CHAPTER V

SUMMARY AND CONCLUSION

It has been estimated that 80% of the populations of developing countries rely on traditional medicines i.e., mostly plant drugs. The increased popularity of herbal remedies for cancer therapy perhaps can be attributed to the belief that herbal drugs provide benefit over that of allopathic medicines while being less toxic.

The study is to discover nano-formulated fig fruit based nanomedicine towards cancer therapeutics. Though many researchers have worked on herbal remedies, very few researchers have reported about the therapeutic efficiency of fig fruits. Nanoformulation is the main criteria for developing fruit based drug. Nanomedicine is simply the practice of nanotechnologies in a healthcare setting and the vast benefits that have been noticed include the use of nanoparticles to better the behaviour of drug substances. This is a novel nanomedical approach to use fig fruit based bioactive agents for analyzing its effect on different cancer cell lines through in vitro and in silico methods.

Many researchers have commented nanoformulation procedures in their work. The Ficus benghalensis fruit extracts have been studied extensively by very few researchers. They have not discussed on their phytochemistry, antimicrobial, anticancer, nanoformulation, bioconjugation and anticancer works. In the present work, an exclusive study on the pharmacological and phytochemical analysis on these fruits were carried out. The metal oxide nanoparticles were synthesized, characterized and bioconjugated with the partially purified compounds from fig. The molecular docking studies were used to dock the cancerous protein with partially purified ligand molecules from fig fruits.

This research work begins with phytochemical, microbiological and pharmacological analysis of Ficus benghalensis fruit extracts. The fruits were collected from in and around Noorul Islam University campus and authenticated. Preliminary screening of extracts was done by phytochemical, microbiological and pharmacological activity. The Ficus benghalensis fruit extracts were qualitatively and
quantitatively analyzed using standard methods. The identified phytochemical constituents such as terpenoids and phenol were effectively present in all the three solvents of fruit extracts. Carbohydrate, reducing sugars, saponins, glycosides and anthroquinones were completely absent in all the three solvents. Flavonoids, alkaloids, tannins, phlobatannins and chloride were present in aqueous and methanolic extracts. Vitamin C and proteins were present in methanolic extracts. Steroids were present only in chloroform extracts. Amino acids were present in aqueous extracts. The result reveals that the methanolic extract of Ficus benghalensis fruits possessed maximum phytochemical constituents. The study also provide a strong evidence for the use of Ficus benghalensis fruit extract to treat various pharmacological activities. The activity may be due to the presence of one or more phytochemical constituents in the extract.

The fruit extracts of Ficus benghalensis were tested for antimicrobial activity against the following bacterial pathogens: Bacillus cereus, Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae. The result revealed that Ficus benghalensis methanolic fruits extracts showed better activity than chloroform and water. The water extract had no antibacterial activity against any of the bacterial pathogens tested. Thus it confirmed that water extract of Ficus benghalensis fruit possessed less or no antibacterial activity.

Based on the phytochemical screening, all the three extracts were subjected to in-vitro antioxidant activity to find out the free radical scavenging potential of individual extract. The in-vitro antioxidant activity was performed by DPPH and Nitric acid radical scavenging method. Comparatively Ficus benghalensis methanolic fruit extract revealed better antioxidant activity in both DPPH and Nitric acid radical scavenging methods.

Results obtained from GC-MS studies confirmed the presence of eleven compounds in methanolic extracts. The flavonoid compounds were isolated and confirmed by TLC techniques. The resulting flavonoid compounds were partially purified using TLC separation and eluted so that the resulting compounds were comparatively studied by GC-MS studies. The result revealed that the obtained compound is eicosane. Eicosane is an alkane with the chemical formula C\textsubscript{20}H\textsubscript{42}.
The iron and zinc oxide nanoparticles were synthesized using both chemical and green methods. Comparatively iron oxide nanoparticles were active, stable and well dispersed than zinc oxide nanoparticles. Iron oxide nano particles were characterized by UV, FTIR, TG/DTA, Raman, FESEM and XRD. The results reveal that the obtained iron oxide nanoparticles as Fe₃O₄ in chemical synthesis and mixture of α-Fe₂O₃ and Fe₃O₄ in green synthesis.

Partially purified eicosane was bioconjugated into iron oxide nanoparticles to enhance adsorption on the carrier surface. The UV results confirmed the presence of bioconjugated molecules and the drug loading efficiency was calculated. With 71.37% of drug loading efficiency the nanoparticles proved to be a better bioconjugated product for further studies.

*In silico* approach proved that, Bcl-2 receptor protein molecule exactly docked with the ligand (eicosane) molecule. The docking score generated by the GOLD commercial software was 49.3. The Bcl-2 gene has been identified as a cause of a number of cancers, including melanoma, breast, prostate, chronic lymphocytic leukemia, and lung cancer, and a possible cause of schizophrenia and autoimmunity.

The *in vitro* anticancer activity was performed against skin cancer, lung cancer, leukemia cancer and breast cancer cell lines with the bioconjugated fig fruit drugs. The result confirms that anticancer activity was high in all cell lines except breast cancer. The comparative results of different cytotoxicity studies in different cancer cell lines confirm the presence of a strong herbal drug combination in the fruit extracts to control the proliferating metastatic cancers.

From this study we conclude that it is possible to develop a fig fruit based anticancer drug and formulate it with iron oxide nano particle to biofunctionalize the compound in to a nanocarrier for effective drug delivery. A novel cancer therapeutic methodology for nanomedicine is introduced and experimentally proved by *in silico* and *in vitro* studies. The drug obtained from this *Ficus benghalensis* fruits is an effective cytotoxic agent for different cell lines. This work will hence pave way for the production and usage of drugs from *Ficus benghalensis* fruits and help the growth of commercially important pharmaceutical drugs.
Future prospects

In present research work we have introduced a novel nano-formulated nanomedicine derived from *Ficus benghalensis* fruits (fig fruits) for cancer therapeutics. The present thesis reported that methanolic extract of *Ficus benghalensis* fruits show better anticancer activity. The same extract could be utilized for the isolation of further bioactive metabolites. The derived eicosane molecule was partially purified and the cytotoxicity results show better results comparable to standard anticancer drugs. We can also extend our work to ultra-high purification of anticancer compounds from *Ficus benghalensis* fruits. So the followings proposed works to be done in future for better results.

- Ultra-high purification of anticancer compounds.
- Further study of crystals obtained from *Ficus benghalensis* methanolic fruits extract.
- Molecular docking study of all possible compounds obtained from *Ficus benghalensis* methanolic fruit extracts.
- *In vivo* studies to analyze the chemotherapeutic effects of compounds isolated.
- Localization of the drugs in the tissues and cells could be imaged for the Pharmacokinetic analysis of the formulated compound.