7. SUMMARY

➢ Turmeric *Curcuma longa* L. was collected from Andhra Pradesh, Maharashtra, Assam, Tamil Nadu and kerala, among these the appearance of rhizome from Assam – Lakadong variety were found to be thick and size of fingers are bigger than other varieties.

➢ Evaluation of curcuminoids from turmeric rhizome collected from different places in India were obtained by HPLC analysis showed presence of varying amount of curcuminoids present in different varieties but Assam variety resulted higher amount of total and individual curcuminoids.

➢ Estimation of individual curcuminoids extracted from turmeric using various solvents from polar to non-polar, acetone extract showed higher yield of individual curcuminoids at 23.9%, 10%, 6.1% of C, DMC, BDMC respectively.

➢ Separation of curcuminoids by TLC method using various solvents resulted better resolution of Rf value in chloroform : methanol 95:5 as mobile phase. The optimum Rf value obtained were 0.69, 0.44, 0.29 for C, DMC, BDMC respectively, representing optimum distance travelled by curcuminoids in mobile phase.

➢ The individual curcuminoids were isolated by column chromatography using chloroform and methanol at increasing polarity. It yields fractions of individual curcuminoids were pooled out and concentrated and purified, resulted in Curcumin as orange-yellow color, Demethoxycurcumin was orange color and Bisdemethoxycurcumin was orange-red color powder.

➢ Purified curcuminoids were characterized by sophisticated analytical technique for confirmation of compounds. Purity of isolated compounds tested in HPLC
showed 90-95% purity compared to retention time and peaks spiking with standard.

- The molecular weight of C, DMC, BDMC were analysed by GC-MS, the full scan mass spectra of each curcuminoids were showed molecular weight of C, DMC and BDMC as 368.31, 337.77, 307.95 respectively.

- FTIR analysis showed the presence of absorption of bands of specific stretching region at their respective frequency confirmed the presence of all functional group in curcuminoids.

- The structure were elucidated by NMR both $^{13}$C NMR and $^1$H NMR showed structure of curcuminoids each curcuminoi ds differs in the presence of methoxyl group on structure.

- Phenomenon of MDR is studied in KB and KBChR8-5 cell lines. Analysis of presence of MDR1 gene expression in KB and KBChR8-5 cells by real time PCR shows higher level of MDR1 gene expression in KBChR8-5 cells at 7.1 fold increase when compared to KB cells, but KB cells showed very mild expression of MDR1 gene.

- Anti-cancer activity of all three curcuminoids on KB and KBChR8-5 cells were evaluated and showed varying activity. DMC and BDMC showed greater anti-cancer activity when compared to curcumin. In terms of drug resistant cell line BDMC kills MDR subline more effectively than parental cells.

- The RR value for C and DMC was $<1$ and BDMC was 1.5 which indicates BDMC kills KBChR8-5 cells more effectively than parental KB cells. This result indicated that BDMC shows collateral sensitivity towards curcuminoids in KBChR8-5 cells.
- Down-regulation of MDR-1 gene expression were studied in three curcuminoids treated with 5µM concentration using real time PCR in KB parental cells resulted greater inhibitory effect by BDMC treatment.

- MDR-1 gene overexpressing KBChR8-5 cells on treatment with three curcuminoids were analysed, which imparts greater inhibitory effect by curcumin treatment. Curcumin showed a fold change of 1.47 range of inhibition when compared to vehicle control. There is no much difference in inhibition were found in DMC treated cells. BDMC showed low difference in inhibition.

- Co-incubation of curcumin with doxorubicin influences increase in cytotoxic effect in KBChR8-5 cells. There is an increased sensitivity of doxorubicin was observed. The IC₅₀ value of doxorubicin treatment was higher but on treatment with co-incubation with curcumin increased the sensitivity of doxorubicin resulting lower IC₅₀ value.

- Apoptosis influenced by curcumin, doxorubicin and co-incubation were studied on confocal microscopy and resulted in increased number of early and late apoptotic cells on treatment with individual drugs and more number of dead cells were viewed on co-incubation treatment under stained condition using AO/EtBr staining, which implies co-incubation increased apoptosis.

- The cell cycle analysis by flow cytometry showed sharp changes in cell cycle arrest at co-incubation of curcumin and doxorubicin with cell cycle arrest at G2-M phase at 24 and 48 hours.

- Curcumin as natural phytochemicals, non toxic to human and potent inhibitor of MDR -1 gene expression in vitro which can act as effective MDR modulator. Co-incubation treatment of curcumin with doxorubicin in KBChR8-5 cells had shown increased sensitivity to doxorubicin therefore can be used in treatment of drug resistance in cancer.