Conclusion and perspectives

Mass spectrometry coupled with pharmaceutical studies can lead to the discovery of novel plant secondary metabolites with antimicrobial, antioxidant, anticancer and other pharmacological potentials. In many of the medicinal plants species used in traditional medicine systems namely In Indian Siddha, Ayurvedic and Unani have not been studied sufficiently. At this juncture, this doctoral thesis has aimed at studying the medicinal properties of *M. emarginata*, one of the medicinal plants reported in Siddha medicinal system.

The present work dealt with various features and was divided into two main fields;

1. Optimization of phenolic compound extraction from *M. emarginata* and profiling them by UPLC-MS/MS.

2. Pharmacological studies
   - Antimicrobial capability of *M. emarginata* with QDs nanoparticle for pathogen detection and reduction.
   - Diuretic effect of *M. emarginata*.
   - Quantification of polyphenolics byproduct by RP-HPLC in the urine sample of rat treated with *M. emarginata* extract to assess the diuretic potential.

All the obtained findings are briefly summarized here.

First chapter has focused on phytochemical screening and *in vitro* antioxidant activity. The *M. emarginata* aqueous extract was used for phytochemical screening and *in vitro* antioxidant activity. The aqueous extract of *M. emarginata* leaves had considerable quantity of total phenolics, flavonoids compounds and phytochemical screening study reveals the presence of alkaloids, steroids, saponin, phenolics, flavonoids, reducing sugars and terpenoids. After confirming the presence of above
mentioned phytochemicals, the *in vitro* antioxidant capability of *M. emarginata* was studied by different *in vitro* assays namely DPPH, ABTS, superoxide anion scavenging activity and inhibitory activity of lipid peroxidation. These assays indicated that the *M. emarginata* plant extract is a significant source of natural antioxidant. The plant *M. emarginata* exhibited high antioxidant and free radical scavenging activities.

Second chapter narrates the antimicrobial activity of *M. emarginata* with fluorescence nanoparticles (QDs). The absorbance spectroscopy study reveals that QDs - *M. emarginata* complex exhibited maximum absorbance spectrum compared to their individual absorption spectrum and the fluorescence spectroscopy study revealed that *M. emarginata* extract can quench the fluorescence emitted by QDs. The antimicrobial activity of *M. emarginata* + CdTe QDs complex was higher with maximum zone of inhibition against *E. coli* compared to plant extract and QDs alone. Further, the fluorescence nature of QDs could be exploited for detecting the microbes. With the perspective of using QDs as a nanocarrier, whether the QDs + *M. emarginata* complex can adhere to the cell membrane or can be taken up by the microbial cell was carried out by fluorescence microscopic study. The presence of QDs + *M. emarginata* complex inside the *E. coli* cell was confirmed by fluorescence emission under the microscopy.

In the third chapter, optimization of phenolic compound extraction and validation of RP-HPLC condition for phenolic compound identification was discussed. Microwave Assisted Extraction (MAE), Conventional Assistant Extraction (CAE) and Ultrasound-Assisted Extraction (UAE) were used to optimize phenolic compound extraction. Among these extraction methods, the combination of ultrasound conventional assisted extraction (UCAE) gave more phenolic compounds in RP-HPLC analysis. For the optimization of RP-HPLC conditions different programmes were tried, compared to isocratic program the gradient programme No. 2 gave more number of phenolic compounds elution with good separation. The detection wavelength was set
between 210 to 500 nm for phenolics detection and most of phenolics showed maximum absorbance at 280 nm.

In fourth chapter, the profiling of phenolic compounds using UPLC-MS/MS was discussed, optimization of RP-HPLC conditions discussed in the previous chapter were adopted for profiling phenolic compounds from *M. emarginata* through UPLC-MS/MS. The UPLC-MS/MS analysis was carried out both positive and negative mode. The phenolic compounds of *M. emarginata* were identified based on molecular mass and MS² fragmentation pattern and λ max. There are 26 known and 19 unknown polyphenolic compounds were identified in the *M. emarginata*. Medicinally important and well known phenolic compounds were identified in *M. emarginata* such as chlorogenic acid isomers, caffeic acid, ferulic acid, protocatechuic acid, dicaffeoquinic acids isomers, rosmeric acid, caffeine and quinic acid.

The final chapter talks about the diuretic activity of *M. emarginata*, which is one of the traditionally reported medicinal properties of *M. emarginata*. The female adult Wistar rats were used in this study; Total volume of urine excretion, total sodium, total potassium, total protein and total glucose concentration were analyzed in the rat urine samples. The total volume of urine excretion data shows *M. emarginata* aqueous extract (MEAE) and furosemide (Lasix) treated animals excreted high volume of urine compared to untreated control group. The MEAE treated rat groups urine excretion had increased in a dose dependant manner. The total protein and glucose levels in urine samples of MEAE treated animal groups were similar to untreated control group. These results clearly indicate that the plant doesn’t produce side effects such as either proteinuria or glycosuria. The *M. emarginata* secondary metabolites might act as diuretic agents. In the previous chapter IV, shows the presence of diuretic responsible compounds caffeine, chlorogenic acid and quinic acid in *M. emarginata* was identified. The chlorogenic acid and other polyphenolics byproduct hippuric acid was identified in
rat urine sample, which can act as a diuretic agent as well as it could be used as a biomarker to detect the polyphenolics induced diuretic activity.

In conclusion, this thesis comprises the first phytochemical study of the herb *M. emarginata*, which is widely used in Indian traditional medicinal system such as siddha and ayurvedha. The aqueous extract of *M. emarginata* leaves possess phytochemicals which can act as good antioxidants, by inhibiting various free radicals which was studied by various *in vitro* antioxidant assays. The *M. emarginata* - QDs complex shows great antibacterial activity against *E. coli*. This property can be exploited for microbial reduction and deduction by using this plant extract along with QDs as a nanocarrier. The plant phenolic compounds profiling study shows the presence of 26 known and 19 unknown phenolic compounds in *M. emarginata*. These compounds might be responsible for traditional medicinal uses. Diuretic is one of the traditional uses of *M. emarginata*. The diuretic effect of *M. emarginata* was studied and exhibited no side effects such as proteinuria and glycosuria. The polyphenolics byproduct hippuric acid was successfully analyzed by RP-HPLC, it can be used as polyphenolics induced diuretics maker. Although these results are of relevance, further study on purifying the active principles and validating their medicinal properties would exert beneficial effect in humans.