CHAPTER 6

SUMMARY AND CONCLUSION
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6.1 SUMMARY

Medicinal plants are the natural store house of phytochemicals & being used to cure different health associated problems in many countries of the world since the prehistoric time. Secondary metabolites synthesized in the plants against defensive mechanism are having valuable therapeutic potential. Hence, these bio-compounds are used in the production and manufacturing of herbal drugs. In recent years most of the synthetic drugs have raised complicated health issues because of their side effect and affirment of multiple resistivities by pathogenic microorganisms. In addition the antibiotics are sometime associated with causing adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. For this reason plant based medicines are highly accepting for health issues across the world.

*Ficus krishnae* belongs to the family Moraceae is used as folklore medicine and commonly known as Makkhann Katori in Hindi and Krishna fig or Krishna’s butter cup in English. It is mainly found in India, tropical Africa and Sri Lanka. The plant has 10 m in height, fast growing tree it spreads branches with aerial roots. The unique character of the tree is that the leaf has the pocket like fold at the base, all the species belongs to this family are medicinally important. Researchers have revealed that the *Ficus krishnae* contains very good amount of phytocompounds that are highly potential to bring pharmacological actions.

In the present study the stem bark of *Ficus krishnae* was selected and the material was collected from Dev Dev Vana Botanical garden, Bidar, Karnataka, INDIA. The bark was allowed to shade dry for three to four weeks. After drying, it was grinded finely and the powder was successively extracted by soxhlet extraction
with solvents of increasing polarity i.e, petroleum ether, chloroform, methanol and aqueous.

The obtained extracts are qualitatively tested for the presence of various primary and secondary metabolites and the result reveals that the methanol extract contains all the secondary metabolites comparatively with other extracts. The antimicrobial activity of all four crude extracts of Ficus krishnae stem bark was evaluated by agar well diffusion method against Enterobacter aerogenes (MTCC 111) Escherichia coli (clinical strain), Shigella dysenteria (clinical isolate), Salmonella typhimurium (MTCC 98). Staphalococcus aureus (ATCC 29122) Enterococcus faecalis (ATCC 29212) and Aspergillus niger (MTCC 282). The result shows that the methanol extract has the potential activity against all pathogens.

Various extracts of Ficus krishnae stem bark was screened for the radical scavenging activities by DPPH, ABTS, reducing power and phosphomolybdenum assay. Among all the four extracts methanol extract has exhibited high efficacy of antioxidant properties.

All the four extracts of Ficus krishnae are further taken for in-vitro anti-inflammatory activity by protein denaturation method. Again the methanol extract has shown promising effect against inflammation, this supports that the methanol extract is having anti-inflammatory properties followed by other extracts.

Considering the highest efficacy of methanol extract this was further used for isolation of pure bioactive compound by preparing the column filled with silica gel of 120-200 mesh size. The column chromatography experiment was repeated to get the pure compound which has been detected by performing the TLC of the fractions.
spectroscopy (GC-MS), FTIR, $^{13}$C and $^1$H NMR studies. Based on the data the compound was identified as Phytol (C$_{20}$H$_{40}$O) having the molecular weight of 296.6.

The isolated compound phytol was subjected for the pharmacological activities like antibacterial, \textit{in-vitro} and \textit{in-vivo} antioxidant activity, in-vitro and in vivo anti-inflammatory activity and anti-ovarian cancer activity by MTT assay, apoptosis studies, cell cycle and gene expression studies.

The in vitro antibacterial activity of phytol by agar well diffusion assay result reveals that the phytol is significantly inhibited the \textit{Shigella dysenteria (clinical isolate)} growth followed by \textit{Enterococcus faecalis (ATCC 29212), Escherichia coli (MTCC 45) and Staphalococcus aureus (ATCC 29122)}.

The phytol was screened for different reactive oxygen species (ROS) scavenging activities including DPPH, ABTS, reducing power assay and phosphomolebdenum assay, it was showing a potential antioxidant activity. Again it is used for \textit{in vivo} antioxidant activity study in CCl$_4$ induced oxidative stress in albino rats. Phytol is exhibited intoxicating effect in the CCl$_4$ induced stress rats by increasing the levels of SOD, CAT and GSH enzyme in liver, whereas MDA level was significantly decreased in a normal control rats when compared with standard silymarin treated rats. The phytol at the dose level of 100 mg/kg body weight was found to be more effective then low dose 50mg/kg b.w. phytol treatment to stress induced animals.

