CHAPTER 7

SUMMARY, CONCLUSION AND RECOMMENDATIONS
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Summary

Leaves, stem bark and fruit peels of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) were collected from the local gardens around Devanahalli, Bangalore, (Karnataka). Coarse powder of Leaves, stem bark and fruit peels *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) was extracted with ethanol, acetone and water solvents.

The *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) subjected to Preliminary qualitative phytochemical investigations and was found to possess alkaloids, carbohydrates, Steroids, Saponins, flavonoids, glycosides, phytosterols, proteins and tannins.

The Ethanol, acetone and water extracts of each of the leaves, stem bark and fruit peels of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) shows significant zone of inhibition against bacteria and fungi.

The ethanol, acetone and water extracts of *Citrus maxima*, *Citrus aurantium* demonstrated the dose dependent antioxidant activity. Ethanol, acetone and water extracts of each of the leaves, stem bark and fruit peels of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) was found to possess superoxide anion radical, DPPH, hydroxyl radical and nitric oxide radical scavenging activities. Therefore, for further studies is ethanolic, acetone and
water extracts of each of the leaves, stem bark and fruit peels of *Citrus maxima* (*Pomelo*) and *Citrus aurantium* (*Bitter orange*) selected for anticancer, antimicrobial, anti-inflammatory, analgesic activities.

The phenolic and flavonoid content analysis showed that, fruit peel contains the maximum amount and between *Citrus maxima* and *Citrus aurantium*, *Citrus aurantium* contains the greater amount of phenolics and flavonoids.

In this study the ethanolic, acetone and water extracts of each of the leaves, stem bark and fruit peels of *Citrus maxima* (*Pomelo*) and *Citrus aurantium* (*Bitter orange*) is evaluated for its Analgesic activity of acetic acid induced writhing in mice, tail flick method in rats and hot plate method in mice.

Anti-inflammatory activity evaluated was acute and chronic models of inflammation. The acute inflammatory property the formalin induced paw oedema in rats was employed and for acute anti-inflammatory activity, formalin-induced paw oedema in rats was used.

The anti cancer activity of LF-ETH, LF-ACET, LF-WATE, BRK-ETH, BRK-ACET, BRK- WATE, FP-ETH, FP-ACET and FP- WATE extracts of *Citrus maxima*, *Citrus aurantium* have been investigated in the present study in experimentally HeLa cell line against by trypan blue dye exclusion method, Ehrlich ascites carcinoma (EAC) inoculated tumor-bearing mice.

The anticancer property of selected plant extracts were analysed using HeLa cell line. The cells were grown in 96 well plates. Among the
extracts ethanol fractions of Citrus aurantium leaf and fruit peel have shown higher percentage of anticancer properties (higher % of dead cells such as 96.5% and 74.5% respectively). Whereas, ethanol fraction of Citrus maxima leaf has shown high percentage of anticancer property as 69.1% of dead cells. At the same time, ethanol fractions of Citrus maxima and Citrus aurantium bark have shown less anticancer property as 15.3% and 23.7% of dead cells respectively. Thus it can be said that, the supplements of leaf, and fruit peel of Citrus aurantium and leaf of Citrus maxima plants are useful as anticancer agents. This study may lead to the formulation of an anticancer drug.

Healthy adult Swiss albino mice weighing 20-25g was obtained from the animal facilities of the Rural College of Pharmacy used for the study. Tumor was induced by Ehrlich ascites carcinoma (EAC) cell lines in mice which was aspirated and reinoculated in fresh mice. About 1x10^6 cells injected intraperitoneally into a new healthy mouse for the present study.

EAC re-inoculated mice were divided into 12 groups consisting of 12 animals in each group and one more group without EAC inoculation served as normal saline control. Group 2-12 were treated with solvent & drug samples from the 6 hrs after the inoculation of EAC for 9 days at 24 hours interval and the observation was continued until the termination of the study.
The antitumor activity was assessed using survival time, body weight analysis, peritoneal cell count, ascites fluid volume, hematological studies. All the three drugs showed the anti cancer activity in EAC bearing animals and demonstrated a significant reduction in body weight, tumor volume, packed cell volume and percentage increase in life span (%ILS), with CM-LF-ETH, CM-BRK-ETH, CM-BRK-WATE, CM-FP-ETH, CM-FP-WATE and CA-LF-ETH, CM-LF-WATE, CA-BRK-ETH, CM-BRK-WATE, CA-FP-ETH, CA-FP-WATE 300 mg/kg b.w. p.o., dose showing maximum activity.

In the EAC control group, the median survival time was 14 days and which increased significantly to 18 days with CM-LF-ETH mg/kg b.w. p.o., to 22 days with CM-FP-ETH, CM-LF-WATE, CM-BRK-ETH, CA-BRK-ETH, CM-FP-WATE and CA-FP-ETH 300 mg/kg b.w. p.o., which showed maximum increase in the life when compared with different doses of Citrus maxima, Citrus aurantium. CM-LF-ACET, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ACET and CM-FP- WATE, CA-LF-ACET, CM-LF-WATE, CA-BRK-ACET, CM-BRK-WATE and CA-FP-ACET showed least effect in increasing the life span among the doses of three drugs on comparison.

