3.1. Chemoprevention and Phytomedicine in the Management of Cancer:

Cancer chemoprevention was first defined as “a strategy of cancer control by administration of synthetic or natural compounds to reverse or suppress the process of carcinogenesis”\(^{39}\).

Chemoprevention strategies target each of the steps including anti-initiation strategies and anti-promotion/anti-progression strategies\(^{40}\).

Herbal medicines, botanicals, dietary supplements, and edible plants have all been suggested as potentially important in cancer chemoprevention due to their long history of human consumption\(^{41}\).

Several known compounds isolated from traditionally used medicinal plants have already been proved to act on newly validated molecular targets, as exemplified by indirubin, which selectively inhibits cyclin dependent kinases\(^{42} \& 43\) and kamebakaurin, which has been shown to inhibit NF kappa B\(^{44} \& 45\).

Anticancer agents from plants currently in clinical use can be categorized into different classes of compounds based on their mechanism of actions: Vinca and camptothecins alkaloids, epipodophyllotoxins & taxanes,. Vinblastine and Vincristine were isolated from *Catharanthus roseus* (L) G. Don (*Apocynaceae*) and have been used clinically for over 40 years\(^{46}\).

The vinca alkaloids and several of their semi-synthetic derivatives block mitosis with metaphase arrest by binding specifically to tubulin resulting in its depolymerisation\(^{47}\).

Podophyllotoxin was isolated from the resin of *Podophyllum peltatum* L. (*Berberidaceae*) but was found to be too toxic in mice so derivatives were made with the first clinically approved drug molecule etoposide. The epipodophyllotoxins bind tubulin, causing DNA strand breaking during the G2 Phase of the cell cycle thereby irreversibly inhibiting DNA topoisomerase II\(^{48}\).
Paclitaxel was originally isolated from *Taxus brevifolia* Nutt. (*Taxaceae*) and was clinically introduced to the US market in the early 1990’s. The taxanes, including paclitaxel and derivatives, act by binding tubulin without allowing depolymerization or interfering with tubulin assembly.

Camptothecin, first isolated and identified from *Camptotherca acuminate* L. was found to kill cancer cells uniquely via topoisomerase I poisoning. Presently, two first-generation analogues of Camptothecin are used to treat ovarian, colorectal and small-cell lung cancers. Togther, the taxanes and the camptothectins accounted for approximately one-third of the global anticancer market in 2002.

There are many researches going on towards the proven anticancer potential of the many plant species. Potential anticancer plants with anticancer molecules are listed below.

- *Vinca rosea* L. (vincristine, vinblastine).
- *Taxus brevifolia* Nutt. (Taxol).
- *Picrorrhizis somnifera* (kurkin).
- *Withania somnifera* L.(Dunal) (withanolides).
- *Daphne mezereum* L.(mezerein).
- *Zingiber officinale* Roscoe. (zingiberol).
- *Hypericum perforatum* (hypericin).
- *Allium sativum* L. (allicin).
- *Astralapus hedysarum* L. (polysaccharide).
- *Silybum marianum* (L.) Gaertn. (silymarin).

The effect of *Semecarpus anacardium* L. on hepatocarcinogenicity of aflatoxin B\(_1\) was evaluated in adult albino male Wistar rats. Aflatoxin B\(_1\) was administered intraperitoneally to induce hepatocellular carcinoma. The cancer bearing animals were treated with *Semecarpus anacardium* L. (nut extract) (200 mg/kg body weight/day) in sunflower oil orally for 14 days. The elevations of plasma concentration of antioxidant enzymes due to the effect of aflatoxin B\(_1\) were restored back to near normal levels in
cancer bearing animals. Lactate dehydrogenase and aminotransferases levels decreased in liver, whereas alkaline phosphatase and $\gamma$-glutamyl transpeptidase increased in cancer conditions. The results clearly depicted antitumor potential of *Semecarpus anacardium* Linn. nut extract on aflatoxin B$_1$ induced hepatocellular carcinoma$^{53}$.

Alcohol and water extract of *Daphna mezereum* L., a plant widely used in folk medicine for treating cancers, showed antileukemic activity against the P388 lymphocytic leukemia in mice$^{54}$.

The polyphenolic extract (PPE) of leaves of *Ichnocarpus frutescens* L. was evaluated for its antitumor activity against Murine Ehrlich ascites carcinoma (EAC) in *In-Vivo model*. PPE cytotoxicity was determined *in vitro* using U-937 monocytoid leukemia and K-562 erythroleukemia cell lines. PPE also have been assessed for the free radical scavenging activity against superoxide and nitric oxide radicals. Acute oral toxicity was performed employing acute toxic classic method. Results of *in vitro* study showed a significant decrease in tumor volume, viable tumor cell count and a significant increase of life span in the PPE treated group as compared to untreated one, the life span of PPE treated animals increased by 53.41% (50 mg PPE/kg) and 73.95% (100 mg PPE/kg). PPE (5, 10 and 20 $\mu$g/mL) effectively inhibited *in vitro* proliferation of U-937 and K-562 cell lines. PPE also exhibited pronounced free radical scavenging activity with an inhibitory concentration (IC$_{50}$) value of 167.46 $\mu$g/mL and 158.52 $\mu$g/mL against superoxide and nitric oxide radicals, respectively$^{55}$.

The anticancer potential of methanol extract of *Mucuna pruriens* L.DC. (seeds) against EAC bearing mice was studied and reported. The extract was administered at a dose level of 125 and 250mg/kg b.w. for 14 days, after 24 hours of tumor inoculation. Decrease in tumor volume, packed cell volume, and viable cell count were observed in MEMP treated animals as compared to EAC treated animals. Treatment increased the mean survival time significantly. The plant extract showed a marked reversal of the Hematological parameters which included a significant increase in RBC count and decrease in WBC count on plant treated animals. The results clearly demonstrate the
antitumor potential of the methanol extract of *Mucuna pruriens* seeds against EAC cell lines\(^5^6\).

Limonin and nomilin were described earlier as being bitter principles from Citrus fruits. Both Limonoids have been found to induce increased activity of the detoxifying enzyme glutathione-S-transferase. The increased enzyme activity was correlated with the inhibiting ability of these compounds to inhibit chemically induced carcinogenesis in laboratory animals\(^5^7\).

