## 7.6. CONCLUSIONS

Dammarane triterpenoid 1 exhibited significant antiproliferative activity on various cancer cell lines. Dammarane triterpenoid 1 showed substantial growth inhibitory activity on MIA PaCa pancreatic and DU145 prostate cancer cells among all tested cell lines. Sub-$G_0$ phase cell population elevation in cell cycle analysis, mitochondria membrane potential loss, increased cytochrome *c* levels, increased Bax levels, decreased Bcl-2 levels, increased caspases-3 & 9 activities, nuclear morphological changes and DNA fragmentation in treated MIA PaCa-2 pancreatic and DU145 prostate cancer cells, which demonstrated the pro-apoptotic potential of dammarane triterpenoid 1. Further investigations are needed to develop novel COX and 5-LOX dual inhibitory dammarane triterpenoid 1 as an anticancer drug.

# SUMMARY AND CONCLUSIONS

Arachidonic acid (AA) pathway is an important metabolic pathway in which phospholipase $A_2$s ($PLA_2$s), cyclooxygenases (COXs) and lipoxygenases (LOXs) and respective metabolites lysophospholipids (LPLs), prostaglandins (PGs), and leukotriens (LTs) are involved. Several studies suggested that AA pathway has a key role in pathophysiology of various diseases *viz*., arthritis, asthma, inflammatory bowel disease, acute respiratory distress syndrome, atherosclerosis and several cancers. AA pathway regulates inflammation, cell growth, survival, invasion and metastasis. AA pathway is involved in several chemical carcinogens, UV-radiation and tobacco-caused carcinogenesis. Hence, AA cascade metabolic enzymes $PLA_2$s, COXs and LOXs and their metabolic products, such as PGs and LTs, have been considered as novel preventive and therapeutic targets in inflammatory and oncologic diseases. Several natural and synthetic inhibitors of AA pathway have been developed by various research groups and have been explored as anti-inflammatory and anticancer agents. Curcumin, resveratrol, ellagic acid, eugenol, ursolic acid, (6)-gingerol, lycopene and genistein are some of the important natural product cancer chemopreventive agents which act by inhibiting multiple pathways, including AA pathway.

*Madhuca longifolia*, *Helicteres isora*, *Borassus flabellifer*, *Punica granatum*, *Abrus precatorius*, *Cocos nucifera* are some of the important Indian medicinal plants. Effect of various parts of these plants on clinically relevant enzymes of AA pathway is not explored even scientific and traditional rationality was found. In this background, *Madhuca longifolia* seed coat and leaves, *Helicteres isora* stem bark, *Borassus flabellifer* seed coat, shoot and flower, *Punica granatum* fruit rind, *Abrus precatorius* inner part of the seed, *Cocos nucifera* fruit fiber husk and tender seed coat were collected from forests of north coastal Andhra Pradesh and screened for AA metabolizing enzyme (PLA2, COX-1, COX-2 and 5-LOX). Among all tested plant extracts, *B. flabellifer* seed coat showed significant 5-LOX inhibitory activity which is comparable or more than positive control NDGA. It exhibited considerable COX-1 and COX-2 inhibitory activity. The study suggests that *B. flabellifer* seed coat extract

is a promising source for isolation of 5-LOX and COX-2 inhibitory compound. Hence, further investigations were carried out on *B. flabellifer* seed coat to isolate and identify active phytochemical which is responsible for its COX and 5-LOX inhibitory activity.

As significant 5-LOX inhibition activity, fractionation of *Borassus flabellifer* seed coat extract was done using silica gel chromatography based 5-LOX inhibition activity. In first separation of seed coat extract using silica gel column chromatography with increasing polarity from hexane to ethyl acetate, yielded seven major fractions (F1 (inactive), F2 ($IC_{50}$ > 100 µg/ml), F3 ($IC_{50}$ = 91.21 µg/ml), F4 ($IC_{50}$ = 7.21µg/ml), F5($IC_{50}$ > 100 µg/m), F6 ($IC_{50}$ > 100 µg/ml) and F7($IC_{50}$ > 100 µg/ml)). F4 fraction showed significant 5-LOX inhibition ($IC_{50}$ = 7.21µg/ml) thus further fractionated using silica gel column chromatography, to yield 3 fractions, $F4_{(1)}$ ($IC_{50}$ = 63.12 µg/ml), $F4_{(2)}$ ($IC_{50}$ = 4.31 µg/ml) and $F4_{(3)}$ ($IC_{50}$ > 100 µg/ml). Among three fractions, $F4_{(2)}$ showed predominant 5-LOX inhibition with $IC_{50}$ of 4.37 µg/ml. $F4_{(2)}$ also inhibited COX (1 & 2) but not inhibited PLA2 activity. Dammarane triterpenoid 1 (Dammara-20,23-diene-3,25-diol) was the compound present in $F4_{(2)}$, determined based on advanced spectroscopic technique, including NMR, MS and elemental analysis.

Dammarane triterpenoid 1 exhibited substantial 5-LOX inhibitory activity as well as considerable COX (1 & 2) inhibitory activity *in vitro*. It was also noted that dammarane triterpenoid 1 did not show any inhibitory effect on PLA2 activity in cell free system. *In silico* studies demonstrated that dammarane triterpenoid 1 strongly binds with 5-LOX and COX (1&2). The strong binding affinity of dammarane triterpenoid 1 on active site amino acids of 5-LOX and COX may be responsible for inhibition of enzyme activity. Dammarane tritepenoid 1 showed comparable or better 5-LOX inhibitory activity than NDGA (positive control).

In anti-inflammatory studies, dammarane triterpenoid 1 inhibited carrageenan-induced paw edema in rats. It exhibits comparable or more antiinflammaotry activity than indomethacin (standard). histopathological studies of tissues of $4^{th}$ h paws (maximum edema inhibition occur in $4^{th}$ h) demonstrated that decreased inflammatory cells populations in tissues of treated rat paws compared to untreated paw tissues. This denotes that COX and 5-LOX inhibitors may block the recruitment of inflammatory cells in site of inflammation during the inflammation. However, detailed investigations are required on influence of COX/LOX inhibitors in recruitment of inflammatory cells

at inflammation site. Dammarane triterpenoid 1 significantly inhibited prostaglandins and leukotrienes as well as pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) secretion in LPS-stimulated THP1 human monocytes. The results of anti-inflammatory studies on dammarane triterpenoid 1 are encouraging and warranted further investigations to develop dammarane triterpenoid 1 as an anti-inflammatory agent.

Dammarane triterpenoid 1 exhibited significant antiproliferative activity on various cancer cell lines. Dammarane triterpenoid 1 showed substantial growth inhibitory activity on MIA PaCa-2 pancreatic and DU145 prostate cancer cells among all tested cell lines. Sub-$G_0$ phase cell population elevation in cell cycle analysis, mitochondria membrane potential loss, increased cytochrome *c* levels, increased Bax levels, decreased Bcl-2 levels, nuclear morphological changes and DNA fragmentation in treated MIA PaCa-2 pancreatic and DU145 prostate cancer cells, which demonstrated the pro-apoptotic potential of dammarane triterpenoid 1. The results of the study warranted further investigations to develop novel COX and 5-LOX dual inhibitory dammarane triterpenoid 1 as an anticancer drug.

Results of the present study warranted further investigations are needed to develop novel COX and 5-LOX dual inhibitory dammarane triterpenoid 1 as an anti-inflammatory and anticancer drug. Our preliminary but encouraging results may facilitate the development of dammarane triterpenoid 1 as an antiinflammatory and anticancer drug.