The aim of this research is to understand the multiple pharmacological potential of *Cephalotaxus griffithii* and *Oroxylum indicum* and to elucidate the active component with respect to anticancer activity. First, the plant materials were extracted and the extracts were tested for antioxidant, antimicrobial and anticancer properties. Further, fractionation of the extracts was carried out for anticancer study. Cytotoxicity guided fractionation was used to find out the anti-neoplastic principle of these two plants.

5.1. *Cephalotaxus griffithii*

Two plant parts of *C. griffithii* i.e. needles and bark were investigated for phytochemical and pharmacological study. The needle and bark were successively extracted with PE, ACE and MeOH to obtain the respective extracts. Besides, the essential oil extracted from of *C. griffithii* needles was investigated for anticancer property.

5.1.1. *Cephalotaxus griffithii needle*

5.1.1.1. Organic extraction

The CGN was extracted with PE, ACE and MeOH, and the extracts were tested for their effect on proliferation, cell cycle regulation, apoptosis induction and telomerase expression on human cancer cells. The antiproliferative activity of the extracts was tested on three different human cancer cell lines, namely HeLa, HepG2 and
ZR751 cells. Among the extracts, the maximum cytotoxicity was induced by PE extract with highest death occurred in breast cancer cells (ZR751 cells). Based on these result, we further carried out mechanism study of CNGP extract induced ZR751 cell death. It was revealed that the CNGP extract induced ZR751 cell death was associated with cell cycle arrest (G2 phase) and apoptosis. Further, molecular mechanism study of CNGP extract treatment on ZR751 cells observed that CNGP extract up-regulated p53 expression in ZR751 cells. When the p53 gene expression was knockdown, there was a significant reduction in the cell death induced by CNGP extract. This suggests that p53 is an essential target for CNGP extract. Furthermore, apoptosis pathway analysis indicated that CNGP extract induces apoptosis in ZR751 cells via both intrinsic and extrinsic apoptotic pathways. CNGP extract down-regulated hTERT and hTR, and up-regulated c-Myc expression. TLC analysis suggests the presence of unique phytochemicals in CNGP extract for inducing maximum antiproliferative effect on cancer cells. Therefore, CNGP extract was further fractionated. Six fractions were obtained and subjected for antiproliferative activity on HeLa and ZR751 cells. Among the fractions tested, fraction 6 exhibited the maximum cell death on both the cancer cells (IC_{50}) followed by fraction 5 > fraction 4 > fraction 3 > fraction 2 > fraction 1. The antiproliferative effect was also higher in fraction 6 than the original CNGP extract in both the cancer cell lines. Fraction 6 induced more growth inhibition to ZR751 cells as compared to HeLa cells. It was also revealed that the cell death induced by fraction 6 was associated with apoptosis. Further, fractionation of fraction 6 yield three compounds CGNC1, CGNC2 and CGNC3, however it was impure. CGNC1, CGNC2 and CGNC3 induced death of ZR751 and HeLa cells. Among the compounds, the maximum antiproliferative activity was observed in CGNC3 followed by CGNC2 and
CHAPTER V: DISCUSSION

CGNC1. Further, the death was more in ZR751 cell with a very low IC$_{50}$ value of 6 ± 1.4 µg/ml and 4.9 ± 1 µg/ml for CGNC2 and CGNC3, respectively indicating a selective cytotoxicity towards breast cancer cells. Further, CGNC2 and CGNC3 induced more antiproliferative activity against ZR751 cells as compared to the fraction 6 and CGNP extract, thus identified the anti-neoplastic principle of *Cephalotaxus griffithii* needles with respect to breast cancer. In case of HeLa cells, only CGNC3 exhibited more antiproliferative activity than fraction 6 and CGNP extract, indicating CGNC3 to be the anti-neoplastic principle of *Cephalotaxus griffithii* needles with respect to cervical cancer. However, further purification is required to elucidate the compound structure.

5.1.1.2. Hydrodistillation

The CGN was hydro distilled to produce CGNO. The CGNO was then investigated for the phytochemical constituents and their effect on human cervical cancer cells in terms of anti-proliferation, anti-migration, cell cycle regulation and apoptosis induction were determined.

GC-MS analysis identified 24 components with β-caryophyllene (28.19%), germacrene D (18.64%), α-pinene (10.24%) and α-humulene (7.24%) as the major constituents of the oil. CGNO treatment significantly reduced the HCC (HeLa, ME-180 and Siha) cell viability as compared to untreated control. CGNO significantly slowed down or inhibited the motility of HeLa cells to close a scratched wound and also reduced the HeLa cell migration towards a chemotactic stimulus. Further, it was revealed that the CGNO induced HCC cell death was mediated through apoptosis.
Apoptosis pathway analysis of HeLa cells indicated the association of both mitochondrial and death receptor-mediated apoptotic pathways.