The antitumor activity of ethanol extract of *Indigofera aspalathoides* VahlexDC. (EIA) was evaluated against the Ehrlich ascites carcinoma (EAC) tumor model. The activity was assessed using survival time, peritoneal cell count, hematological studies, solid tumor mass and *in vitro* cytotoxicity. Oral administration of EIA increased the survival time and normal peritoneal cell count. The altered parameters on tumour bearing animals were restored on treatment with EIA. Short time *In-Vitro* cytotoxic studies also established the anticancer potential of the plant extract\(^5^8\).

Withaferin-A showed marked tumor-inhibitory activity when tested *in-vitro* against cells derived from human carcinoma of nasopharynx (KB). It also acted as a mitotic poison arresting the division of cultured human larynx carcinoma cells at metaphase and in HeLa cultures similar to star-metaphase. It also produced significant retardation of the growth of Ehrlich ascites carcinoma, Sarcoma 180, Sarcoma Black (SBL), and E 0771 mammary adenocarcinoma in mice at the dose levels of 10, 12, 15 mg/kg body weight. Growth of Ehrlich ascites carcinoma was completely inhibited in more than half of the mice, which survived for 100 days without the evidence of growth of the tumor. Withaferin-A caused mitotic arrest in embryonal chicken fibroblast cells\(^5^9\).

A number of studies have indicated that β-sitosterol may show powerful anticancer properties as well. β-sitosterol was found to reduce growth of human prostate and colon cancer cells, and also exhibited antitumor activity against lymphocytic leukemia\(^6^0\).
The methanol extract of *Caesalpinia bonducella* F. (*Caesalpiniaceae*) leaves (MECB) were evaluated for antitumor activity against Ehrlich ascites carcinoma (EAC)-bearing Swiss albino mice. Various doses of the plant extract were administered *viz.*, 50, 100, and 200 mg/kg body weight per day for 14 days after 24 h of tumor inoculation. After the last dose and 18 h fasting, the mice were sacrificed. The effect of MECB on the growth of transplantable murine tumor, life span of EAC-bearing hosts, hematological profile, and antioxidant parameters were analysed. MECB caused a significant decrease in tumor volume, packed cell volume, and viable cell count. Treatment also shows an increase in the life span of EAC-tumor bearing mice. Hematological profiles were reverted back to near normal levels in extract-treated mice. MECB restored the altered levels of antioxidant parameters. MECB was found to be devoid of conspicuous short-term toxicity in the mice when administered intraperitoneally for 14 days. The results indicated that MECB possess significant antitumor and antioxidant activity in EAC-bearing mice.

Methanolic extract obtained from *Acridocarpus* Oliv. were found to be having new triterpenoids, acridocarpuric acid and three known triterpenoids moronic acid, Ursolic acid and oleanolic acid. These compounds showed significant cytotoxic activity in the A 2780 assay with IC 50 value as 0.7 mg/ml.

Crude extracts of *Piper longum* L. fruits, were tested for short term *in vitro* and *in vivo* cytotoxic activity using Ehrlich ascites carcinoma cells. Swiss albino mice were used for the *in vivo* studies. Ehrlich ascites carcinoma cells were inoculated intraperitoneally. Petroleum ether and chloroform extracts had shown significant cytotoxic action on the EAC cell lines. Petroleum ether extract treated animals showed an increase in life span up to 44.44%. But, chloroform extract increased the life span of tumor bearing animals only by 33.33%. An increase in life span up to 87.5% was observed in animals which were treated with both the extracts.
Bruceantin isolated from *Brucea antidysentrica* Lam. was screened for anticancer activity against B16 melanoma, colon 38 and L120 and P388 leukemia in mice and showed elevated level of apoptosis\(^64\).

The effect of *Acanthus ilicifolius* L. against tumor progression in carcinogen induced skin papilloma formation in mice and Ehrlich Ascites Carcinoma was analysed. Oral administration of the extract reduced the tumour volume and increased the life span by 75% in ascites tumour (EAC cells). The extract also significantly delayed the onset of dimethylbenzanthrazene (DMBA)/Croton oil induced skin papilloma in mice\(^65\).

Methanolic extract of *Peganum harmala* L. yielded alkaloids harmine and harmaline, which revealed cytotoxic activity against human cancer cell lines *viz.*, TK-10, MCF 7 and UAAC-62\(^66\).

The anticancer effect of hydroalcoholic extract of *Aegle marmelos* (L.) Corr. (AME) was studied in the Ehrlich Ascites carcinoma bearing Swiss albino mice. The study was aimed to find out the LD10 and LD50 of the plant drug under study. The AME treatment resulted in a dose dependent elevation in the median survival time (MST) was observed up to 400 mg/kg and declined thereafter. The acute toxicity study of AME showed that the drug was non-toxic up to a dose of 1750 mg/kg b. wt. The LD10 and LD50 was found to be 2000 and 2250 mg/kg respectively\(^67\).

Effect of methanol extract of *Cassia fistula* F. seeds on Ehrlich ascites carcinoma (EAC) was studied. Extract treatment showed an increase in life span and a decrease in the tumor volume and viable tumor cell count in the EAC bearing animals. A significant effect is found on the hematological parameters on treatment with plant extract. The results clearly depict the anticancer potential of *Cassia fistula* seed extract on EAC bearing mice\(^68\).
The anticancer potentials of Methanol Extract of *Careya arborea* Roxb. (MEC) (bark) was evaluated against Dalton's lymphoma ascites (DLA)-induced tumors. Methanol extract was administered orally to mice at the dose of 250 and 500 mg/kg body weight for 10 days. There was a significant reduction in body weight, viable tumor cell count and, packed cell volume as compared to the mice of control group. On administration of the drug, restoration of altered haematological and biochemical parameters were observed. Histological observations of liver and kidney also supported the anticancer activity of the plant extract\(^69\).

The antitumor and anti-invasion activity of taxane, harringtonine, homoharringtonione and camptothecin on highly metastatic melanoma B-16 and B1-16 and human fibro sarcoma HT-1080 cells were studied. Results revealed that taxol, harringtonine, homoharringtonione and camptothecin exhibited significant inhibition of cell growth and also found to be effective in inhibiting cell invasion and migration of B-16 and B1-16 cells\(^70\).

