since long and proved to be an anti-inflammatory agent (Pawar *et al.*, 2000; Nwosu *et al.*, 2008). It is also known as Indian almond, Malabar almond and Tropical almond. The aqueous and cold extracts of leaves of the *Terminalia catappa* have been reported to be antioxidant, hepatoprotective, and antidiabetic (Ahmed *et al.*, 2005).

Nowadays people are running after the readymade chemical drugs for a timely relief without caring for their harmful side effects. Now man has no time to go in search of leaves and roots of plants of medicinal importance even though they are harmless. He even neglects the medicinal plants around him. All these factors persuaded us for the medicinal plant study. In particular *C. dactylon* and *T. catappa* received a lot of attention in the research world wide because of their potent antioxidant activity. So far, no studies were undertaken to explore he antioxidative, antitumor and immunomodulatory
activity of protein fractions of *C. dactylon* (*C. dactylonPF*) and *T. catappa* (*T. catappaPF*) leaves. This made us to investigate and examine the “Antioxidative, antitumor and immunomodulatory efficacy of protein fraction of *Cynodon dactylon* and *Terminalia catappa* leaves on experimentally implanted ELA cells in Swiss albino mice”.

The present study was organized in four phases. The first phase involved the precipitation of proteins by 10-100 per cent ammonium sulphate fractionation using 20 per cent Phosphate Buffered Saline (PBS) extract of fresh leaves of *C. dactylon* and *T. catappa*, to predict the protein content and to select the fraction of each with maximum protein content. These selected protein fractions were dialyzed, purified by Polyacrylamide Gel Electrophoresis (PAGE) and the major bands of each protein fraction were eluted. In the second phase, eluted fractions were subjected to *in vitro* cytotoxic studies to identify the minimum concentration of protein fractions with 50 per cent cytotoxic activity (EC$_{50}$) against ELA and *in vitro* antioxidative studies by DPPH radical, NO radical and H$_2$O$_2$ scavenging assays. In the third phase, the selected EC$_{50}$ protein fractions of *C. dactylon* and *T. catappa* were evaluated for their antioxidative role by assessing the activities of enzymic antioxidants, the levels of nonenzymic antioxidants and the rate of lipid peroxidation against standard antioxidant silymarin. Antitumorigenic effect was assessed against intraperitoneally propagated ELA tumor cells. In the fourth phase, the selected EC$_{50}$ protein fractions were evaluated for the immunomodulatory role with the following specific objectives:

**PHASE I**

1. To precipitate the proteins of the PBS extracts of *C. dactylon* and *T. catappa* from 10 to 100 per cent saturation of ammonium sulphate.

2. To assess the protein content of precipitates obtained by ammonium sulphate fractionation procedure.

3. To select the precipitate with maximum protein content of *C. dactylon* and *T. catappa*.

4. To purify and separate the ammonium sulphate protein fractions by polyacrylamide gel electrophoresis (PAGE).

5. To elute and characterize the major bands of the selected protein fractions.
PHASE II

6. To assess the \textit{in vitro} antioxidative potential of \textit{C. dactylon} PF and \textit{T. catappa} PF by DPPH radical, NO radical and H$_2$O$_2$ scavenging assays.

7. To assess the \textit{in vitro} antitumorigenic effect against ELA and to identify the minimum concentration of protein fractions with 50 per cent cytotoxic activity.

PHASE III

8. To evaluate the \textit{in vivo} antitumorigenic activity and antioxidative activity of \textit{C. dactylon} PF and \textit{T. catappa} PF against standard antioxidant silymarin and the intraperitoneally propagated ELA tumor cells respectively.

PHASE IV

9. To evaluate the immunomodulatory activities of \textit{C. dactylon} PF and \textit{T. catappa} PF.