Abstract:

Hypercholesterolemia is an important contributor to cardiovascular diseases and reduction of cholesterol can lead to reduction of mortality due to atherosclerosis and other related diseases. Hence statins are used as therapeutic agents for reduction of cholesterol levels in patients, however the side effects of these synthetic drugs in patients made the researchers to explore herbal drugs for the treatment since herbal drugs are perceived as safe compared to synthetic drugs. Many of medicinal plants are being used to manage hypercholesterolemic condition. The extracts of Mangifera indica Linn and Moringa oleifera Lam have been reported to have hypocholesterolemic activity. However, no studies seem to have been done on identification of chemical constituents of M. indica and M. oleifera responsible for cholesterol lowering activity of these plants. Thus, the current study was undertaken to isolate and identify the active principles from M. indica and M. oleifera through bioactivity-guided fractionation (BAGF) and to standardize the extracts using the 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 indicated that methanol extract of leaves of M. indica at the dose of 90 mg/kg (po), significantly reduced the cholesterol levels. The extract showed IC$_{50}$ value in the range of 11-21 µg/mL in in vitro cholesterol esterase inhibition assay and the same assay was used in BAGF study. On further fractionation, 3β-taraxerol and iriflophenone-3-C-β-glucoside were isolated from active sub-fractions of ethyl acetate fraction. These results established that the nonpolar compounds present in the extract are responsible for cholesterol esterase inhibition than polar compounds. Four compounds were isolated using High Performance Counter Current Chromatography method in the pure form from less active butanol fraction and were identified as iriflophenone 3-C-(2-O-p-hydroxybenzoyl-β-D-glucose;1,2,3,4,6-Penta-O-galloyl-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 data. The newly developed HPCCC method was found to be reproducible in
isolating the above four compounds along with mangiferin and iriflophenone-3-C-β-glucoside. A mixture of hyperoside and isoquercitrin were also isolated from butanol fraction using adsorption chromatography.

Two new analytical HPLC methods were developed for determination of the isolated constituents in extracts of leaves of *M. indica*. In one of the method, 3β-taraxerol was quantified using a non-polar mobile phase whereas, in the other method, the eight identified compounds were quantified using a reverse phase HPLC method.

Methanol extract of leaves of *M. oleifera* showed maximum inhibition in lipase inhibition assay *in vitro* compared to other bioassays and the same was used in BAGF. On further fractionation α-linolenic acid (IC$_{50}$ 1.75 µg/mL) and niazirin (not active) were isolated from an active ethyl acetate fraction of methanol extract of leaf. The chromatographic separation of less active butanol fraction afforded five polar compounds viz. neochlorogenic acid, cryptochlorogenic acid, 4-p-coumaroylquinic acid, vicenin-2, isoquercitrin and along with known rutin compound. Fractional inhibitory concentration (FIC) index of both *M. oleifera* leaf and fruit extracts (MOLF) was determined using lipase inhibition assay. A 1:1 combination of methanol extract of leaves and fruits of *M. oleifera* (MOLF) were found to be additive. The MOLF was further evaluated using *in-vivo* studies. MOLF exhibited cholesterol-lowering activity in Triton WR1339 induced hypercholesteromic albino Wistar rats at the oral dose of 45 mg/kg. In chronic high cholesterol diet *in vivo* model, MOLF exhibited a mild cholesterol-lowering activity at tested dose levels. α-linolenic acid was quantified using GC method by methylation process. Other eight polar compounds were estimated simultaneously using newly developed HPLC method.

The thirteen compounds isolated from butanol fraction of both the plants were screened using *in vitro* cholesterol esterase and lipase inhibition assay. 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. 1,2,3,4,6-penta-O-galloyl-β-D-glucose (IC$_{50}$ Value-0.43 µM) was identified as the potent among the tested compounds.
Both the methanol extracts of *M. indica* and *M. oleifera* have shown cholesterol-lowering activity in *in-vitro* assay as well as *in vivo* studies. The bioactive compounds isolated through BAGF indicate the contribution of secondary metabolites of these plants for the cholesterol lowering activity. Our results provide the scientific usefulness of these plants for hypercholesterolemia. The isolated constituents and the analytical methods developed in this work can be used for manufacturing standardized herbal extracts and formulations from these plants.

Keywords: *M. indica, M. oleifera*, hypocholesterolemic activity, BAGF, 3β-taraxerol, α-linolenic acid, standardization.