Summary

Hypercholesterolemia is one of the reasons for major health issues such as coronary heart disease and atherosclerosis. Controlling the plasma cholesterol level has become one of the mandatory therapeutic strategies to control these diseases. The available synthetic drugs such as statins, which are used for the treatment of coronary heart disease, are known to have various side effects. These side effects lead to the demand for alternative drugs developed from traditional sources. Various plants as well as drugs derived from traditional sources are found to have hypocholesterolemic activity.

The extracts of *Mangifera indica* Linn. and *Moringa oleifera* Lam. are reported to have hypocholesterolemic activity. Presently, there are no studies available to explain the active principles of *M. indica* and *M. oleifera* responsible for hypocholesterolemic activity of these plant extracts. Therefore, the present study was undertaken to isolate and identify the active principles from both the plants using bioactivity-guided fractionation (BAGF) for standardizing the extracts using these isolated active principles.

The methanol extract of leaves of *M. indica* was evaluated in an *in vivo* model using Wistar rats fed with high cholesterol diet. The results of the study showed that methanol extract of leaves of *M. indica* significantly reduced the cholesterol levels at the dose of 90 mg/kg. Thus, the methanol extract of leaves of *M. indica* was subjected to BAGF study using *in vitro* cholesterol esterase inhibition assay. The methanol extract was found to be active compared to polar compounds. In addition, ethyl acetate fraction of methanol extract of leaf of *M. indica* showed highest activity than butanol and water fraction. The fractionation of ethyl acetate fraction yielded two compounds viz. 3β-taraxerol, a bioactive sterol- related compound and iriflophenone-3-C-β-glucoside a polar compound, which was not active in cholesterol esterase inhibition assay. The results are also confirmed that the nonpolar compounds present in the extract are responsible for cholesterol esterase inhibition. The less active butanol fraction was subjected for various separation techniques to isolate and characterize the compounds. A mixture of hyperoside and isoquercitrin was isolated using adsorption chromatography. Further, four compounds were isolated using High Performance Counter Current Chromatography(HPCCC) in the pure form and identified as iriflophenone 3-C-(2-O-p-
hydroxybenzoyl-β-D-glucose; 1,2,3,4,6-Penta-O-gallolyl-β-D-glucose, iriflophenone-3-C-(2"-galloyl)-β-D-glucoside and iriflophenone-3-C-(6"-galloyl)-β-D-glucoside by spectral studies and by matching with the literature. The newly developed HPCCC method was found to be reproducible to isolate the above four compounds.

*M. indica* extract was quantified by two different reverse phase HPLC methods. The bioactive compound 3β-taraxerol was quantified in first method, whereas eight identified compounds were quantified using second method. These two newly developed and optimized methods can be utilized to standardize the herbal extracts or the formulations of leaves of *M. indica*.

In the case of *M. oleifera*, various parts of this plant were screened in different *in vitro* assays. Methanol extracts of both leaves and fruits showed maximum inhibition in lipase inhibition assay compared to other assays. Hence, the fractions and extract were screened in lipase inhibition assay. Among the fractions screened, ethyl acetate fraction was found to be more potent and hence, it was subjected for bioactivity guided fractionations, which lead to the isolation of α-linolenic acid and niazirin. Alpha-linolenic acid showed potent inhibition of cholesterol esterase whereas niazirin isolated from active fraction did not show any inhibition. Comparatively less active butanol fraction was subjected for chromatographic isolation which resulted in the isolation of five compounds viz. neochlorogenic acid, cryptochlorogenic acid, 4-p-coumaroylquinic acid, vicenin-2 and isoquercitrin. These compounds were identified with the help of spectroscopic studies and by comparing with the previous published literature.

Fractional inhibitory concentration (FIC) index of extracts of leaves and fruits of *M. oleifera* was determined using *in vitro* lipase inhibition assay. Additive effect was observed at 1:1 combination of these two methanol extracts. The blended extract (MOLF) was further studied in *in-vivo* studies. MOLF exhibited cholesterol-lowering activity in Triton WR139 induced hypercholesteromic albino Wistar rats at the oral dose of 45 mg/kg. In chronic high cholesterol diet *in vivo* model, the extract of *M. oleifera* showed a mild cholesterol-lowering activity at tested dose levels, which provides evidence for the complementary action of phytoconstituents present in leaf and fruit in manifesting cholesterol-lowering activity effect.
Two different chromatographic methods were used for standardization of *M. oleifera* extracts and raw materials. A lipase inhibitor, ALA was quantified in MOL and MOF by GC method and eight identified phenolic compounds were estimated by a newly developed HPLC method.

In the present study, for the first time, three new analytical HPLC methods were developed for standardization of extracts of both plants. These chromatographic fingerprint profiles can be used for quality control to establish identity, purity and quantification of constituents in the extracts, raw materials and their formulations. Various compounds isolated from both *M. indica* and *M. oleifera* were screened for hypocholesterolemic activity by lipase and cholesterol esterase inhibition assays. Among thirteen compounds screened, neochlorogenic acid from *M. oleifera* and 1,2,3,4,6-penta-O-galloyl-β-D-glucose, mangiferin and iriflophenone-3-C-(2-O-p-hydroxybenzoyl)-β-D-glucose from *M. indica* were found to possess potent lipase inhibition activity. Among the tested compounds 1,2,3,4,6-penta-O-galloyl-β-D-glucose was emerged as the potent lipase inhibitor. However, none of these compounds showed any significant inhibition of cholesterol esterase.

This is the first study to report the hypocholesterolemic activity of all the isolated compounds from the two plants except mangiferin. These findings also provided the scientific proof to support and defend the use of *M. indica* and *M. oleifera* in the treatment of hypercholesterolemia as mentioned in ayurvedic literature. Both the plants viz. *M. indica* and *M. oleifera* showed cholesterol-lowering activity in *in-vitro* assay as well as *in vivo* studies. The bioactive compounds isolated using bioassay guided fractionation indicated that the secondary metabolites contribute to the bioactivity of the extracts. The newly developed analytical methods can be utilized for the standardization of these herbs, their extracts and their formulations. This study also paved a path for the development of standardized herbal formulation for the treatment of hypercholesterolemia. A human clinical validation may be considered as the future scope and prospects of the present project.