Different extracts and fractions of *Bidens pilosa* L. were evaluated for their anticancer potential employing *In-Vitro* methods. The Methanolic extract of *Bidens pilosa* showed a significant cytotoxic activity against HeLa cells\(^71\).
Essential oil components showing anticancer activity:

Essential oils are the volatile fraction of aromatic and medicinal plants after extraction by steam or water distillation. They have been used for their pharmaceutical potential since early times, and even now are still subject to a great deal of attention.

The essential oils obtained from different officinal plants of Lebanon, belonging to the Magnoliophyta division, have been tested for their antiproliferative activity on human erythroleukemic K562 cells. *Satureja montana* showed the most interesting biological activity in inhibiting the cell growth and inducing erythroid differentiation of K562 cells. The essential oil of *Satureja montana* was therefore analyzed using a GC/MS (gas chromatography/mass spectrometry) system in order to identify the major constituents and the results were compared with *Satureja hortensis*. The essential oil composition was found to be varied between the species, the major constituent of *Satureja hortensis* being carvacrol (50.61%) and that of *Satureja montana* being α-terpineol (12.66%). In order to identify molecules which could be possibly responsible for the biological activity, commercially available derivatives have been assayed on the K562 cell line. *Satureja montana* essential oil displayed different natural derivatives characterized by higher activity than those present in *Satureja hortensis*. The common active principles are α-pinene, γ-terpinene, 4-terpineol, α-terpineol, τ-cadinene, τ-cadinol and caryophyllene. Both caryophyllene and α-terpineol showed promising antiproliferative effects on K562 cells.72

Review on essential oil by Amr E. Edris73 presents both fundamental and recent studies carried out on anticancer potential of essential oils of *Nigella* whose major constituents are thymoquinone and β-elemene, potential chemotherapeutic and chemopreventive anti-cancer agents. The mechanism of action and the factors which determine the concentrations of these major constituents in the essential oil are also reviewed in this article. The essential oil, when injected directly into the tumor, is found to be useful in reducing tumor volume, inhibiting metastasis development and delaying mortality of P815 tumor-bearing mice. These activities were attributed to the thymoquinone (TQ) component of the essential oil. Thymoquinone is generally the
major constituent of *N. sativa* essential oils. TQ shows promising in vitro and in vivo antineoplastic growth inhibition against various tumor cell lines. This activity may be attributed to its inhibitory effects on cancer cell growth and its capability of inducing apoptosis. The growth inhibiting activity of TQ is associated with the induction of cell cycle arrest and inhibition of DNA synthesis, and the apoptotic activity which were shown to be mediated via p53-independent and p53-dependent pathways. In addition, TQ has been reported to be active against many multidrug-resistant variants of different human cancer cell lines.

**Fig 3: Chemical structure of Thymoquinone**

Some species of *Nigella* contains no TQ at all, for example *N. orientalis*, *N. damascene* and *N. arvensis*. Despite the absence of TQ in these species, their essential oils still possessed anti-tumor activity, and this is mainly due to the presence of a sesquiterpene hydrocarbon called β-elemene. The concentration of β-elemene can reach up to 69.0%–73.0% in the essential oils of *N. orientalis* and *N. damascene* respectively. β-Elemene was recently investigated as a novel anticancer plant-based drug. Combinations of β-elemene with clinically used anti-cancer drugs like paclitaxel or docetaxel can lead to synergistic interactions. The authors of this study concluded that β-elemene induced an alteration of cell membrane permeability which may enhance cellular uptake of the drug. β-Elemene was found to inhibit the growth of laryngeal cancer cells both *in vitro* and *in vivo*, probably by enhancing caspase-3 activity, thus inhibiting protein expression of eIFs, bFGF, and VEGF. β–elemene also had differential inhibitory effects on cell growth between non-small-cell lung cancer
cell lines and lung fibroblast and bronchial epithelial cell lines. The study indicated that the effect of β-elemene on lung cancer cell death may be through the cytochrome c-mediated apoptotic pathway.\(^75\)

**Fig 4: Chemical structure of β-elemene**

The molecular events involved in the antineoplastic activities of Thermoquinone (TQ), an important compound present in essential oil of most of the plants of *Asteraceae* family, in prostate cancer cells have now been revealed: the compound effectively blocks G1-phase prostate cancer cells from entering the S phase, and thus may prove to be useful in treating prostate cancer, particularly in hormone refractory cases. In the same investigation it was also observed that TQ possesses a high selectivity for inhibiting proliferation and viability of only cancerous prostate epithelial cells. The selective anti-tumor activity of TQ compared with that of 5-fluorouracil suggests that this compound may be a safer alternative for the treatment of human colon cancer. Sustained delivery of TQ produced significant cellular destruction and interference of cellular metabolic functions of SW-626 human colon cancer cells, which was comparable to the effect of standard 5-fluorouracil (the gold-standard for treatment of human colon cancer). TQ also triggers apoptotic cell death in human colorectal cancer cells, which correlates with the G1 phase arrest of the cell cycle.\(^76\)

The essential oils isolated by hydro-distillation from *Pelargonium graveolens* (geranium) and *Citrus reticulate Blanco*. (petitgrain mandarin) were analyzed by GC/MS and assessed for their antioxidant and anticancer activities. Twenty five components of petitgrain mandarin essential oil were identified and the major components being γ-terpinene (47.89%), methyl N-methyl anthranilate (13.17%), α-terpinene (7.40%), β-phellandrene (6.26%) trans-isolimonene (5.87%), α-terpinolene
PHARMACOGNOSTIC STANDARDIZATION AND BIOCHEMICAL EVALUATION OF TRADITIONAL ANTICANCER DRUG SOURCES - *ARTEMISIA* SPP.
were α -caryophyllene, terpinen-4-ol, myrtenol and borneol, which were proven anticancer agents\textsuperscript{78}.

