CHAPTER 7

SUMMARY AND CONCLUSION

Plants have provided enormous medicinal contributions for human well-being and have been the insight for drug development. Drugs from natural sources have always proved better than the synthetic drugs. This will help to develop an effective alternate drug from natural source in place of existing chemical based drugs. Inflammatory Bowel Disease (IBD) circumscribed with ulcerative colitis (UC) and inflammation in chronic part (Chrons diseases). The inflammation starts in the inner lining or mucosa which leads to ulceration. The etiology for UC is not clearly mentioned by researchers, but genetic factors, environmental factors and free radicals are the major reasons for UC. The available medication for the UC is less and surgery disturbs the other regions. The severity of the UC leads to the colon cancer. The medication available for the cancer have adverse side effects. The recent understanding is that, the best way to overcome the problem of UC and cancer is to treat with natural products mainly using plants having more antioxidant activities. Plants contain various natural compounds which can fight against wide varieties of diseases without any side effects and provide complete cure. Medicinal plants have always been an undeniable source for treatment and prevention of several diseases since time immemorial. The medicinal attributes of plants are being explored since the civilization of mankind.

From the above study, it is apparent that the Averrhoa bilimbi L. fruit extract is a rich source of phytochemicals with biological activity.
Traditional healers in different regions in India mainly Kerala Thodu hill region tribes have routinely used *Averrhoa bilimbi* L. to treat several disorders and they used this as their medicine in the form of juice or paste. Among the 151 compounds identified in GC–MS, 15 compounds are found to have diverse biological activity. We also observed that the *Averrhoa bilimbi* L. fruit extract has high level of total phenolic compounds at a concentration of 209.25 GAE mg/g. Presence of phenolic compound apparently explains the antioxidant activity of the plant. Antioxidant activity of *Averrhoa bilimbi* L. fruit extract is proven from its high level of NO scavenging activity with an IC50 value of 108.10 µg. The pre-administration of *Averrhoa bilimbi* L. fruit extract has reduced the inflammation in acetic acid induced UC in rats. The mechanism involved in this might be the antioxidant activity by free radical scavenging action as well as anti-inflammatory activity by inhibiting thei-NOS and COX2. The cytokines TNF-α, IL6, and IL1b levels also were found to be reduced by the fruit extract. The effects were even better than that of the standard drug sulfasalazine. The *Averrhoa bilimbi* L. fruit extract showed promising anti-lymphoma activity in Swiss albino mice. Treatment of *Averrhoa bilimbi* L. normalized the hematological parameters significantly (**P ≤ 0.01) by decreasing the high level of white blood cell and by increasing the levels of red blood cells and hemoglobin content when compared with DAL control mice. The treated mice group with *Averrhoa bilimbi* L. fruit extract resulted in significant (P ≤ 0.01) decrease in body weight when compared with the control. The MTT assay also has shown a significant growth inhibition percent (97.96% ± 0.02) for the fruit extract treatment on DAL cell lines.

The phytochemicals which we have reported in *Averrhoa bilimbi* L. having antioxidant role and chemopreventive role in cancer is squalene which act through effects on signal transduction in cell proliferation. The *in vitro* studies on COLO-205 cells by MTT and tryphan blue assays have shown a
significant cytotoxic effect. Further more the apoptosis induction and migration inhibition were observed on COLO-205 cells by the fruit extract. Thus *Averrhoa bilimbi* L. could be an efficient drug from natural source for ulcerative colitis, lymphoma and colon cancer therapy. It is clear that the compounds present in *Averrhoa bilimbi* L. methanolic extract are significant and can be isolated for further study at molecular level in the treatment of Ulcerative Colitis and colon cancer.

The outcome of this research work clearly explains that the *Averrhoa bilimbi* L. fruit extract contain active compounds with antioxidant potential which helps in the Anti-ulcerative colitis and Anticancer properties. The GC-MS analysis for identification of major bioactive compounds by us is the first reported study. The results directly enlighten and are helpful for the use of *Averrhoa bilimbi* L. for UC and colon cancer treatment.

**SCOPE OF THE STUDY**

The current study will bestow wide perspective of the *Averrhoa bilimbi* L. fruit in terms of its medicinal effects against the widespread ulcerative colitis and colon cancer. Although tribal people and people from few areas of Kerala use this fruit for many medical effects against the wide spread diseases, UC and colon cancer. By this study we wanted to probe the *Averrhoa bilimbi* L. fruit for its pharmacologic importance for the human benefits. This study will reveal the importance of the *Averrhoa bilimbi* L. fruit against ulcerative colitis, lymphoma and colon cancer through our study both at *in vivo* and *in vitro* level. The current study results and outcomes would encourage further the future researchers to work on the notable phytochemical compounds present in the *Averrhoa bilimbi* L. fruit extract for the therapeutic uses and drug development.
Flowchart of the (outlines) working chapters

*Averrhoa bilimbi* L.

The *Averrhoa bilimbi* L. fruit extract showed presence of Phenols, Alkaloids, Flavonoids, Saponins, glycosides. Hexadecanoic acid, Squalene, Erucic acid, Oleic acid, Chimaneine D, Boronic acid, Mannitol, Desulphosinigrin

IL-1β, IL-6, TNF-α, iNOS and COX-2 attained the normal values by the *Averrhoa bilimbi* L. fruit extract

The Dalton Ascitic Lymphoma (DAL) inhibited by the *Averrhoa bilimbi* L. fruit extract (MTT and Trypan blue assay)

The Colon cancer cells COLO-205 inhibited by the *Averrhoa bilimbi* L. fruit (MTT and Trypan blue assay)

The *Averrhoa bilimbi* L. fruit extract showed promising Antioxidant activity in both *in vitro* and *in vivo*

The *Averrhoa bilimbi* L. fruit extract showed normal weight of the colon by the *Averrhoa bilimbi* L. fruit extract

The haematological parameters were attained normal values due to the *Averrhoa bilimbi* L. fruit.

The *Averrhoa bilimbi* L. fragmented the DNA and restricted the moment of the COLO-205 cells which showed anti-metastasis activity

The *Averrhoa bilimbi* L. fruit extract showed promising Antioxidant activity in both *in vitro* and *in vivo*
**Possible Mechanism**

Averrhoa bilimbi L.

Induction of apoptosis via activation of caspases-3 mediated proapoptotic signaling

and inducing cell cycle arrest at sub-G0/G1 phase

Possible mechanism of action

Averrhoa bilimbi L.

Stimulate the IL-2

IL-2 stimulates NK cell & T cell proliferation

IFN-γ exerts direct antitumor activity

Triggers TH1 cytokine producing T-cells

Inhibits Angiogenesis

Protective effect against metastasis and angiogenesis