CHAPTER 6

SUMMARY AND CONCLUSIONS
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Plants and their parts are used as crude drugs in many medicinal preparations from centuries. Proper identification of drugs and their evaluation on a scientific basis is therefore of prime significance. The present investigation is on the two ethno-pharmacologically important plants *Catunaregum spinosa* Thunb and *Pavonia zeylanica* Cav. for antioxidant and anticancer activity has lead us to explore these plants thoroughly to get a novel lead molecule for various diseases.

However, despite these many important past contributions from the plant kingdom, a great many plant species have never been described and remain unknown to science, and relatively few have been surveyed systematically to any extent for biologically active chemical constituents. Thus, it is reasonable to expect that new plant sources of valuable and pharmaceutical interesting materials remain to be discovered and developed. Regrettably, if the current trends of destruction of tropical forests and general biotic simplification continue at their present rates, scientists interested and involved in medicinal plant research may have only a few decades remaining in which to investigate much of the rich diversity of the plant kingdom for useful new bioactive compounds, and many opportunities for successful drug development will almost certainly be lost. It is therefore imperative that endangered, fragile, and over-exploited genetic resources be preserved to the greatest extent possible for future generations which may have at their disposal the tools (both technical and intellectual) necessary to successfully exploit and manage these species more intelligently.

In our investigation, an attempt has been made to outline the most important aspects of the empirical approach to find new lead compounds from plants. The two ethnopharmacologically important plants *Catunaregum spinosa* Thunb. and *Pavonia*
zeylanica Cav. have been studied for their physicochemical and phytochemical analysis, antioxidant potentiality and *in-vitro* anticancer activity.

- The extracts of *C. spinosa* and *P. Zeylanica* was analysed for Physicochemical values and Fluorescence characters of the plant powder under ordinary light and UV light (UV 366 nm) which gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values were also determined which determined whether it’s an exhausted or adulterated drug. The water soluble and alcohol soluble extractive values, the fluorescence characteristics under ordinary and UV light (366nm), the preliminary phyto–profiling for the leaves extracts of *C. spinosa* and *P. Zeylanica* was carried out. The percentage yield w/w of the extracts was also analyzed wherein the highest yield was found to be in the ethanolic extract.

- The preliminary phytochemical for the ethanolic leaf extract of *P.zeylanica* when subjected to phytochemical analysis, showed the presence of proteins in ethanolic extract alone and carbohydrates in all the extract. Ethanolic extracts showed positive tests for phenols, flavonoids, terpenoids, tannins and alkaloids. Aqueous extracts showed positive for Alkaloids and terpenoids where as in *C.spinosa* the presence of the triterpenoids was seen in all the solvent extracts except for the aqueous extract. The glycosides, carbohydrates, phenols/tannins and flavonoids were present in the ethanolic, methanolic and acetone extracts.

- The antioxidant activity by DPPH and FRAP assays showed very high IC50 value in the *C.spinosa* followed by *P.zeylanica* when compared with the standard.

- Anti-cancer activity was evaluated against *HeLa* cells, PC- 12 and MCF-7 cell lines (breast cancer cells) wherein it was found that the ethanolic leaf extract of *P.zeylanica*
showed inhibition for the 24hrs of trypan blue assay. The cells show positive Hoechst assay as the DNA has acquired the Hoechst stain and the same is seen under the fluorescence microscope. The results analysis for MTT assay shows that the cell viability decreases with increase in concentration of the drug. The inhibition concentration value (IC50) for the MTT assay at 24hrs shows to be 5.1µg/ml and for 48hrs at 5.4µg/ml. *C.spinosa* showed that cell proliferation has been arrested at very low concentrations of the plant extract. According to the results obtained the cells are not undergoing apoptosis neither necrosis but their growth is getting arrested, this indicates that there can be bioactive compound which is stopping the cells to divide in all the 3 cancer cell lines. The interesting thing is that this cell growth arrest is at very low concentrations of the plant extract. In *HeLa* cells the concentration of the plant drug added was 0.000001%, whereas for the *PC12 & MCF7* was 0.0000001% respectively. This data hence gives way to other future works that can be done for cell cycle analysis by using various other techniques like flow cytometry and identify the mechanism of action of the plant drug.

- TLC performed with the crude leaf extracts of *Pavonia zeylanica* showed the movement of different biomolecules. It was seen that chloroform extract moved to the maximum distance followed by ethyl acetate extract and Ethanolic extract moved the least distance. Whereas, the aqueous extract did not show any movement on the stationary phase.

The *C.spinosa* ethanolic leaf extract was subjected to TLC wherein the solvent used for the separation of the compounds for *C. spinosa* was in the combination of Hexane: Methanol: Acetic acid with a ratio of 8:1:1. The TLC sheet showed three spots which were analyzed under the UV light.
It is thereby apparent and promising, to state the obtained research findings of phytochemistry, antioxidant activity and anticancer activity for both the selected plant species clearly reveals that, the plants are potential and challenging enough to be worked out further. The inhibition of proliferation can be caused by various mechanisms such as the activation of cell cycle suppressors, the down-regulation of positive cell cycle regulators, or the abrogation of mitotic signaling. These have to be further explored through various cell cycle assays using standard methods.

As a result of these new technologies, it has become current fashion to guide fractionation of plant extracts towards rapid isolation and identification of the pure bioactive compounds. Since more and more approaches to automating the de-replication of these bioactive natural products are developed, it is not too presumptuous to expect that in the near future several new lead chemical entities from nature will be placed in the research and development pipelines for new drugs.

The herbal medicines took great importance in the treatment of many diseases. Since herbal medicines are mainly used by Chinese, but now gaining acceptance all over the world. Attraction over the complementary and alternative medicines is often more as they are widely available, non-prescription and “natural”. Herbal plants and their derivatives are widely used in the treatment of cancer. The treatment of cancer must include the benefits of botanical medicines. There are many classes of plant-derived cytotoxic natural products and the structural modification studies for further improvement and development of drug.

New anticancer drugs derived from research on plant will be continuously discovered. The activities of phytochemicals and the synergistic action shown by them make them ideal in alternative cancer therapies. The chemopreventive effects that most phytochemicals exert are likely to be the sum of their effect on several distinct mechanisms working inside the cell. The
phytochemicals have been focused for the research since 1930’s but many of them have been used in traditional medicines for thousands of years. Proper identification of drugs and their evaluation on a scientific basis is therefore of prime significance. This can certainly help to rejuvenate the ancient system of medicine like ayurveda.