Sweet marjoram (*Majorana hortensis* Moench.) is a perennial herb native to Cyprus and eastern Mediterranean countries\textsuperscript{79}. Hydro-distilled essential oil of *Majorana hortensis*, grown in the lower region of Kumaon Himalaya was analysed by GC and GC-MS. Thirty-two constituents accounting 96.32 % of the oil were identified. The oil was mainly composed of monoterpenes and to a small extent sesquiterpenes. The major constituents of this oil were (Z)-sabinene hydrate (30.81 %), terpinen-4-ol (22.02 %), (E)-sabinene hydrate (9.16 %), sabinene (6.73 %) and p-cymene (5.15 %). The plant is reported to possess anticancer\textsuperscript{80}, antioxidant\textsuperscript{81} and antifungal properties\textsuperscript{82} & \textsuperscript{83}.

The search for innovative therapeutic approaches based on the use of new substances is gaining more interest in clinical oncology. An *in vitro* study depicted the potential anti-tumoral activity of tea tree oil, distilled from *Melaleuca alternifolia* (Maiden & Betche) Cheel, this study was against human melanoma M14 WT cells and their drug-resistant counterparts, M14 adriamycin-resistant cells. Both sensitive and resistant cells were grown in the presence of tea tree oil at concentrations ranging from 0.005 to 0.03%. Both the complex oil (tea tree oil) and its main active component terpinen-4-ol were able to induce caspase-dependent apoptosis of melanoma cells and this effect was more evident in the resistant variant cell population. Freeze-fracturing and scanning electron microscopy analyses suggested that the effect of the crude oil and of the terpinen-4-ol was mediated by their interaction with plasma membrane and subsequent reorganization of membrane lipids. In conclusion, tea tree oil and terpinen-4-ol are capable of impairing the growth of human M14 melanoma cells and appear to be more effective on their resistant variants, which express high levels of P-glycoprotein in the plasma membrane, overcoming resistance to caspase-dependent apoptosis exerted by P-glycoprotein-positive tumor cells. TTO and terpinen-4-ol, at concentrations lower than 0.03%, after disrupting molecular architecture of the plasma membrane partially stimulated programmed cell death\textsuperscript{84}.
Present literature review suggested that plant species are rich sources and are highly useful in developing anticancer drugs. In the present dissertation *Asteraceae* members are selected being one of the largest flowering family existing around and are also rich in anticancer molecules. With a view to understand the anticancer potentials of this family, a review was carried out on the anticancer screening studies conducted on these members which will be presented and discussed in sequel.
3.2. Review on Anticancer Studies Carried out on the Asteraceae Members:

Sesquiterpene lactone is an important class of naturally occurring substances generally found in Asteraceae family. Many of these compounds are endowed with an impressively rich spectrum of biological activity including anticancer potential\textsuperscript{85}. The discovery and structural elucidation of approximately 100 new sesquiterpene lactones during a systematic study of Artemisia and other genera of the family of Asteraceae have provided the opportunity for further investigations on the therapeutic potentials of these sesquiterpene lactones as new drug molecules. Much of the earlier studies on sesquiterpene lactones for medicinal purposes were focussed mainly on santonin, a santanolide, and its derivatives, which are well known as important anthelmintic and ascaricidal agents. Many sesquiterpene lactones bearing an α-methylene-7-lactone grouping have occasionally been isolated and shown to contain significant antitumor or cytotoxic activity. These include the germacrnanolides, elephantin, elephandopin, costunolide, and tulipinolide; the guaianolides, gaillardin, euparotin acetate, and its companions, the pseudoguaianolide, damsid; and the elemanolide, vernolepin. Although the structural requirements for the antitumor or cytotoxic activity of some of the above sesquiterpene lactones have been postulated, and attempts have been made to explain their mechanism of action, there has been a need for further study of other structural types of the sesquiterpene lactones, such as santanolides and xanthanolides, the antitumor or cytotoxic activities of which have not yet been reported. Variety of structural types were selected for investigation for cytotoxicity screening, since this type of preliminary screening can be used as an invaluable guide for selecting possible antitumor agents. The results obtained show that the most immediate and direct factor responsible for cytotoxicity of the compounds studied is the introduction of the 0=C- C=CH2 system in their structure\textsuperscript{86}.

Antiproliferative effect of chloroform soluble extract obtained by re-extraction of the methanol extract of whole plant of Carpesium rosulatum MIQ. (Asteraceae) was studied against five types of human cancer cell lines (A549, SK-OV-3, SKMEL- 2, XF498, HCT15). Four germacrane sesquiterpene lactones 2 α,5-epoxy-5,10-dihydroxy-6-angeloyl-oxy-9 β -isobutyloxy-germacran-8 α,12-olide, 2 α,5-epoxy-5,10-dihydroxy-6
α, 9 β-diangeloyloxy-germacran-8 α,12-olide, 2 α,5-epoxy-5,10-dihydroxy-6 α -angeloyloxy- 9β -(3-methyl-butanoxyloxy)-germacran-8 α,12-olide, and 2β,5-epoxy-5,10-dihydroxy-6 α,9 β - diangeloyloxy-germacran-8 α,12-olide were isolated from the chloroform extract of C. rosulatum, and 2 α,5- epoxy-5,10-dihydroxy-6 α, 9 β - diangeloyloxy-germacran-8 α,12-olide showed the most potent cytotoxicity with IC50 value of 6.01 μM against SK-MEL-287.

The molecules responsible for the anticancer potential of four important members of Asteraceae family were reported below:

**Table 2: Proven anticancer molecules from Asteraceae members**

<table>
<thead>
<tr>
<th>Plant source</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Baccharis megapotamica</em> var weirii.</td>
<td>Baccharin</td>
</tr>
<tr>
<td><em>Helenium autumnale</em> L.</td>
<td>Helenalin</td>
</tr>
<tr>
<td><em>Liatris chapmanii</em> Torrey &amp; A. Gray.</td>
<td>Liatrin</td>
</tr>
<tr>
<td><em>Vernonia hymenolepis</em> A. Rich.</td>
<td>Vernolepin</td>
</tr>
</tbody>
</table>

The investigators demonstrate the anticancer activity by blocking various stages viz., initiation, promotion and progression of rapidly growing cancer cells88.

