Section 2

Executive Summary of the Project:

Traditional remedies have a long-standing history in Sri Lanka and still popular for treating many ailments including cancer. Induction of apoptosis is a cancer preventive process and related studies were carried out with six prescriptions provided by traditional doctors. Decoction 1 (*Terminalia bellerica, Terminalia chebula, Phyllanthus emblica* and *Commiphora mukul*), Decoction 2 (*Terminalia bellerica, Terminalia chebula, Phyllanthus emblica, Commiphora mukul, Smilax china* and *Nigella sativa*), Decoction 3 (*Munronia Pumila, Azadirachta indica, Solanum surattense, Solanum xanthocarpum, Rubia cordifolia, Picrorhiza kurroa , Trichosanthes cucumerin* and *Pterocarpus santalinus*), Decoction 4 (*Boerhavia diffusa, Toddalia asiatica, Anacyclus pyrethrum, Ricinus communis, Crateva adensonii, Bombax ceiba*), Decoction 5 (*Adenanthera pavonina, Adenanthera pavonina. L*), Decoction 6 (*Flueggea leucopyrus*) and `pranajeewa oil' are the herbal drugs used in this study. Antioxidant activity, phenolic and gallic acid were determined for all decoctions. RD or *HEp* 2 cell lines were treated for 24 hours with respective drug and cytotoxicity was determined by LDH, MTT assays and protein synthesis. Morphological changes were determined by light microscopy and fluorescent microscopy.

Decoction 1 showed the highest phenolic and gallic acid contents. The antioxidant activity and phenolic content is in order of D1>D2≈D5>D6>D4. *Bombax ceiba ,Anacyclus pyrethrum* are two other herbs which showed high phenolic content and antioxidant activity. The EC50 values for DPPH scavenging activity were very low with D1, D2 and D5 and comparable to the ascorbic acid. The phenolic content was exponentially correlated [$y = 1896.3x^{-1.5149}$ ($r^2 = 0.7719$)] with the EC50 for DPPH radical scavenging activity. The mean EC50 for LDH assay for D1, D3, D4, D5 and D6 were 94.4, 129.7, 1060, 178.8 and 254.5µg/ml respectively. It is observed that the EC50 values for both LDH and MTT assays were linearly correlated ($y = 1.192x - 37.56$ $R^2 = 0.999$) each other, and exponentially correlated ($R^2 > 0.96$) with the phenol content of the decoctions. The morphological changes were appeared gradually with increasing concentration at different levels for each decoction. The results obtained for LDH, MTT and protein synthesis were compatible with the morphological changes observed for each decoction. The morphological changes show that the cell death induced by decoctions is mediated through apoptosis.

Among the six decoctions investigated, D1, D2, D5 and D6 are very effective in cytotoxicty and polyphenols play an important role in this regard.

Section 3

3.1 Introduction

Traditional remedies have a long-standing history in Sri Lanka and still popular for treating many ailments. Many plant derived chemicals are the basis of conventional drug therapies. Over 3000 species of plants have been reported to have anticancer properties (Graham *et.al.* 2000).

Some of the screening tests have been carried out on the basis of apoptosis process or a programmed cell death. It is a highly regulated process that involves the activation of a series of molecular events, leading to cell death. Apoptosis is characterized by cellular morphological change such as membrane blebbing, cell shrinkage, protein fragmentation, chromatin condensation and DNA degradation followed by rapid engulfment of cell debris by neighbouring cells (Elmore, 2007). It plays an important role as a protective mechanism in the organism by removing damaged cells or over-proliferating cells due to an improper mitotic stimulus (Wyllie, 1999). It is therefore possible to take advantage of this intrinsic mechanism by manipulating the apoptotic process for therapeutic gains in cancer treatment.

Reactive oxygen species (ROS) are constantly being produced in animal cells and associated with DNA damages , inducing premutagenic modifications of nucleotides and promoting oxidation of proteins and lipids.. Data support that increased formation of ROS may play an important role in carcinogenesis, atherosclerosis, diabetes, emphysema, cataracts, and neurodegenerative diseases (Gago-Dominguez, 2005). ROS seem to be mediators or triggers of protective mechanisms, such as apoptosis, phagocytosis, and detoxification reactions.

The traditional drugs used for cancer treatments in Sri Lanka are made of decoctions comprising several medicinal plants or a single plant. Only countable studies on cytotoxicity have been reported so far regarding traditional preparations of drugs used in Sri Lanka for cancer treatment. We initiated investigations for the first time in Sri Lanka to screen and study the cytotoxic properties of drugs currently used in cancer treatment. In addition to decoctions investigated, different types of Sri Lankan tea products also investigated for cytotoxicity using cancer cell lines.

3.2 Objectives

Objectives of this study were to:

(1) evaluate and compare the total antioxidant capacity by common antioxidant activity methods, and phenolic content

(2) identify and quantify gallic acid present by RP-HPLC;

(3) determine the relationship between antioxidant activity and phenolic compounds

(4) Cytotoxic activity and capacity to induce apoptosis
of selected decoctions which is used for cancer therapy in Sri Lanka