Synopsis of the Ph.D. Thesis entitled

EVALUATION OF PHYTOCHEMICAL PROSPECTS, PHARMACOLOGICAL EFFECT AND ANTI-CANCER PROPERTY OF SOPHORA INTERRUPITA

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SYNOPSIS

Herbal medicines often complement the conventional modern treatments providing safe, well-tolerated remedies for many chronic diseases such as liver disorder, rheumatoid arthritis, diabetes, obesity, cardiovascular, neurological disorders and cancer. The ability of herbal medicines to affect the body systems depends on the chemical constituents they contain. Research into the isolated plant constituents is of great importance. Many of today’s medicines are either obtained directly from a natural source or were developed from a lead compound originally obtained from a natural source (Handa, 2008). The use of traditional medicine has increased in developed countries, mainly due to the failure of modern medicine to provide effective treatment for chronic diseases and emergence of multi-drug resistant pathogens. The adverse effects of chemical drugs, questioning of the approaches and assumptions of allopathic medicine, their increasing costs and greater public access to information on traditional medicine also led to an increase interest in alternative treatments (WHO, 2002). In the last few decades research explore the number of medicinal plants for various biomedical applications, especially 

*Tripterygium wilfordii* Hook (Liu et al., 2011), *Betula alba* (Fulda et al., 2008), *Hydrastis canadensis* L. (Wang et al., 2011), *Berberineeris* sp (Patel et al., 2010), *Wikstroemia indica* (Lu et al., 2011), *Curcuma longa* (Sa et al., 2010), *Salvia prionitis* Hance (Deng et al., 2011) *Catharanthus roseus* (Cragg et al., 2005), *Podophyllum species* (Sarker et al., 2006), *Taxus brevifolia* (Hait et al., 2007) and many others. However, scientists are still attempting to explore the anti-cancerous compounds in unexplored plant species.

*Sophora interrupta* belongs to the family; *Fabaceae* (*Leguminaceae, Papilonaceae*) is commonly called as *Edwardsia maderaspatana* Wight, Pili Girgoli. There are approximately 219 species in this genus Sophora. *Sophora interrupta* is available exclusively in Seshachalam
Hill ranges of Tirumala. This plant is endemic with very limited area of distribution. This plant is a woody perennial shrub with pinnate leaves, sub-opposite leaflets, broadly ovate and golden yellow flowers. It has multifarious medicinal properties including antibacterial (Cha et al., 2007), anticancer (Ma et al., 2008) and anti inflammatory (Zhou et al., 2009). Very there is little literature available on this plant.

Therefore, the present study was undertaken to investigate the antioxidant and anti cancer properties of crude extracts of *Sophora interrupta*.

**Specific objectives**

- An extraction of leaf extracts of *Sophora interrupta* phytochemicals in water, methanol and ethanol solvents.
- Phytochemical screening and qualitative analysis of bioactive constituents present in methanol, ethanolic and aqueous leaf extracts of *Sophora interrupta*.
- Evaluation of *in vitro* free radical scavenging and antioxidative properties of extracts using various *in vitro* methods.
- Comparison of *in vitro* DNA protection and DNA nicking activity of methanol, ethanolic and aqueous leaf extracts of *Sophora interrupta*.
- Assessment of *in vitro* anticancer property of leaf extract of *Sophora interrupta* leaves against MCF-7 breast cancer cells.
- To evaluate protective role and chemotherapeutic efficacy of aqueous extract of *Sophora interrupta leaves* against cadmium nitrate induced toxicity in wistar strain albino rats.

This chapter contains preamble of herbal medicine, importance of herbal medicine and necessity to explore new medicinal plants for scientific community for various biomedical applications.
*Sophora interrupta* was collected from the Seshachalam hill ranges from Tirumala, A.P, India. The leaves of the plant were used in the current investigation. The dried leaves were made into a fine powder. 10 grams of the dried powder was weighed and soaked in either water, ethanol or methanol solutions at a concentration of 10% w/v for 8 hours and filtered to get a clear solution. These extracts were labeled as water, ethanolic and methanolic extracts and stored at -4°C in a refrigerator.