The chromatographic separation of the MeOH extract of the aerial parts of *Solidago virgaurea* var. gigantea MIQ. (Asteraceae) led to the isolation of six terpenoids and four phenolic compounds, trans-phytol, ent-germacra-4(15),5,10(14)-trien-1α-ol, β-amyrin acetate, ent-germacra-4(15),5,10(14)-trien-1β-ol, β-dictyopterol, oleanolic acid, kaempferol, kaempferol-3-O-rutinoside, methyl 3,5-di-O-caffeoyl quinate, and 3,5-di-O-cafeoyl quinic acid. Their structures were established by chemical and spectroscopic methods. Among them 5,10(14)-trien-1β-ol, β-dictyopterol, and 3,5-di-O-cafeoyl quinic acid showed moderate cytotoxicity against five cultured human tumor cell lines in vitro with its ED50 values ranging from 1.52~18.57 mg/ml89.

*Rhaponticum carthamoides* (Willd.) Iljin, syn. *Leuzea carthamoides* (Willd.) DC, is a large perennial herb belonging to the *Asteraceae* family. A study was
conducted to demonstrate the cancer inhibitory effects of adaptogenic herbs on rats, including extracts of *Panax ginseng*, *Leutherococcus* and *R. carthamoides*(Wildd) Iljin (*Asteraceae*) roots. The specific study involved the cancer induction by transplacental administration of N-nitrosoethylurea. The herbal extracts were given orally for over a year. The administration of the adaptogens increased the survival time of the rats and lowered the occurrence or multiplicity of tumors mainly those of the central nervous system90.

The chloroform soluble fractions of ethanolic extracts of five *Inula* species namely *I. Granitoides* Boiss., *I. vulgare* LAM., *I. thapsoides* M.B.ex WILLD., *I. oculus christi* L., and *I. Salicina* L. subsp. *aspera* POIR., were evaluated for cytotoxic activity against five different cell lines such as CACO2 (human colon adenocarcinoma), MCF7 (human breast adenocarcinoma), HEPG2 (human hepatocellular carcinoma), VERO (green African monkey kidney) and WEHI164 (balb c mouse fibrosarcoma). Cytotoxicity was assessed by MTT assay. Among these five species, *Inula oculus christi*, exhibited better cytotoxic effects91.

Aqueous and ethanol extracts of *Youngia japonica* (L.) DC. (also known as Oriental hawksbeard) were tested in vitro for anti-tumor activity against three cell lines, human promyelocytic leukaemia (HL-60), human myelogenous leukaemia (chronic K-562) and mouse Sarcoma 180 (S-180). Hot water extract of *Youngia japonica* inhibited cell proliferation and growth of all cancer cell lines to various extents. K-562 cells were most sensitive to the extract whereas S-180 cells were the least. It did not show any significant cytotoxic effects on normal mammalian Vero cells up to the concentration of 450 μg/ml92.

*Centratherum punctatum* Cass. is an important medicinal plant belonging to *Asteraceae*. Isocentratherin, a germacranoide, was isolated from the aerial parts of *Centratherum punctatum* and studied for its cytotoxic potential. Isocentratherin was found to be cytotoxic against various cell lines. It is also found that Isocentratherin is responsible for the anticancer activity of *Centratherum punctatum* Cass.93.
The antioxidant and free radical scavenging activity of *Cichorium intybus* L., a plant which is commonly found in China, was studied and reported. The methanolic crude extracts of the plant were screened for its antioxidant and free radical scavenging properties using \( \alpha \)-tocopherol and butylated hydroxy toluene (BHT) as standard antioxidants. Antioxidant activity was measured by ferric thiocyanate (FTC) assay and compared with the thiobarbituric acid (TBA) method. Free radical scavenging activity was evaluated using diphenyl picryl hydrazyl (DPPH) radicals. The plant material was found to be showing free radical scavenging activity whose level changed drastically in the case of various clinical conditions, especially cancer.\(^9\)

Phytochemical analysis of the aerial parts of *Aster oharai* Nakai led to the isolation of a new sesquiterpene hydroperoxide, \( 7 \alpha \)-hydroperoxy-3,11-eudesmadiene and seven known compounds, teucdiol B, \( \alpha \)-spinasterol, oleanolic acid, \( \alpha \)-soinasterol 3-\( O \)-b-D-glucopyranoside, methyl 3,5-di-\( O \)-caffeoyl quinate, 3,5-di-\( O \)-cafeoylquinic acid, 3,4-di-\( O \)-cafeoylquinic acid. \( 7 \alpha \)-hydroperoxy-3,11-eudesmadiene which showed cytotoxicity against, SK-OV-3 (Ovarian), SK-MEL-2 (Skin melanoma), and HCT 15 (Colon) with \( ED_{50} \) values ranging from 3.86-17.21\( \mu \)g/ml.\(^9\)

*Gentianella alborosea* (‘Hercampure’) is a Peruvian species used in folk medicine for treating a variety of health disorders. This study is focused to test the efficacy of the methanol extract of *Gentianella alborosea* for its free radical scavenging (DPPH) and for its apoptosis induction potential on a human uterus tumor cell line (HeLa). The data of the results revealed a noticeable free radical scavenging activity and a dose-dependent apoptotic effect.\(^9\)

A sesquiterpene lactone isolated from Brazilian Asteraceae, *Eremanthus elaeagnus* (Mart. ex DC.) Schultz-Bip and *Vanillosmopsis erythropappa* Schul. BIP was converted to the diol, a potential precursor for the synthesis of micheliolide, a potential anticancer sesquiterpene lactone isolated from *Michelia compressa* (Maxim.) Sargent and *Michelia champaca* L.\(^9\)
A new sesquiterpene, named baccharisketone, and a new monoterpene, p-methoxythymol acetate, were isolated from the leaves of *Baccharis dracunculifolia* DC along with seventeen known compounds. The growth inhibitory activity of the isolated compounds against leukemia cells (L 1210) was tested and three terpene phenols and five sesquiterpene alcohols were found to exhibit strong cytotoxic activity. Sesquiterpene dimmer isolated from ethyl acetate soluble extract of the *Inula Britannica* (L) DC. exhibited a strong anticancer activity by inhibiting the NF-kappa B and TNF-α production.

The inhibitory activity of artemisinin an active principle of *Artemisia annua* L. and its derivative’s showed activity against cancer cells in the nano- to micromolar range. Artesunate, another active principle, triggers apoptosis both by p53-dependent and independent pathways. The anticancer activity of artesunate has also been shown in human genograft tumours in mice.

