

Summary and Conclusion

5.0 SUMMARY AND CONCLUSION

The results of the present study entitled “**Extraction and purification of withanolides from dry roots of *Withania somnifera***” were summarized as follows.

Secondary metabolite extraction from the roots of *Withania somnifera* was obtained sequentially using solvents petroleum ether, chloroform, ethyl acetate and ethanol in the order of increasing and decreasing polarity.

The thin layer chromatogram pattern and scanning at 254nm using double beam spectrophotometer revealed the presence of a number of secondary metabolites from dry roots of *Withania somnifera*.

Petroleum ether extract (Extract A) upon TLC analysis with the solvent system chloroform: methanol in the ratio 9:1 revealed the presence of five non polar compounds corresponding to R_f values 0.67, 0.55, 0.5, 0.4 and 0.35. Therefore petroleum ether was employed for defatting the powdered *Withania somnifera* roots prior to extraction with suitable solvent (chloroform, ethyl acetate and ethanol).

Extraction of Withanolides from *Withania somnifera* roots carried out using various solvents namely 50% ethanol, 100% ethanol and 100% chloroform upon TLC analysis revealed that sugars along with other polar compounds may have been extracted predominantly in 50% ethanol extract (Extract I) which hindered the resolution compared to 100% chloroform extracts (Extract K). And thus 50% ethanol extract (Extract I) was subjected to fractionation using chloroform (Extract L).

Chloroform fraction (Extract L) upon TLC analysis revealed the presence of spots with clear resolution and hydro alcoholic extract after fractionation (fraction M) revealed the presence of only sugars with no spots corresponding to withanolides.

The chloroform extract (Extract K) from 15g of *Withania somnifera* when subjected to purification by silica gel column chromatography followed by elution using solvents namely chloroform and ethyl acetate in the ratio 100:0,75:25,50:50 and 0:100 respectively resulted in fractions indicated that polarity of the mobile phase when increased increases the elution of more polar compounds. Fractions containing polar compounds with R_f values 0.17

obtained revealed that the polar saponins of *Withania somnifera* roots was also obtained in considerable quantity using chloroform extract (Extract K).

The chloroform fraction (extract L) obtained from 50% ethanol extract of *Withania somnifera* roots when subjected to silica gel column chromatographic analysis as mentioned in method 1 and method 2 confirmed that the compounds corresponding to the R_f values 0.1 and 0.28 can be obtained upon elution with chloroform: ethyl acetate 40:60 followed by step wise gradient increase of ethyl acetate.

In future, the fraction containing two compounds with R_f values 0.1 and 0.28 can be further subjected to silica gel column in order to obtain the single purified compound which can be then analyzed by HPLC and NMR to determine the structure of the purified compound.