Phytochemical screening of the crude extract in the three solvents was performed in order to characterize alkaloids flavonoids, tannins, phenolics, proteins and amino acids, carbohydrates, steroids, saponins, cardio glycosides, terpenoids, fats and oils. Final quantitative spectroscopic estimation total flavonoids, phenolic compounds and total protein levels were performed. All extracts were subjected to *in vitro* radical evaluation, such as DPPH radical assay for antioxidant estimation. Inhibition of membrane damage was assayed in terms of oxidative hemolysis and lipid peroxidation assays. Antioxidant and radical quenching efficiency were assayed by β-carotene bleaching and hydroxyl radical scavenging method and results were compared with vitamin C. The electron quenching ability of leaf extracts was assayed by DPPH and reducing power assays. All the assays were performed in a dose dependent manner from 10-250 µg/ml concentration and 50% Inhibitory concentration (IC₅₀) values along with correlation co-efficient (r²) were carried out for all the assays. The *in vitro* free radical quenching and antioxidant results correlated with *in vitro* DNA protection assay.

This cell line model was used in the present study to identify potential anti cancer activity of *Sophora interrupta*. Further *Sophora interrupta* leaf extract was analyzed for the anti-proliferative properties against MCF-7 breast cancer cell line. The cells were cultured in the presence of aqueous extract in the following concentrations: 100, 250, 500, 1000 (µg/ml) for 24
hr and these concentrations were compared with standard positive control, i.e., doxorubicin (1µg/ml). The percentage of cell viability and membrane leakage were evaluated by MTT and LDH assays in a dose dependent manner. The anti proliferative effect of water extract of *Sophora interrupta* on MCF-7 cells was associated with induction of apoptosis, morphological changes and DNA fragmentation. All these methods demonstrate the anticancer efficacy. The morphology of water extract of *Sophora interrupta* on MCF-7 cells was observed by inverted phase contrast microscope in a time dependent manner such as a 3 hrs, 6 hrs, 12 hrs, and 24 hrs cell. The apoptotic cell morphology was observed using AO/EtBr (Acridine orange/Ethidium bromide) and apoptotic cell nucleus was stained with Hoechst 33258. The apoptotic cell DNA fragmentation was analyzed using agarose gel electrophoresis.

It is well acknowledged that cadmium is a metal pollutant which eventually cause a variety of oxidative stress mediated deleterious effects. It is believed that, ROS (reactive oxygen species) like superoxide, hydroxyl radicals can attack many cellular substances including lipids, proteins and nucleic acids which gets oxidized resulting in loss of function. In a preliminary *in vitro* antioxidant studies water extract of *Sophora interrupta* exhibited potential radical quenching propriety. Therefore, *Sophora interrupta* aqueous extract was evaluated as a therapeutic agent against cadmium nitrate induced acute toxicity.

**Total flavonoid and phenol content**

The preliminary phytochemical screening of methanol, ethanol and aqueous extracts of *S. interrupta* were carried out. The aqueous extract of *S. interrupta* contains almost all phytochemicals except terpenoids and fat oils. The total phenols were estimated spectrophotometrically using Folin - Ciocalteu’s reagent. Total phenol content of leaf extracts were in the following order WE > EE > ME respectively. The solubility of total phenols was
increased by decreasing the polarity of solvents. Total flavonoid content of leaf extracts was estimated using NaNO$_2$ and 10% AlCl$_3$ reagents. The extraction of yield of total flavonoid for different extracts of *Sophora interrupta* leaves are as follows: WE > EE > ME which is 16.47; 14.28; 5.75 $\mu$ moles of CE per gram of leaf respectively. The solubility of total flavonoid increased as the polarity of solvents decreased. Significant quantities of flavonoid contents were observed in water extract. Studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of various parts of plants.

**In vitro free radical scavenging and antioxidant assays**

The measure of antioxidant potential in leaves and other parts of plant products are influenced by many factors, which includes extract composition, solvents used for extraction and conditions of test system. Due to the complex nature of phytochemicals, no single standard method can be used to determine the antioxidant capacity accurately. To overcome this, the antioxidant property of *S. interrupta* extract was assayed by different methods.

**Inhibition of $\beta$-carotene bleaching.**

The percentage of $\beta$-carotene bleaching inhibition ranged from 55.5% to 76.92% for both standards and leaf extracts of *S. interrupta*. The following hierarchy for inhibition of $\beta$-carotene bleaching for the different extracts of *S. interrupta* was as follows: WE > EE > ME.

