Chapter 9

Summary & Conclusion
SUMMARY AND CONCLUSION

8.1 SUMMARY

The present study has been designed to scientifically investigate the plant *Zanthoxylum alatum* for its antioxidant and cytotoxic potential against various cancer cell lines using *in vitro* methods. The most potential plant extract(s) was selected for further isolation of cytotoxic active compounds by column chromatography.

The following salient features can be summarized on the basis of results obtained in the present study:

- On the basis of ethnomedical information and literature survey, plant material *Zanthoxylum alatum* was selected for the present study.
- Plant material (stem bark) was collected and authenticated from authorized and approved authority.
- The physicochemical parameters such as ash and extractive values were determined.
- Extracts (petroleum ether, chloroform, ethyl acetate and methanol) were prepared using soxhlet apparatus and subjected to preliminary chemical screening which shows the presence of steroids, phenolic compounds, alkaloids and flavonoids in it.
- All prepared extracts in different concentrations (1-150 mg/ml) were screened for cytotoxic activity using MTT assay on different cell lines such as A-549, MIA-PaCa, MCF-7 and CaCo-2. Docetaxel was used as a standard.
SUMMARY AND CONCLUSION

- The petroleum ether and ethyl acetate extracts have shown more cytotoxic potential as compared to other extracts. They showed most promising results in MIA-PaCa and A-549 cancer cell lines.

- The extract(s) having cytotoxic potential were evaluated for their free radical inhibition activity using *in vitro* assays such as DPPH and NO radical scavenging assay, reducing power assay. Results were expressed in IC$_{50}$ values. Petroleum ether and ethyl acetate extracts were also showed significant antioxidant potential. The antioxidant activity increased with increasing concentration in all the models.

- The petroleum ether and ethyl acetate extracts were evaluated for its total phenolic content on the basis of its gallic acid equivalent using Folin-Ciocalteu phenol assay. The phenolic content found in petroleum ether and ethyl acetate extract was 5.12 and 4.36 mg/g GAE respectively.

- Both petroleum ether and ethyl acetate extracts were further taken to column chromatography as they both showed significant antioxidant and cytotoxic activity as compared to another extracts. Column was packed with silica gel (60-120 mesh) by wet packing method.

- From petroleum ether extract β-sitosterol (A) and three lignans; sesamin (B), kobusin (C), 4’O demethyl magnolin (D) were isolated and from ethyl acetate extract two flavonoids; apigenin (E) and kaempferol-7-O-glucoside (F) were isolated.

- Isolated compounds were also subjected to cytotoxic assay on MIA-PaCa and A-549 cancer cell lines. They showed cytotoxic potential in different ranges but the most potent inhibition of cell proliferation was observed with lignan 4’O
demethyl magnolin in both cell lines. The IC\(_{50}\) value of 4’O demethyl magnolin was found to be 21.72 ± 1.50 and 26.47 ± 1.87 in MIA-PaCa and A-549 cancer cell lines respectively.

- So, only 4’O demethyl magnolin (compound D) was subjected to apoptosis study by acridine orange/ethidium bromide method. Results of AO/EB indicates that 4’O demethyl magnolin enhance the apoptosis at IC\(_{50}\) dose (21.72mg/ml), however showing necrotic cell death at higher dose after 24 h.

**8.2 CONCLUSION**

The results of the present study support and justify the traditional and folklore therapeutic claims attributed to *Zanthoxylum alatum* in the treatment of tumour. The conducted studies indicated that petroleum ether and ethyl acetate extracts of the plant has cytotoxic potential. Thus, traditional value of the plant has been scientifically proved by MTT and AO/EB assay on human cancer cell lines. It has been proved that the cytotoxic potential of plant may be attributed to the combined effects of the phenolic compounds particularly, lignans and flavonoids. *Zanthoxylum alatum* has excellent antioxidant activity supporting the use of the plant as a natural source of phenolic for antioxidant and anticancer benifits.