5.1.2. *Cephalotaxus griffithii* bark

The three extracts of *C. griffithii* bark were tested for the total phenolic and flavonoid content, antioxidant, antibacterial and anticancer property. Polyphenols are well documented for antioxidant, antibacterial and anticancer activities (Yuan and Walsh, 2006; Rao et al., 2010; Carvalho et al., 2010; Scalbert, 1991; Cowan, 1999; Lamoral-Theys et al., 2010). Therefore, the TPC and TFC were determined. The TPC and TFC were highest in the ACE extract followed by the MeOH and PE extracts. Among the three extracts, the highest antioxidant activity was observed in ACE extract followed by the MeOH and PE extract. The DPPH free radical scavenging capability of ACE and MeOH extracts was comparable with that of ascorbic acid (positive control). Surprisingly, ACE and MeOH extracts possessed higher SORS capacity than ascorbic acid. These clearly indicate that *C. griffithii* bark contains potential antioxidants. Further, CGB extracts exhibited differential bacterial inhibitory effects and both the ACE and MeOH extracts inhibited only three minor pathogens, namely *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*, out of the six organisms tested. The PE extract inhibited only one strain, *Klebsiella pneumoniae*. The ACE extract was the most effective among the extracts as it produced a thicker inhibition zone (10.8-15.6 mm) and smaller MID (31.2-62.5 µg/disc) compared with that produced by the other extracts. Earlier, Cho et al. (2009) reported an inhibitory effect of Korean plum yew (*C. koreana*) extract on gram-positive bacteria, gram-negative bacteria, yeasts, and molds. Similarly, Watanabe and Fukao (2009) reported that an extract of
unripe Japanese plum yew fruit (C. harringtonia) possessed, among 101 edible plants, the highest inhibitory effect against *Bacillus cereus* and *Leuconostoc mesenteroides*. Our results on the antibacterial effect of CGB and those of Korean and Japanese plum yew confirm the antibacterial potential of plants in the *Cephalotaxus* genus. Application of CGB extract also inhibited the growth of HeLa cells. Among the extracts, ACE extract was most effective in inducing cytotoxicity of HeLa cells. Cell death mechanism study revealed that the HeLa cell death induced by CGB extract was mediated through apoptosis. Apoptosis was also highest in ACE extract treated HeLa cells. Correlation analysis suggested that phenolic and flavonoid content present in stem bark of *C. griffithii* extracts was responsible for the high antioxidant, cytotoxic, and apoptotic activity.

5.2. *Oroxylum indicum* bark

The *Oroxylum indicum* bark was successively extracted with PE, DCM and MeOH. These extracts were then tested for antioxidant, antimicrobial and anticancer activity. The antioxidant activity was highest in OIBM extract. Further, OIBM and OIBM extract displayed a broad antibacterial spectrum against three gram negative (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*) and gram positive (*Bacillus subtilis, Bacillus cereus* and *Staphylococcus aureus*) bacteria while OIBP extract showed antibacterial activity only against *Escherichia coli* and *Staphylococcus aureus*. OIBM extract inhibited two pathogenic fungi (*Aspergillus fumigatus* and *Macrofomina phaeolina*). In contrast, the HeLa cell death was highest after treatment with OIBP extract. It was observed that the HeLa cell death induced by OIB extracts were associated with apoptosis. Correlation analysis showed a high correlation between
antioxidant activity and phenolic/flavonoid content. However, a negative correlation was observed between antiproliferative activity and phenolic/flavonoid content.

OIBP extract which showed the maximum antiproliferative activity was fractionated. Five fractions were obtained and subjected for human tumor cell growth inhibitory effect on three human cancer cells (HeLa, HepG2 and ZR751). The magnitude of effect on antiproliferative activity varied among the fractions. Among the fractions, fraction 4 and 5 induced maximum death in all the tested cancer cells.

From fraction 5, a compound was obtained and identified to be oroxylin A. Treatment of oroxylin A induced death of human cancer cells (ZR751 and HeLa cells). However, the antiproliferative activity was higher than fraction 5 and OIBP extract suggesting that oroxylin A is not the main active antineoplastic component present in fraction 5 or OIBP extract or OIB associated with the antiproliferative activity. This result suggests that components other than oroxylin A were mainly responsible for the anti-neoplastic effect induced by fraction 5.

One major challenge in chemotherapy is to increase new drugs that can selectivity kill cancer cells sparing the normal cells. Therefore, oroxylin A was tested for cytotoxicity on normal cells (WI-38 cell). Compared to cancerous cells, the normal human fibroblast was less sensitive to the cytotoxic effect of oroxylin A. oroxylin A was 2.3 times more active in killing ZR751 cells, and 1.7 times more active in killing HeLa cells than normal human fibroblast indicating the ability of oroxylin A to induce selective cytotoxicity towards cancer cells.