The histopathological studies of transverse section of liver in control group exhibits a normal texture of cells and vessels. Whereas the T.S of liver of rats treated with CCl$_4$ induced stress in rats has shown morphologically irregular hepatocytes with more haemorrhage and necrosis. However the T.S of liver of rats received CCl$_4$
treated with phytol at the dose of 50 and 100 mg/kg b.w. has showed normal hepatocytes and vessels with healthy hepatocord, less or no haemorrhage and distinct hepatocytes as seen in normal rat liver compared to the standard drug sylimarin treatment. This observation reveals that the phytol has reversed the structure and function of CCl₄ damaged cells significantly by acting as hepatoprotective agent.

In vitro anti-inflammatory activity of phytol has shown significant inhibition and IC₅₀ value is 98µg/mL when compared with standard diclofenac sodium. In-vivo anti-inflammatory activity of phytol also exhibited the moderate activity when compared with standard.

In the present study the cytotoxicity activity of phytol at different concentration ranging from 10 to 320 µg/mL for ovarian cancer cell line was evaluated. The phytol has decreased the cell viability in a dose dependent manner; this clearly demonstrates that the phytol has significant role in inhibiting the ovarian cancer cell growth.

The apoptosis activity of phytol by using flow cytometry at 160 and 320µg/mL concentration on ovarian cancer cell lines was tested. The results exhibited that the phytol at 320µg/mL has showed the potential property to induce the apoptosis in ovarian cancer cells when compared to standard cisplatine. Whereas phytol at 160 µg/mL has shown the moderate effect on ovarian cancer cells.

Cell cycle study of ovarian cancer cells are evaluated with phytol at 160 and 320 µg/mL concentration. The result reveals that the phytol at 320 µg/mL concentration has shown moderate effect in expressing G₂M phase and SubG₀ phase of SKOV-3 cells. Whereas phytol at 160µg/mL has exhibited the mild effect in SKOV-3 cells, when compared with standard cisplatine. Mean while the phytol initiated the apoptosis in the SKOV-3 cells.
The gene expression study for CASPASE 3, CASPASE 8, BRCA1 and BRCA2 of SKOV-3 cells, indicated that there is a significant observation made with expression of CASPASE 3 which induces the apoptosis in the ovarian cancer cells, where as CASPASE 8 has less expression at 32µg/mL of phytol. The same trend is followed at 160µg/mL concentration of phytol. Whole expression the GAPDH was used as housekeeping gene.

6.2 CONCLUSION

_Ficus krishnae_ (Moraceae, commonly called as ‘krishna’s butter cup’ is used extensively in Indian folk medicine for the treatment of various ailments. Preliminary phytochemical screening of petroleum ether, chloroform, methanol and aqueous extract _Ficus krishnae_ indicated that the methanol extract contains all types of secondary metabolites. The methanol extract exhibited high pharmacological activities like antimicrobial activity, _in-vitro_ antioxidant and _in-vitro_ anti-inflammatory activity. Based on the potential activity shown by methanol extract, it was subjected for analytical and physico-chemical techniques for isolation, purification and characterization of bioactive compound. The isolated compound found to be phytol a terpene it has demonstrated a great antibacterial, _in-vitro_ and _invivo_ antioxidant, _in-vitro_ and _in-vivo_ anti-inflammatory and anticancer activities in ovarian cancer cells. In anticancer investigation it has induced apoptosis, cell cycle and gene expression studies in SKOV-3 cells treated with phytol has shown the remarkable activity by inhibiting cell cycle and over expression of apoptotic genes compared with standards. Consequently, it is noticed that whole screening and exploratory strategies methods carried out in present research work has provided the detailed profile of pharmacological property of _Ficus krishnae_ and the separated compound phytol has acted as anticancer agent on ovarian cancer cells.
Thus it can be concluded that the bioactive compound phytol isolated from stem bark of *Ficus krishnae* has shown a significant effect in inducing apoptosis in cancer cells. Hence this can be used as a drug to treat ovarian cancer.