The percentage decrease in the body weight after treatment with Citrus maxima, Citrus aurantium for 14 days was found to be 45.81%, 52.88%, 48.32%, 56.23%, 61.11%, 54.18%, 53.63%, and 79.33% in EAC induced mice treated with CM-LF-ETH, CM-BRK-ETH, CM-BRK-WATE, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH, CA-FP-ETH 300 mg/kg b.w. p.o., and Cyclophosphamide 25 mg/kg respectively when
compared with vehicle treated cancerous animals. Treatment with CM-LF-ETH, CM-BRK-ETH, CM-BRK-WATE, CM-FP-ETH, CM-FP-WATE, CA-LF-ETH, CM-LF-WATE, CA-BRK-ETH, CM-BRK-WATE, CA-FP-ETH, CA-FP-WATE 300 mg/kg b.w. p.o., significantly decreased the tumor volume and packed cell volume when compared to that of EAC control group.

The effect of different doses of LF-ETH, LF-ACET, LF-WATE, BRK-ETH, BRK-ACET, BRK-WATE, FP-ETH, FP-ACET and FP-WATE extracts of *Citrus maxima*, *Citrus aurantium* on hematological parameters against EAC induced animals estimated on 14th day of treatment. CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH 300 mg/kg b.w. p.o., showed better improvement in the hematological parameters than the rest of the doses among the compared groups.

The total WBC count found significantly increased in the EAC control group. All the eighteen drugs when administered to the EAC bearing mice showed the significant decrease in the WBC count when compared with the EAC control group, with CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH 300 mg/kg b.w. p.o., showing the significant decrease in the WBC when compared to the EAC control group. RBC count and Hb content in the EAC groups were significantly decreased as compared to the normal group. Treatment with CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH 300 mg/kg b.w. p.o., significantly increased the RBC and Hb content when compared with
the EAC control. All the eighteen drugs have showed the significant increase but CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH 300 mg/kg b.w. p.o., showed the better activity compared to rest of the drugs and doses.

Overall the Leaves, stem bark and fruit peels of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) are having *in vitro* antioxidant, anti microbial, anti inflammatory, analgesic and anti-cancerous properties against HeLa cell line by trypan blue dye exclusion method and also against Ehrlich ascites carcinoma (EAC) inoculated tumor-bearing mice.

**Conclusion**

In the present study indicate that ethanolic, acetone and water extracts of each of the leaves, stem bark and fruit peels of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) demonstrated the anti-cancer activity in HeLa cell line against by trypan blue dye exclusion method. Ethanol fraction of *Citrus maxima* leaf has 300 mg/kg b.w. p.o., shown high percentage of anticancer property as 69.1% of dead cells. *Citrus aurantium* leaf and fruit peel have 300 mg/kg b.w. p.o., shown higher percentage of anticancer properties. (Higher % of dead cells such as 96.5% and 74.5% respectively).

The effect of ethanol, acetone and water extracts of each of the leaves, stem bark and fruit peels extracts of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) demonstrated the anti-cancer activity in EAC cell inoculated swiss albino mice. With CM-LF-ETH,
CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH at the dose of 300 mg/kg b.w. p.o. has shown significant prolongation of lifespan, reduction in tumor volume, improvement in the hematological parameters when compared to the rest of groups. Thereby it can be concluded that CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH at the dose of 300 mg/kg b.w. p.o. possesses better anticancer activity than CM-LF-ACET, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ACET and CM-FP-WATE, CA-LF-ACET, CM-LF-WATE, CA-BRK-ACET, CM-BRK-WATE and CA-FP-ACET and rest of the doses.

The acute toxicity study indicated that, the ethanolic, acetone and water extracts of each of the leaves, stem bark and fruit peels extracts of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) was devoid of major toxic effects.

The LF-ETH, LF-ACET, LF-WATE, BRK-ETH, BRK-ACET, BRK-WATE, FP-ETH, FP-ACET and FP-WATE extracts of *Citrus maxima*, *Citrus aurantium* showed dose dependent superoxide anion radical, DPPH, hydroxyl radical and nitric oxide radical scavenging activities.

The effect of ethanolic, acetone and water extracts of each of the leaves, stem bark and fruit peels extracts of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) exhibited significantly has 300 mg/kg b.w. p.o., showed analgesic, anti-inflammatory activity.
Overall observed significant activity may be due to presence of bioactive constituents present in ethanolic, acetone and water extracts of each of the leaves, stem bark and fruit peels extracts of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange).

**Recommendations**

These observations concluded that, the ethanolic, acetone and water extracts of each of the leaves, stem bark and fruit peels extracts of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) posses’ anti-cancer, analgesic, anti-inflammatory antimicrobial and *In-vitro* antioxidant activity. More detailed toxicity study can also be conducted to determine the safety of the herbal formulation. Further, the work could be extended to evaluate the effectiveness of the marker compounds for the treatment of anti-cancer at its cellular level to elucidate its exact mechanism for the traditional claim.

The probable bioactive components such as flavones like hesperidin and acridone alkaloids having anticancer effect, Flavones like nobelitin and acridone alkaloids having antiinflammatory and antioxidant effect, flavonoids having antimicrobial effect. Thus, these components of leaf, stem bark and fruit peel of *Citrus maxima* (Pomelo) and *Citrus aurantium* (Bitter orange) can be used to formulate the most potent, inexpensive and health promoting herbal based drugs in pharmaceutical industry.