7. SUMMARY AND CONCLUSION

Traditional plant based system of medicine or herbal medicine is an important part of the health care system in most of the countries. Majority of the people depend on the traditional system of medicine for their primary health care needs. Nature is a reservoir gifted with thousands of medicinal plants and a large number of modern drugs have been originated from different natural sources. Due to the high cost and side effects of the synthetic drugs, plant derived medicine has served as a good source of alternative medicine. Moreover, combination therapy is sometimes used to make the treatment more effective and there is a real need for a next generation of safer and more potent drugs.

*Rubus* is one of the important genera with significant pharmacological activities, of which many species has been used traditionally for the treatment of various ailments. Based on the strong evidences of traditional uses of this species, the present study was focused on pharmacological evaluation of *R. ellipticus*, *R. niveus* and *R. fairholmianus* with special reference to inflammation and cancer. The total phenolics, flavonoids, tannins contents and *in vitro* antioxidant activities of the extracts were evaluated. Then, the pharmacologically active extract was subjected to the isolation of compounds.

The RFRA extract showed highest total phenolics (63.53 g GAE/100 g extract) and flavonoid contents (981.33 mg RE/g extract), whereas acetone extract of *R. ellipticus* leaves showed higher tannin content (46.47 g GAE/100 g extract). Among the various extracts tested for *in vitro* antiradical activities, RFRA extract was observed for greater DPPH radical (IC\textsubscript{50} 3.55 μg/mL), superoxide radical scavenging (72.88%) and phosphomolybdenum reduction (94.09 g AAE/100 g extract). On the other hand, RELM extract was found to be the best scavenger of ABTS\textsuperscript{++} and nitric oxide radical. Moreover, RNRA extract showed higher ferric reducing antioxidant activity (8024.44 mM Fe(II)/mg extract).

The toxicity studies of RELM, RNRA and RFRA extracts showed the safety and non toxicity of the drug up to 2000 mg/kg in acute and 400 mg/kg in sub acute in 14 days extract treated animals. The 5% extract did not show any toxicity in dermal toxicity studies. The enzymatic and non enzymatic antioxidant levels of blood and liver were observed in mice treated with 200 and 400 mg/kg of RELM, RNRA and RFRA.
after 30 days and was compared with normal untreated animals. Levels of CAT (119.55 U/Hb), SOD (790.32 U/Hb), GSH (54.17 nM/mg protein) and GR (8.43 nM of NADPH consumed/min/mg protein) were increased significantly ($p < 0.01$ and $p < 0.05$) in blood of the RFRA (400 mg/kg) treated mice. Similarly, levels of CAT (6.51 U/mg protein), SOD (1.54 U/mg protein), GPx (21.16 U/mg protein), GR (114.03 nM of NADPH consumed/min/mg protein), GST (105.16 nM/mg protein) and GSH (14.7 nM/mg protein) were also increased significantly in the liver of RFRA (400 mg/kg) extract treated group.

The acute anti-inflammatory activity was evaluated using carrageenan induced paw edema in rats and croton oil induced ear edema in mice. In the first model, RFRA extract (400 mg/kg) was most active with significant inhibitory effect of 77.98% whereas in croton oil induced model, RNRA (400 mg/kg) extract reduced the edematous response by 54.53%. The analgesic study revealed that RFRA extract at 400 mg/kg possesses 62.69% inhibition in acetic acid induced writhing test whereas, RELM showed significant activity in Eddy’s hot plate mediated analgesia. In antipyretic studies, the RNRA extracts seems to be more potent in reducing the yeast induced fever in rats, when compared to RFRA and RELM.

In excision wound model, the percentage of wound contraction was higher in 2% RFRA extract treated animals, with shorter period of epithelization (13.02 days); this was comparable with betadine (12.62 days). SOD activities of the granuloma tissue also showed more or less same for RFRA extract (1.26 U/mg protein) and standard treated group (1.28 U/mg protein). RFRA and RELM at higher doses showed comparable wound healing activity in infected model, with an epithelization period of 15.02 and 15.00 days respectively. The levels of SOD in the granuloma tissue taken from RELM and RFRA (2%) treated animals were also found to be the same (1.15 U/mg protein). The RFRA extract at 1 and 2% showed significant results in incision wound model, with a skin breaking strength of 95.31 and 70.28 N/cm² respectively, followed by RNRA and RELM.

