CHAPTER 4

HYDROTROPIC EXTRACTION OF MANGIFERIN

4.1 INTRODUCTION

Extraction of phenolic compounds present in natural sources has attracted a special interest in recent days. This is mainly due to its ability to scavenge free radicals responsible for the deterioration of lipid-containing food and to reduce the great number of diseases. There are many bioactive compounds extracted from plant source which can be used as phytomedicines. Mangifera indica L. is the most popular tree originated in Southeast Asia and it is commonly known as Mango tree. The various parts such as stem bark, leaves and flowers of the mango tree have been used in traditional medicine. The leaves of Mangifera indica L. have long been used as a traditional Chinese medicine to treat many diseases (Jieping Qin et al, 2008).

The most important constituent of this plant is mangiferin, a C-glucosyl xanthone, which is present in different amounts in different parts of the plant. Mangiferin has been reported to have important and broad pharmacological activities such as antidiabetes (Muruganandan et al, 2005), antiviral (Zakaria et al, 2006), antitumor, immunomodulatory, antioxidant and anti-inflammatory. Hence the extraction and purification of mangiferin from mango leaves have become important for its utilization as phytochemical.

There are several methods used to extract phytochemicals from plants. It has been reported that the organic solvents such as methanol or
ethanol is used to extract mangiferin from mango leaves. These solvents used for the extraction are not selective towards mangiferin and repeated extraction of raw materials for long durations results in the extraction of other components also. The crude extract obtained has to be purified by various steps. The poor extract quality, excess usage volatile organic solvents and residual solvent traces remaining in the final product limit the usage of organic solvents for the extraction of mangiferin.

Supercritical fluid extraction using carbon dioxide is another option for the extraction of mangiferin. The major advantage of this method is that the products obtained will be completely free from residual solvent. This extraction technique normally eliminates the problem of product recovery. However the main limitation of supercritical extraction method is the need of high-pressure equipment required to maintain the supercritical pressure of the solvent, which add significantly to the cost of separation.

High-pressure steam treatment can also enhance extraction rates by an osmotic shock; however, this technique is relatively slow and requires a large amount of steam. Hence there is a need for effective and clean method for extraction of highly valuable mangiferin from mango leaves. In the present work an effective process based on the phenomenon of hydrotropy is presented for the selective extraction of mangiferin from mango leaves.

4.2 EXTRACTION USING HYDROTROPE SOLUTIONS

Hydrotropy refers to the ability of certain compounds termed hydrotropes to increase the solubility of sparingly soluble or water soluble organic solutes in aqueous solutions. The effect of hydrotropes on the solubility and mass transfer coefficients are discussed in Chapter 7. This technique is a collective molecular phenomenon with a much higher capacity.
It is a consequence of the tendency of amphiphilic hydrotrope molecules to aggregate among themselves and probably with other hydrophobic molecules.

Amphiphilic organic substances with a short linear or branched alkyl chain or an aromatic ring with a short alkyl chain, attached to a strongly polar/ionic group can be used as hydrotropes. These hydrotropes could also be successfully utilized in the extractive separation and extractive distillation of close-boiling mixtures. (Girija Raman and Gaikar, 2002, 2003, Mishra and Gaikar, 2009).

4.2.1 Mechanism of Hydrotropic Extraction

The phenomena of hydrotropic extraction can be initiated by penetration of hydrotropes on the plant cell wall, penetration into the cells followed by solubilization of the active compound. The hydrotrope solution should either partly dissolve the cell membrane or at least destabilize the cell wall structure during the process. Surfactant solutions at fairly high concentrations form lamellar crystalline structures in the solutions. Hydrotropes have tendency to destabilize lamellar liquid crystalline phases of a conventional surfactant in aqueous solutions. The destabilizations of lamellar liquid crystalline structures of surfactants in aqueous solutions by hydrotropes have been extensively studied because of their similarity with the natural cell membrane structure.

The double phospholipid layers on the cell membrane resembles as same as lamellar crystalline structure of surfactant in aqueous solutions. Hence the hydrotrope which shows the tendency to disrupt lamellar crystalline structure will also be capable of destabilizing the cell wall structure to extract bioactive compounds. The amphiphilic nature of the hydrotrope molecules also imparts the well-known characteristic of semipermeability to the cell membranes. Moreover the hydrotrope helps the
reduction of surface forces on the cell wall and improves the permeability and thus hydrotrope molecules penetrate into cell wall easily to extract bioactive compounds (Dandekar et al, 2003).

The hydrotrope molecules penetrating the cell wall at higher concentration aggregate to form stack like structure. The nonpolar bioactive compounds would enter the hydrophobic layers of these assemblies, adding themselves between the layering molecules to form stable structure. These hydrotropic solutions precipitates the bioactive compounds out of the solution on dilution with distilled water thus enable the easy recovery of the extracted solute. Since hydrotropes are highly water soluble, the use of aqueous hydrotrope solution for extraction avoids the presence of residual solvent remaining with the final product. The use of hydrotropes in extraction was investigated by few research groups because of its high selectivity in separation and easy recovery of natural compounds.

4.2.2 Selection of Hydrotropes

Hydrotropes are amphiphilic organic substances with a linear or branched alkyl chain or an aromatic ring with a short alkyl chain, attached to a strongly ionic group. The enhancement of solubility of sparingly soluble organic compounds will be different for different hydrotropes. This difference in hydrotropic solubilization is mainly due to the different sizes of their hydrophobic parts, number of –CH₂- groups in the hydrocarbon side chains (Srinivas et al, 1991). The hydrophobic region of the hydrotrope aggregates helps in enhancing the solubility of the dissolved solutes.

The hydrophobic region provided by a hydrotrope can be estimated from its effective carbon chain length. Since the solubilization of organic solutes depends on the hydrophobicity of hydrotropes, sodium cumene sulfonate is used for the extraction of mangiferin from mango leaves. Sodium
salicylate is another hydrotrope selected for this extraction study which is widely used in pharmaceutical formulations to dissolve insoluble drugs. It consists of no side chains but the hydroxyl group in ortho position probably supports the aggregation process.

Factors such as hydrotrope concentration, temperature and raw material loading affect the extraction of mangiferin from mango leaves. To study the effect of these factors on the extraction process, response surface method is used.

4.3 RESPONSE SURFACE METHODOLOGY

The experimental design was carried out using Minitab software. Central composite design (CCD) was used to identify the optimum hydrotrope concentration, system temperature and raw material loading, in order to obtain maximum yield of mangiferin. The design consisted of eight factorial points, six axial points and six centre points thus giving total set of 20 experimentations. The collection of experiments provides an effective means for optimisation through these process variables. Besides, the design permits the estimation of all main and interaction effects. On the other hand, the purpose of the centre point is to estimate the pure error and curvature. The quadratic model for predicting the optimal point was expressed according to the following equation:

\[ Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j \]  (4.1)

where \( Y \) - response variables;

\( b_0 \) - constant,

\( b_i \) - linear coefficient,

\( b_{ii} \) - quadratic coefficient
$b_{ij}$ - cross-product coefficient

$X$ - coded levels of the independent variables

The experiment was conducted in a randomized order to avoid systematic bias. Interpretation of results was analysed by analysis of variance (ANOVA) as appropriate to the experimental design used. The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of linear, quadratic and interaction terms were determined. The significances of all terms in the polynomial were judged statistically by computing the F-value at a probability (P) of 0.05. The regression coefficients were then used to make statistical calculations to generate contour maps from the regression models.