6. Summary and Conclusions

Phytochemical markers are pivotal in the current practice of quality control since they are used as indicator at various stages of development and manufacturing of herbal medicine, such as authentication and differentiation of species, collecting, harvesting, quality, stability assessment and discovery of lead compounds. Lack of chemical markers remains a major problem in the quality control of herbal medicines.

Chromatographic fingerprint can successfully demonstrate both similarities and differences between various samples. The authentication and identification of herbal medicines can be accurately obtained even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples of herbal medicine. Chemical fingerprints obtained by chromatographic techniques such as HPLC-UV (DAD), HPLC-ELSD, HPLC-MS, GC-MS, HPTLC-densitometry are strongly recommended for the purpose of quality control of complex herbal medicines.

Recent approaches of applying hyphenated chromatography and spectroscopy such as High-Performance liquid chromatography- diode array detection (HPLC-DAD), capillary electrophoresis-diode array detection (CE-DAD), Liquid chromatography-mass spectroscopy (LC-MS) and HPLC-NMR could provide the additional spectral information, which will be very helpful for the qualitative analysis and even for the on-line structural elucidation. A chemical fingerprint obtained by hyphenated chromatography, can become the primary tool for quality control of herbal medicines

The objectives of the present study was to

- Isolate phytoconstituents from the *Enicostemma littorale* and *Phyllanthus emblica* using new commercially viable isolation and purification methods/ techniques.
- Method development and validation of isolated marker compound by HPLC, HPTLC and LC-MS/MS method.
- Safety and Antidiabetic study of *Enicostemma littorale* and *Phyllanthus emblica*.  

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The study involved

- Collection and authentication of plant materials.
- Standardization of extract of Amla and Mamejva
- Extraction, fractionation and phytochemical screening of plant material
- TLC and HPTLC fingerprinting
- Isolation and Purification of marker
- Characterization and structural elucidation
- HPLC/ HPTLC method development
- HPLC-LC/MS/MS analysis
- Safety studies.
- Antidiabetic activity

Detailed pharmacognostical study, physicochemical parameters and preliminary phytochemical screening of *Enicostemma littorale* (Mamejva) and *Emblica officinalis* (Amla) were carried out. *Enicostemma littorale* and *Emblica officinalis* showed presence of alkaloids, carbohydrates, flavonoids and tannins. Foreign organic matter, ash values, extractive values, and moisture content of *Enicostemma littorale* and *Phyllanthus emblica* were found to be within Pharmacopoeial limits.

HPTLC profiling of *Enicostemma littorale* was carried out. Seven spots with Rf values 0.12, 0.21, 0.33, 0.42, 0.53, 0.59 and 0.78 as indicating the occurrence of at least seven different components in the methanol extract. The component with Rf value 0.33 and component at Rf value 0.42 were found to be more predominant as the percentage area was more with 52.52% and 18.95% respectively.

HPTLC profiling of *Phyllanthus emblica* was carried out. Seven spots with Rf values 0.13, 0.17, 0.25, 0.29, 0.41, 0.50, 0.58, 0.64, and 0.73 as indicating the occurrence of at least nine different components in the freeze dried extract. Thus the developed chromatogram will be specific with selected solvent system and constant Rf values, and can serve as a better tool for standardization of *Phyllanthus emblica*. 
Summary and Conclusions

Four marker compounds were isolated from Mamejva, which were **Swertiamarin, Isoswertisin-5-O- β-D-glucoside, Quercetin and Swertisin**. Eight marker compounds were isolated from Amla, which were **Chlorogenic acid, Rutin, Cinnamic acid, Methyl gallate, Gallic acid, Ethyl gallate, Ellagic acid and Chebulagic acid**. Characterization and structural elucidation of isolated marker compounds were carried out by different spectroscopic methods: UV, IR, NMR, Mass and LC-MS/MS. HPLC method was developed and validated for isolated marker compounds from *Enicostemma littorale* and *Emblica officinalis*.

New HPLC method was developed for the quantification of phtyocconstituents in *Enicostemma littorale*. Chromatographic separation was achieved on a Kromasil -C18, (250 x 4.6 mm; 5Μ) reversed-phase column. The mobile phase consisted of Acetonitrile (A): 0.2% formic acid and pH 3.5 adjusted with ammonia at a flow rate of 1 ml/min in the gradient mode to keep short run time of 40 min. This method was validated for good accuracy, repeatability and precision, and can be used to evaluate the quality of the drug. This multi-phytoconstituents assay method will be helpful in quality control and stability studies of *Enicostemma littorale*.

New HPLC method was developed for the quantification of phtyocconstituents in *Phyllanthus*. The chromatographic separation was achieved on a Thermo Scientific BDS HYPERSIL Phenyl reversed-phase column (250mm×4.6mm, 5µm) The mobile phase consisted of 0.1% ortho-phosphoric acid: Methanol (95:05v/v) (A) and acetonitrile (B) at a flow rate of 1.5 ml/min (A) and acetonitrile (B) in the gradient mode to keep short run time of 35 min. This method was validated for good accuracy, repeatability and precision, and can be used to evaluate the quality of the drug. This multi-phytoconstituents assay method will be helpful in quality control and stability studies of *Enicostemma littorale*.

A densitometric HPTLC method for simultaneous qualitative and quantitative analysis of Isoswertisin-5-O- β-D-glucoside, Swertiamarin and Swertisin in the methanolic extract of *Enicostemma littorale* Blume is established. Ethyl acetate: methanol: water (8.0:1.0:0.5)
mobile phase was used for separation. The method is simple, precise, specific, sensitive, and accurate. Very low concentration of Isoswertsisin -5-O- β-D- glucoside was present in the methanol extract. The method established in this work can be used as quality-control method for quantification of Isoswertsisin-5-O- β-D-glucoside, Swertiamarin and Swertisin in the plant powder of *Enicostemma littorale* Blume.

HPTLC method used in this research work is accurate, sensitive, and can be used as a routine quality-control method for quantification of Ascorbic acid, Ellagic acid, Gallic acid, Methyl gallate and Cinnamic acid in the *Phyllanthus emblica*. This method is validated for good accuracy, repeatability and precision, and can be used to evaluate the quality of the drug. This multi-phytoconstituents assay method will be helpful in quality control and stability studies of *Phyllanthus emblica*.

LC/MS/MS permitted the detection of known and novel phenolic compounds, while gradient HPLC permitted the separation of several phenolic compounds (including isomers). This method is suitable for the routine analysis of samples containing prenylated flavonoids, as well as for the separation and identification Swertiamarin and Swertisin from this family Gentianaceae with potential biological and/or pharmacological activity. Ethyl acetate extracts of *Enicostemma littorale* and *Emblica officinalis* were found safe in acute and repeated dose toxicity studies. Ethyl acetate extracts were tested for their antidiabetic activity using streptozotocin induced diabetes model and showed significant antidiabetic activity in the selected model.

In conclusion of this work “Novel isolation method was developed for phytoconstituents from *Enicostemma littorale* and *Emblica officinalis*. A new, simple HPLC and HPTLC method was developed for isolated phytoconstituents. The extracts of *Enicostemma littorale* and *Emblica officinalis* were found to be safe and it was confirmed by acute and repeated dose toxicity studies. The extracts also showed a comparative antidiabetic activity.