Artemisinins reveal promising anti-cancer activities when tested in vitro and in vivo. Artemisinins contain an endoperoxide group that is essential for their antimalarial and anticancer activities. Like hydrogen peroxide, artemisinin reacts with ferrous iron, to generate radical species. The short-lived artemisinin-generated radical species which have been linked to its anti-parasitic and anti-cancer activities. The anti-cancer activity of artemisinin derivatives can significantly increase when iron complexes are added in the cell culture medium. A covalent conjugate of artemisinin and transferrin (ART-Tf), an iron transport protein in human, is actively taken up by cancer cells through the transferrin receptor (TfR)-mediated endocytosis pathway, and shows significantly higher anti-cancer activity than unconjugated artemisinin. Like ART-Tf, artemisinin-peptide conjugates that are designed to target TfR also showed highly potent and selective anti-cancer activity. These studies show the importance of iron metabolism in determining the effectiveness of artemisinin derivatives in killing cancer cells.

Artemisinin derivatives induced programmed cell death of cancer cells by activating the intrinsic or the cytochrome C-mediated pathway for apoptosis, but the
initial protein targets of artemisinin derivatives for apoptosis induction in human cancer cells have not yet been identified. Although the generation of free radicals originating from the reaction of artemisinin with molecular iron is mentioned as one of the main mechanism for its anticancer activity, other mechanisms that are crucial for cancer proliferation and survival are also affected by artemisinins\textsuperscript{102}.

Apigenin, isolated and found in *Artemisia annua* L. has been extensively studied for its anti-cancer effect. When tested on A2780 (human ovarian cancer) cells, apigenin arrested cells at G2/M mitotic phase, and induced apoptosis at the concentration of 40 μM. Intraperitoneal injection of apigenin administered daily to a mouse xenograft model with subcutaneous implantation of A2780 cells significantly reduced tumor mass compared to the control group. Western blot analyses showed that apigenin suppressed the expression of Id1 (inhibitor of differentiation or DNA binding protein through activation transcription factor 3 or ATF3). Apigenin was also recently tested on S2-013 and CD18 (human pancreatic cancer) cell lines. In the study, apigenin was shown to inhibit the expression of GLUT-1 glucose transporter at a concentration of 25 μM. Glucose transporters are generally overexpressed in cancer tissues, and its over-expression is a poor prognosis factor in colorectal, breast, ovarian and gastric carcinomas. Apigenin also inhibited the phosphoinositol 3-kinase (PI3K)/Akt pathway, inducing the growth inhibition of the pancreatic cancer cells\textsuperscript{103}.

Eupatorin present in *A. Annua* revealed a moderate cytotoxic effect on MK-1 (human gastric adenocarcinoma), HeLa (human uterus carcinoma), B16F10 (murine melanoma), and 26-L5 (murine colon cancer) cell lines. Interestingly, the same compound was totally inactive against P-388 (lymphatic leukemia) cell line. Eupatorin inhibited the growth of MDA-MB-468 (human breast carcinoma) cell line in a dose dependent manner with an IC50 of 0.5 μM, eupatorin showed only weak inhibitory effect against MCF-10 (normal mammary tissue) cells. Eupatorin was found to be metabolized to the flavone cirsiliol and two other unidentified metabolites by a CYP450 enzyme, CYP1A1. The enzyme is present in MDA-MB0468 cells, but not expressed in MCF-10A healthy mammary cells. A similar activation of eupatorin by a CYP450 enzyme has also been observed in MCF-7 (human breast adenocarcinoma) cells\textsuperscript{104}.
Luteolin is another widely distributed flavone among the *Asteraceae* family. Its anti-cancer activity has been reviewed extensively. Luteolin induces apoptosis and inhibits cell proliferation, metastasis and angiogenesis. When tested *in vitro*, IC50 values ranged between 3 and 50 mM, and luteolin was active when tested in xenograft cancer models. Luteolin's cytotoxicity appeared to be associated with suppression of PI3K/Akt pathway, nuclear factor kappa B (NF-kB), and X-linked inhibitor of apoptosis protein (XIAP). Luteolin was shown to increase intracellular reactive oxygen species (ROS) in human hepatic cancer cells. Proteomics analyses showed that peroxiredoxin (PRDX6) and prohibitin (PHB) are key targets of luteolin. These two proteins are involved in ROS metabolism and apoptosis induction\textsuperscript{105}.

Cirsilineol from *A. annua* was tested on three cancer cell lines, HeLa, MK-1 and B16F10. Although cirsilineol was more potent than eupatorin against MK-1 cells, it was not effective against HeLa cells. Both cirsilineol and eupatorin showed similar activity against B16F10 cells. In a separate study, cirsilineol inhibited the growth of four cancer cell lines, Caov-3, Skov-3, HeLa, PC3 and HepG2. Interestingly, cirsilineol had no effect on a normal human liver cell line (L02). Cirsilineol induced apoptosis in Caov-3 cells by releasing cytochrome-c from mitochondria, followed by activation of caspase-9, -3 and PARP proteins\textsuperscript{106}.

Eupatin was tested on the NCI 60-cell line *in vitro* screen, and the mean GI50 value was 4 μM. The growth inhibition data of structurally similar flavonoids suggest that anti-mitotic mechanism may be responsible for the anticancer activity of eupatin although eupatin itself is a poor inhibitor of tubulin polymerization. Eupatin was also highly active as a free radical scavenger when tested against the oxidizer 1,1-diphenyl-2-picrylhydrazyl (DPPH). Eupatin has been found to be a moderately potent inhibitor of ABCG2, breast cancer resistance protein (BCRP) or mitoxantrone resistance protein (MXR). The IC50 value of eupatin for ABCG2 inhibition was 2.2 μM against ABCG2 or BCRP. ABCG2 appears to be involved in the resistance to several chemotherapeutic agents, and eupatin and its derivatives may be useful as synergistic flavonoid in reversing the drug resistance of cancer cells\textsuperscript{107}.
Quercetin another common flavones of *Asteraceae* member has been extensively studied for its biological activities including anticancer activity. Quercetin inhibited the growth of MCF-7 breast cancer cell line with an IC50 value of 5.2 mg/mL. Quercetin has been shown to inhibit protein kinase C and to bind to type-II estrogen binding sites. Interestingly, structurally similar flavonoids, myricetin and epicatechin, did not show significant inhibitory activity against MCF-7, indicating some specific structure-related interactions between quercetin and its potential cellular target(s) \(^{108}\). In the presence of 150 mM of quercetin, surface expression of alkaline phosphatase was increased along with a marked increase in caspase-3 activity that lead to apoptosis.

