CHAPTER 6
CONCLUSION
6. Conclusion:

Simple extraction method have been developed for the isolation of oleanolic acid from the seeds of *Achyranthes aspera* Linn. The isolated oleanolic acid was purified and the structure was confirmed by spectral analysis. Further, HPTLC method was developed and validated for the estimation of oleanolic acid. The proposed method was successfully applied for the quantification of oleanolic acid in the seeds of *Achyranthes aspera* Linn. The percentage w/w of oleanolic acid in the seeds of *A. aspera* is found to be $0.343 \pm 0.0071$. Literature survey revealed that germination of seeds of the various plants affects the content of active ingredient present. The developed method was further explored for the quantification of oleanolic acid in the germinated seeds of *A. aspera* Linn. The amount of oleanolic acid in the seeds of *A. aspera* after germination for 48 hrs is found to be $0.625 \pm 0.018 \%w/w$. The increase in the content of oleanolic acid in the seeds of *A. aspera* after germination was about 82.21%. The proposed concept of germination of seeds for the increase of the content of oleanolic acid can be used as satisfactory tool for the large scale production of oleanolic acid from *Achyranthes aspera* Linn.

*Sapindus mukorossi* is a plant with various pharmacological activities. Different parts of the plant is used for different activities. The most widely used part of the plant is the pericarp of the fruit. Reports have revealed that saponins is the major constituent present in the pericarp of *Sapindus mukorossi*. A simple extraction procedure was developed for the isolation of saponins from the fruit pericarp of *S. mukorossi*. The crude saponin isolated was purified and identified as mukorosside, a pentacyclic triterpenoid, by its melting point and TLC study. This pentacyclic triterpenoid (mukorosside) was further hydrolyzed with 8% sulphuric acid under optimized condition to yield an aglycone. This

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aglycone was identified by spectral analysis as hederagenin. HPTLC method was
developed and validated for the estimation of hederagenin. The proposed method was
further applied for the quantification of hederagenin in the dried pericarp of *Sapindus mukorossi*. The amount of hederagenin found in the dried pericarp of *S. mukorossi* is $3.4 \pm 0.09\% w/w$.

The mukorosside, a pentacyclic triterpenoid, is a mixture of seven different saponins. These saponins were confirmed as mukorossides A-G by TLC study and comparing it with the reports of Sexana et al., 2004. The solvent system chloroform: methanol: water (10:2.8:0.2v/v) was selected for the separation of mukorossides A-G. Seven different spots at Rf values 0.21, 0.35, 0.45, 0.56, 0.66 and 0.75 corresponding to Sapindosides A-G respectively were obtained. HPTLC method was developed for the estimation of total saponins in the pericarp of *S. mukorossi*. An area normalization method was adopted for the estimation of total saponins in the pericarp of *Sapindus mukorossi*. The areas of the individual mukorosside A-G were measured and the total was considered equal to the total mukorosside present in the applied reference standard. The amount of each mukorosside present in standard was calculated using the following formula.

\[
\text{Concentration of mukorosside } Y = \frac{\text{area of mukorosside } Y \times \text{amount of reference mukorosside applied}}{\text{total area of all mukorosside}}
\]

Where mukorosside $Y$ is mukorosside A, B, C, D, E, F, G.
Conclusion

This is based on the assumption that the all mukorosside (A-G) had the same molar extinction co-efficient. The total content of mukorosside in the Sapindus samples were determined by the above formula.

The amount of total saponins in the dried pericarp of Sapindus mukorossi is found to be 15.18 ± 0.34%w/w

Ursolic acid, a pentacyclic triterpene acid, is used for various pharmacological actions like hepatoprotective, antimalarial, anti-tumor etc. It is one of the constituent of the leaves of the plant Alstoma scholaris R. Br. This plant was selected for the isolation of ursolic acid. A simple procedure was developed for the isolation of ursolic acid from the dried leaves of Alstonia scholaris R. Br. The isolated ursolic acid was purified by recrystallization and subjected to spectral analysis. The spectral datas were compared with the standard values. The identity of ursolic acid was thus confirmed. HPTLC method was developed and validated for the estimation of ursolic acid. The proposed method was further applied for the quantification of ursolic acid in the dried leaves of Alstonia scholaris R. Br. The amount of ursolic acid present in the dried leaves of A. scholaris is found to be 3.10 ± 0.01%w/w.

Thus to conclude it can be stated that a simple procedure for isolation of oleanolic acid, hederagenin and ursolic acid from seeds of A. aspera, from the fruit pericarp of S. mukorossi and leaves of A. scholaris respectively was developed. HPTLC method was developed and validated for the estimation of oleanolic acid, hederagenin and ursolic acid from the seeds of A. aspera, pericarp of S. mukorossi and leaves of A. scholaris respectively. Germination of seeds of A. aspera to find out the increase in the content of oleanolic acid was selected as another aspect of study. The proposed HPTLC method was

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used to study the effect of germination of *A. aspera* seeds on the yield of oleanolic acid. The results showed an increase in the content of oleanolic acid upon germination of the seeds. For the estimation of saponins, the pericarp of *S. mukorossi* was selected. The mukorosside mixture isolated was further subjected to TLC study for the separation of mukorosside A-G. HPTLC method was developed and validated for the estimation of total saponins by an area normalization method. The amount of total saponins was determined.