The antitumor activities of the extracts have been evaluated by ascites and solid tumor models using mice whereas, *in vitro* cytotoxic activities were checked using short term trypan blue dye exclusion method and long term MTT assay. The RFRA extract was
highly toxic to the DLA and EAC cell lines inhibiting 100% proliferation by 200 μg/mL of the extracts. In MTT assay, RFRA extracts decreased the cell viability of MCF-7, Jurkat, HeLa and Vero cells in a dose dependant manner. RFRA extracts was more toxic to MCF-7 breast cancer cells (IC$_{50}$ 29.36 μg/mL). The RFRA extract also found to possess significant activity against DLA and EAC induced solid and ascites tumors respectively. The solid tumor volume in mice was significantly reduced by the treatment of 250 mg/kg of RFRA extract (1.94 cm$^3$ on 38$^{th}$ day) compared with other extracts, which also increased the life span of the tumor bearing mice (76.57%) by significantly reducing the ascites tumor development in mice. At a concentration of 20 μg/mL, RFRA extract found to induce apoptosis in EAC cells which was observed by morphological changes and DNA ladder formation. The potent anticancer activity of RFRA extract was confirmed in this research suggesting that further studies are needed to figure out the exact mechanism of action behind it.

RFRA extract exhibited a concentration dependant activity in all in vitro and in vivo pharmacological activities. The most promising activity of RFRA was an indication of its potential for further phytochemical studies. The activity guided column chromatographic separation of this extract yielded relatively small amounts of six bioactive compounds such as; 1-(2-hydroxy phenyl)-4- methyl pentan-1-one (1), 2-[(3-methylbutoxy) carbonyl] benzoic acid (2), 2-(5-methylhexyl) benzoic acid (3), 4- methylpentyl benzoate (4), 3-(iminomethyl)-2, 4-dimethylphenol (5) and isopentyl benzoate or 3- methyl benzoate (6). All of these compounds possessed significant in vitro antioxidant activity against DPPH$^\ddagger$ and ABTS$^{+\ddagger}$. Among the isolated compounds, 1-(2-hydroxyphenyl)-4-methylpentan-1-one showed maximum DPPH radical scavenging activity (IC$_{50}$ 3.23 μg/mL) followed by 2-[(3-methylbutoxy) carbonyl] benzoic acid (IC$_{50}$ 5.09 μg/mL) whereas, highest ABTS radical cation scavenging was observed for 2-[(3-methylbutoxy) carbonyl] benzoic acid (9233.20 μM TE/g) followed by 4- methylpentyl benzoate (8754.78 μM TE/g).

The molecular docking studies of target proteins (BRCA1, BRCA2, COX1 and COX2) with isolated compounds were performed using Schrodinger maestro module. The compound 1-(2-hydroxyphenyl)-4-methylpentan-1-one was found to be firmly bounded with active pockets of BRCA1, BRCA2, COX1 and COX2 target proteins.
with G-scores of -4.7, -4.5, -5.8 and -2.4 respectively. Moreover, the compound 2-[(3-methylbutoxy) carbonyl] benzoic acid was found to have significant G-scores for binding with the active sites of the target proteins such as BRCA1 (-3.86), BRCA2 (-3.1), COX1 (-4.4) and COX2 (-2.9). Besides, 4-methylpentyl benzoate showed specific binding affinity towards BRCA1 and BRCA2 with G-score of -3.83 and -3.8 respectively. These docking methods have been intentionally developed in this study to find out the inhibitory activities of the isolated compounds against oncoproteins and inflammatory target proteins. Out of 6 compounds, three were found to be docked more firmly and highly specific to the active pockets of target proteins recommending them as promising inhibitors for the development of new drugs.

Present study highlights the remarkable antioxidant and pharmacological properties of three Rubus species, among which the versatile plant, *R. fairholmianus* stood as the best source of various types of compounds with diverse chemical structure. Moreover, this study has also demonstrated for the first time that, *R. fairholmianus* possess excellent pharmacological effects. Medicinal applications of the most active compounds isolated from the root acetone extract of this plant should be evaluated to explore the therapeutic efficacy against various diseases. With the changing global scenario towards the use of non-toxic herbal products, there is an urgent need for the development of much safer drugs from *R. fairholmianus* for the control of cancer and inflammation related diseases. This study should also encourage the scientific search for more medicinal properties from these species so as to combat the ever increasing demand of medicine for the mankind.