Quercetagetin 6,7,3’4’ tetramethyl ether (differ from artemetin only by having a OH) was reported to be extremely effective against tumor cell lines P-388 (murine lymphocytic leukemia), A-549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), HT-29 (human colon adenocarcinoma), and KB (human nasopharynx carcinoma) with ED50 values of 4.9 × 10−1, 4.81 × 10−1, 2.47, 1.25, and 6.80 × 10−1 μg/mL, respectively, while its 3-O-methyl analog artemetin was not effective against any of these tumor cells. Anticancer effects of Quercetagetin was recently confirmed when it was reported as a potent and selective inhibitor (IC50 = 0.34 μM) of a serine-threonine kinase (PIM1), implicated in the development of leukemia, lymphoma, and prostate cancer. In the same study, it was found that apigenin, quercetin, kaempferol, and luteolin (all present in *A. annua*) also inhibited PIM1 with IC50 of 0.94, 1.1, 1.3, and 1.6 μM, respectively \(^{109}\).

Casticin from *A. annua* has exhibited a potent anticancer activity against PC-12 (GI50 = 114 nM) and HCT116 (GI50 = 119 nM). The activity was comparable to that of cisplatin. Casticin was also tested on two human epidermoid carcinoma cell lines, KB and A431. While the growth of KB cells was significantly inhibited by casticin (IC50 = 0.23 μM), the compound had only a minimal effect on the growth of A431 cells. Two normal cell lines, 3T3 Swiss Albino (mouse) and TIG-103, were not affected by casticin. Flow cytometry analyses showed that casticin induced significant arrest of KB cells at the mitotic phase G2-M. Casticin disrupts mitotic spindles that may be responsible for the G2-M arrest of KB cells. Similar observations have been reported
with MCF-7 breast cancer sublines MN1 and MDD2. Casticin treatment increased the expression of p21, resulting in the inhibition of cyclin-dependent kinases (Cdk). Furthermore, casticin inhibited the expression of cyclin-A and Bcl-2 proteins which induced apoptosis.

Artemetin is a compound which is structurally very similar to casticin, but differs only by a hydroxyl group on the 3' position of the B ring, which is occupied by a methyl group in artemetin. Artemetin showed a moderate anticancer activity (IC50 = 16 μM) when tested on two human epidermoid carcinoma cell lines, KB and A43. The activity was 7–8 times lower than that of casticin. Artemetin has shown a significant anti-cancer activity against HL60 (human promyelocytic leukemia) cells with an IC50 = 6.44 μM.

Chrysosplenol-D inhibited the growth of KB cells with an IC50 = 13.95 μg/mL. In a study with HeLa cells, chrysosplenol-D markedly inhibited the incorporation of 32P into phospholipids when the cells are stimulated by 12-O-tetradecanoylphobol-13-acetate (TPA). Chrysosplenol-D is one of the active ingredients in Fructus Viticis (Vitex trifolia), a traditional Chinese medicine, that has been used to treat human cancer. Chrysosplenol-D inhibited the growth of tsFT210 (a mouse cdc2 mutant) cells with an IC50 = 3.5 μg/mL by inducing apoptosis.

Kaempferol, present in A. annua, inhibited the growth of ovarian cancer cell lines, OVCAR-3 and A2780/CP70. Kaempferol at the dose levels of 20 μM and 40 μM showed 91% inhibition of OVCAR-3 cells and 94% inhibition of A2780/CP70 cells. Interestingly, kaempferol significantly reduced both angiogenesis and VEGF gene expression at both mRNA (transcriptional) and protein (translational) levels. Both hypoxia inducible factor-1α (HIF-1α) and ESRRα were also down-regulated by kaempferol. Both these proteins are involved in regulating VEGF expression. Kaempferol induces apoptosis in MCF-7 cells at a concentration of 50 μM. Western blot analyses show a cleavage of PARP and activation of caspases-7 and 9 as well as an increase in Bax expression. In a separate study with multidrug resistant cells, MCF-7/ADR, kaempferol has shown p-glycoprotein inhibition with an efficacy similar to that
of verapamil. The growth of another breast cancer cell line, MDA-MB-453, was also inhibited by kaempferol. When the cells were exposed to kaempferol, cell cycle arrest occurred at G2/M phase, which may be due to the down-regulation of CDK1 and cyclin A and B.

Kaempferol and other two flavonoids, quercetin and myricetin, prevented the cell migration and metastasis of DAOY (medulloblastoma) cells by inhibiting hepatocyte growth factor (HGF)/Met signaling. Among the three flavonoids tested, kaempferol was found to be most effective with an IC$_{50}$ value of 0.5 μM. Kaempferol was ineffective in inhibiting the growth of human glioma cells U251 and U87. However, exposure to kaempferol sensitized these cell lines to tumor necrosis factor-related apoptosis ligand (TRAIL). Kaempferol induced the proteosomal degradation of survivin, thus increasing the sensitivity of the treated cells to TRAIL-induced apoptosis. Kaempferol was also recently shown to be moderately-active against pancreatic cancer, prostate cancer, and lung non-small cell carcinoma in vitro.

Isorhamnetin occurs in plants both as a secondary metabolite as well as an immediate metabolite of quercetin. Isorhamnetin inhibited the growth of BEL-7402 (human hepatocellular carcinoma) cells with an IC$_{50}$ = 74.4 μM/mL at 72 h. Incubation with 50 mg/mL of isorhamnetin induced apoptosis in 13.77% of BEL-7402 cells. The growth of Eca-109 (human esophageal squamous carcinoma) cells was inhibited by isorhamnetin, with an IC$_{50}$ = 40 μg/mL. Western blot analyses showed that incubation with isorhamnetin decreased the expression of Bcl-2, c-myc and H-ras while the expressions of Bax, c-fos and p53 increased. Isorhamnetin induced apoptosis in LLC (Lewis lung cancer) cells, and inhibited the cellular growth with an IC$_{50}$ value of 40 μM. The apoptosis was mediated by the release of cytochrome-c from mitochondria, and subsequent activation of caspase enzymes. Mouse xenograft model with LLC cells showed that i.p. injections of isorhamnetin (0.5 mg/kg/day) significantly reduced the tumor volume. The efficacy was about 10 times better than that of quercetin.

