CHAPTER 5

SUMMARY AND CONCLUSION

In the present study, four medicinal plants such as Acalypha alnifolia, Blepharis maderaspatensis, Ipomoea staphylina, and Dregea volubilis were screened for the phytoconstituents content namely, flavanoid, phenolic, tannin, carotenoid and saponin. Different solvent extracted fractions were tested for the efficiency of the components. Among the four plants, Blepharis maderaspatensis showed higher phytoconstituents content extracted with methanol and this plant was selected for further studies. The anti-inflammatory and antioxidant studies of Blepharis maderaspatensis were studied. The anti-oxidant compound was purified and characterized using various techniques like Silica gel column chromatography, TLC, UV-VIS, HPLC, FTIR, LC-MS, NMR and GC-MS. The compound was found to be Protocatachuic acid which was then checked for its anticancer activity on cell lines. The In vitro cytotoxic activity was checked on vero cell lines on four different time period namely 12hr, 24hr, 48hr and 72hr respectively. The anti-proliferative effect of protocatachuic acid on three cell lines such as MCF-7, HCT-116 and SNU-182 were studied. The apoptotic activity of the cells by flow cytometer confirmed the anti-proliferative activity of the purified Protocatachuic acid.

- Among the medicinal plants tested, Blepharis maderaspatensis methanol leaf extract showed the maximum amount of total saponin, tannin, phenol, carotenoid and flavanoid compounds.
In the antioxidant activity, *Blepharis maderaspatensis* showed the maximum ABTS, DPPH and NO scavenging activity in the methanol fraction.

In the *In Vitro* cytotoxicity assay on vero cell line, 50µg/ml showed the least growth.

The purified antioxidant compound was confirmed the purity by HPLC and the structure of the compound has been elucidated by IR, NMR and MS the compound was found to be protocatachuic acid (PCA).

In 20µg of PCA was found to reduce the expression levels of cytokinins: iNOS, COX2, TNF-α, IL1 and IL-6 after LPS induced RAW 264.7 cell lines.

In the Western blot analysis of cytokinin expression was reduced at 20µg of PCA treated RAW 264.7 cell lines.

The increasing concentration of protocatachuic acid and as well as incubation period showed cytotoxic activity using MTT assay.

Acridine orange/ethidium bromide stained microscopic photography showed apoptotic activity of protocatachuic acid under fluorescent microscopy.

Among the cancer cell line, SNU-182 cells showed good ladder formation on gel electrophoresis than the other two cell lines in IC₅₀ cells.
• Flow cytometry data also showed the SNU-182 cancer cells apparent variation as pro and late apoptotic cells and as well as dead cells while compared with rest two cells.

• The proteolytic Zn ion depended enzymes on zymogram shows the SNU-182 cells greatly inhibited.

• MMP-9 was highly inhibited than the MMP-2 enzyme.

1. Possible pathway for lowering the inflammatory cytokines of protocatachuic acid

Description: IKK: IB kinase; COX-2: cyclooxygenase-2; p50/p65: subunits of NF-B; IL1: Interleukin 1; iNOS: inducible nitric oxide synthase; TNF α: Tumour necrosis factor α; IB: Inhibitor of B in cytoplasm; IL6: interleukin 6; NFkB: nuclear factor kappa-light-chain-enhancer of activated B cells; TLR-4 : Toll-like receptor 4; IKK: IkB kinase enzyme complex; p38: Mitogen-activated protein kinase; p50, p65: NF-kB subunits; JNK : c-Jun N-terminal kinases; I-kB: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor; ERK: Extracellular signal-regulated protein kinase;
2. Possible pathway for anticancer activity of protocatechuic acid

Protocatechuic acid (C₇H₆O₄/154.12 g/mol)

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Cancer cell

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DNA fragmentation

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Apoptosis