An organic expression of oxidative lipid damage can be done by measuring TBA-MDA abduct. The capacity of inhibition of lipid peroxidation is proportional to the antioxidant concentration in the extracts. WE of *S. interrupta* leaves showed highest percentage of peroxidation inhibition compared to ME and EE. The inhibition of lipid peroxidation was
observed more in increasing polarity of solvents of leaf extracts, with IC_{50} values of WE > EE > ME, Catechin 52.2, 50.2, 43.2 and 252 µg/ml respectively

**DNA Protecting Activity**

Inhibitory effect of the extracts from *S. interrupta* on the oxidative DNA damage caused by H_{2}O_{2} was evaluated by *in vitro* DNA cleavage assay using pBR322 plasmid DNA and Lambda phage DNA.

**Anti-cancer studies**

Prevention of cancer through phyto therapeutics’ intervention recently has received an increasing interest, since synthetic drugs have various and severe adverse effects on adjacent cells of normal cells. Therefore, products of natural origin with no or very few side effects are desirable as substitutes for chemotherapy. Natural compounds have been reported to interfere at the initiation, promotion and progression of cancer.

Effect of *Sophora interrupta* aqueous extract on MCF-7 breast cancer cells was studied: The MCF-7 was incubated with the aqueous extract at a concentration of 100, 250, 500 and 1000 µg/ml for 24 hours. Doxorubicin was used as a standard positive control for all the assays.

The morphology of MCF-7 cells treated with *Sophora interrupta* aqueous extract were observed to lose cell integrity significantly in the course of time, at 3, 6, 12 and 24 hours, under an inverted microscope.

Analysis of nuclear chromatin condensation and apoptotic cell in MCF-7 cells treated with *Sophora interrupta* leaf extracts were demonstrated by typical morphology
of apoptotic nuclei stained with the DNA-binding fluorophore Hoechst 33258. The nuclear condensation and fragmentation in the form of apoptotic nucleic bodies were clearly seen in a dose dependent manner from 100 µg/ml to 1000 µg/ml concentration. The shape of the nucleolus completely vanished at higher concentration of *S. interrupta* extract and doxorubicin.

MCF-7 cells treated with extracts of *S. interrupta* showed increased percentages of early apoptotic cells for 24 h treatment, increased percentages of early apoptotic (-12.22%), late apoptotic (-51.11%) and necrotic cells (-17.78%) after 24 h at higher concentration by Ao / EtBr staining. The results were compared with positive control doxorubicin.

**Chemo protective studies**

Highly natural stress inducer, Cadmium (Cd), promotes an early oxidative stress and afterward contributes to the development of serious pathological consequences because of its long retention in some tissues (Bagchi *et al.*, 2000).

**Changes in the Activities of Liver Marker Enzymes**

The activities of alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) (liver markers) were increased significantly in plasma of cadmium treated groups when compared to normal groups. Low dose of *S. interrupta* treatment (250 mg/kg bwt) did not show any significant reduction in the activities of these enzymes.

Cadmium induced nephrotoxicity was assessed by Serum Creatinine and urea concentrations levels which was elevated by 50% in comparison to control group (p < 0.05).
(*Sophora interrupta* leaf aqueous extract could protect the cadmium induced toxicity in liver and kidney of animals.

**Changes in the Levels of Reduced Glutathione and Glutathione Metabolism**

The significant decrease in the levels of GSH in Liver and Kidney of Cadmium treated groups, upon treatment with plant extract showed a significant increase compared to the group that was treated with Cadmium alone.

**Changes in the Activities of antioxidant enzymes**

The activities of SOD showed a significant decrease in tissues of liver and kidney of cadmium treated groups. Treatment with plant extract significantly increased their levels when compared to controls.

**Lipid peroxidation status**

The changes in the levels of TBARS in liver and kidney of plant extract treated groups and cadmium treated groups showed that there was a significant increase in the cadmium treated group when compared to normal. Plant extract (500 and 750 mg/kg b wt.) treatment significantly decreased their levels, when compared to the above groups.

**Histopathological changes in the liver**

Treatment with plant extract (500 and 750 mg/kg b wt) to cadmium treated animals effectively reduced pathological changes, such as sinusoidal dilatation, vascular congestion, thickening of blood vessels and nuclear disintegration when compared to cadmium treated and control groups.
Histopathological Changes in Kidney

Extensive fatty infiltrate, cloudy swelling and hemorrhage were observed in cadmium treated rats. However, cadmium and plant extract supplemented rats showed cell regenerative processes which was very effective in preventing pathological abnormalities induced by cadmium.

All the above studies highlighted, the pharmacological (therapeutic) importance of aqueous extracts of *Sophora interrupta* leaves. This can be a potential anti-cancer, hepato and nephro protective agent.