Astragalin is a 3-O-β-D-glucoside of kaempferol. Astragalin was tested on several cancer cell lines, including DU-145, GLC4 (human small cell lung carcinoma), and
COLO 320 (human colorectal cancer), with only a weak growth inhibitory effect. Isoquercitrin and quercimeritrin are the 3-O-β-D-glucoside and 7-O-β-D-glucoside of quercetin, respectively. In an ex vivo angiogenesis assay, isoquercitrin had the strongest activity, completely inhibiting microvessel growth at 100 μM. Both quercetin and quercimeritrin had a weaker effect on angiogenesis. Quercetin, isoquercitrin and quercimeritrin inhibited the growth of HUVEC cells at 100 μM, but only quercetin and isoquercitrin were able to inhibit the HUVEC tube formation. Other flavonoids including flavolin, luteolin 7-methyl ether, tomentin, isokampferide, luteolin-7- methyl ether, quercetagetin 3-methyl ether, luteolin 7-methyl ether have been isolated from Artemisia annua, but their anti-cancer potentials have not been well studied.

Artemisia, a large genus of the Anthemideae, has been the subject of numerous chemical and biological studies, yielding primarily sesquiterpene lactones, coumarins and acetylenes as the main metabolites. In addition to the production of artemisinin and related sesquiterpenes that are produced in specialized plastids in the apical and subapical cells of capitate glandular trichomes, A. Annua and most of the Artemisia spp. yielded an aromatic essential oil that is rich in monoterpenes. β-Caryophyllene, a common sesquiterpene widely distributed in plants possessing anti-inflammatory and anticarcinogenic effects.

The genus Tanacetum, totaling over 200 species distributed over Europe and West Asia and growing up to altitudes of 2,000 meters contains several strongly scented annual and perennial. Interest is increasing in species of Tanacetum due to their essential oil content, bitter substances and sesquiterpene lactones, which exhibit cytotoxicity and growth regulating activity.

A previous study reported that the AIP1 fraction, a fraction of water soluble polysaccharides smaller than 2 kDa purified from Artemisia iwayomogi Pampan, increased antibody production and suppressed transplanted tumor cell growth. It was also suggested that the AIP1 fraction might be involved in the survival of immune cells either by suppressing apoptotic death or by stimulating cell proliferation thereby contributing towards the inhibition of cancer growth.
Ethanol extract of *Artemisia vestita* Wall., a Jacoesidin containing plant, showed an inhibitory effect on endotoxin-induced sepsis by suppressing MAPKs and NF-κB signaling in macrophages. Few reports states the inhibition of COX-2 and MMP-9 in human mammary epithelial cells, suppression of E6 and E7 oncoproteins of HPV 16, and induction of apoptosis in ras-transformed human breast epithelial cells by Jacoesidin\(^{120}\).

Antiproliferation effect of Jaceosidin (4 α, 5, 7-trihydroxy-3 α, 6-dimethoxyflavone) was well demonstrated on several human cancer cell lines. Jaceosidin significantly reduced the proliferation of CAOV-3, SKOV-3, HeLa, and PC3 cells in a dose dependent manner. A time-dependent inhibition was also observed in CAOV-3 cells. By flow cytometric analysis, it was reported that Jaceosidin treatment resulted in an increased apoptosis in CAOV-3 cells. The cancer cells treated with Jaceosidin exhibited a decreased mitochondrial membrane potential. Jaceosidin also increased the level of cleaved caspase-9 and induced the cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP), in CAOV-3 cells. Moreover, Jaceosidin elevated the level of cytochrome c in cytosol. These findings suggest that the anticancer effect of Jaceosidin may be due to its apoptosis induction involving cytochrome c release from mitochondria to cytosol\(^{121}\).

Artemisinin derivatives are well-tolerated as anti-malarial drugs, besides they also exert anti-cancer activity. Artemisinin and its derivatives dihydroartemisinin and artemesunate were tested against chemosensitive and chemoresistant human neuroblastoma cells as well as in primary neuroblastoma cultures. Only dihydroartemisinin and artemesunate affected neuroblastoma cell viability with artemesunate being more active. Artesunate-induced apoptosis and reactive oxygen species in neuroblastoma cells. Of 16 cell lines and two primary cultures screened, only UKF-NB-3rCDDP1000 showed low sensitivity to artemesunate. Characteristic gene expression signatures based on a previous analysis of artemesunate resistance in the NCI60 cell line panel clearly separated UKF-NB-3rCDDP1000 from the other cell lines. L-Buthionine-S,R-sulfoximine, an inhibitor of GCL (glutamate–cysteine ligase), resensitised in part UKF-NB-3rCDDP1000 cells to artemesunate. This, together with bioinformatic findings of expression of genes involved in...
glutathione metabolism suggested that this pathway is involved in artesunate resistance. These data indicate that neuroblastoma represents an artesunate-sensitive cancer entity and artesunate is also effective in chemoresistant neuroblastoma cells\textsuperscript{122}.

From the review of the literature it is clearly evident that \textit{Asteraceae} members possess anticancer effect working through one of the following molecular mechanism of actions:

- Alterations in components of the downstream signal transduction pathways which leads to the activation of MAP kinase cascade.
- Inhibition of the Bax protein by Bcl\textsubscript{2} and Bclx proteins. Alterations in the PI-3 kinase-AKT/PKB pathway, which transmit antiapoptotic survival signals, leading to cancer cells evading apoptosis.
- Inhibition of inhibitor thrombospondin and activation of transcription of \textit{VEGF} gene.
- Activation of \textit{Ras}, Myc and Vhl oncogene family are also reported to play a key role in angiogenesis.
- Loss in the function of E-cadherin and b-catenin genes and transcriptional repression or proteolysis of the extracellular cadherin domain which leads to tissue invasion and metastasis.
- Alter the activity of p53, Ras and Src proteins.

Hence in the present dissertation common \textit{Asteraceae} members in and around Trichy were selected and screened for their anticancer activity. Attempts were also made to understand the mechanism of action and to identify the active molecule responsible for cancer prevention.