



# NOVEL AND CONVENTIONAL METHODS FOR EXTRACTION OF MANGIFERIN FROM MANGIFERA INDICA (MANGO) LEAVES

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**Abstract:** We have here studied some conventional and novel method approaches for extraction of Mangiferin from leaves of Mangifera Indica (Mango). The one of the common methods used for extraction of Mangiferin is three phase partitioning. The other methods such as Batch extraction, Soxhlet extraction were also studied to see which method gives the optimum yield of Mangiferin. Three phase partitioning (TPP) is the method of extraction in which two phases viz. organic phase and aqueous phase are formed by salt (mostly Ammonium sulphate) saturation, continuous addition of solvent (mostly t-butanol) and adjusting the pH respectively.

TPP is the most simple, economical and quick method for recovering Mangiferin from Mangifera Indica leaves as it requires less amount of solvent for extraction and also takes less time. Various parameters which have effects on TPP extraction of Mangiferin such as right salt selection, extraction time, slurry to t-butanol ratio, solute to solvent ratio and right pH were tested to get the optimum yield of Mangiferin. In the study, it was observed that in Soxhlet extraction, the mangiferin content was found to be 57mg/g in 5 hr. time, while in TPP the mangiferin content was 28mg/g in 2 hr. time. Also, conventional batch extraction using water as solvent gave the total yield of 23 mg/g in 2 h 40 min.

**Keywords - Three phase partitioning, extraction, mangiferin, Mangifera indica leaves, batch extraction, Soxhlet extraction.**

## •Introduction:

India is a rich country in collection of registered and widely popular medicinal plants. In recent years, the use of Ayurvedic (herbal) medicines to treat diseases has also attracted people's interest in western countries. Mango (*Mangifera indica*), which is the national fruit of India, is cultivated in several tropical and subtropical regions. Mangiferin is a major component of mango leaves and important natural drug which has wide range of applications in pharmaceutical & other related industries. It has antioxidant, antitumor and antiviral properties which is effective in treatment of cancer, obesity, diabetes, etc. Different methods are used for extraction of Mangiferin such as Soxhlet extraction, supercritical extraction, Microwave assisted extraction (MAE), Ultrasonic assisted extraction (UAE), etc. Vrushali Kulkarni and Virendra Rathod have published the reports on the methods for extraction of Mangiferin such as Batch extraction, Soxhlet extraction and TPP extraction. Reason for preferring TPP extraction over conventional methods like batch extraction or Soxhlet extraction is that these methods require large amount of solvent and time for extraction. However, in India, advanced methods like microwave-assisted extraction and ultrasound-assisted extraction are still at the early stage for extraction and do not have much research and also have issues in scalability. Thus, it is important to use simple extraction methods that require less power, space and simple setup and give sufficient yield. As limited work is reported in the literature on the extraction of mangiferin using TPP, the present study reports the TPP method for extraction of mangiferin from *Mangifera indica* leaves with comparison to Soxhlet extraction process.

**•Materials and Chemicals:**

Leaves of *Mangifera Indica* were obtained from the Institute of Chemical Technology Garden at Matunga, Mumbai, India. The leaves were washed in tap water and dried under the sunlight for 48 h. The dried leaves were then powdered in a grinder and then stored in an airtight container in a cool place. The moisture content of leaves was around 9%, and the powder size was about 1-2 mm. The solvents used in this extraction such as t-butanol, deionised acidified water and methanol were of analytical grade. Also the ammonium sulphate of analytical grade was used in extraction because of its effective contribution of getting optimum Mangiferin yield. All experiments were performed several times and average values were taken with a specific standard deviation.

**•Methods:****1. Three Phase Partitioning (TPP) extraction:**

Slurry of 0.5 gm of powdered *Mangifera Indica* (mango) leaves was made by adding 20ml of deionised distilled water. Then 3.4 gm (30% w/v) of ammonium sulphate was added to it part by part with stirring at some time intervals in 250ml reactor. Then 20ml of t-butanol was added for formation of organic and aqueous phase. This mixture was then stirred at 500 rpm for 60 minutes in glass reactor and temperature was maintained at  $30\pm 2^\circ\text{C}$ . Mixture was then allowed to stand for 1hour to form three phases at room temperature. Organic phase containing t-butanol and Mangiferin was then analyzed to determine the actual Mangiferin content with the help of HPLC which is Agilent 1260 infinity high performance auto sampler.

**2. Soxhlet extraction:**

In this method, 5 gm powdered leaves were placed in timple-holder and filled with water as solvent from distillation flask. When water reaches overflow level, siphoning action extracts solution of timple-holder and unloads it back into distillation flask. This stream carries extracted solutes which are separated from solvent by distillation in solvent flask. Solute remains in flask and solvent is sent again for extraction. This extraction was carried out for 5 hours. Samples were withdrawn from distillation flask and analyzed for finding Mangiferin content. It was observed that Mangiferin content extracted in Soxhlet extraction was 57mg/g in 5 hour whereas in TPP it was 28mg/g in 120min.

**3. Batch extraction:**

Batch extraction was performed in a glass reactor of 250 ml capacity consisting of a six-bladed glass turbine for agitation. Powder of *Mangifera Indica* leaves of about 0.5 g was put in a glass reactor, and 40 ml of water was added to it. The temperature of experiment was maintained at  $30\pm 2^\circ\text{C}$ . The mixture was then agitated for about 3 hour and 40 minutes at a speed of 500 rpm. Samples of 0.1 ml were withdrawn at regular intervals of 20 min and then diluted and centrifuged for analysis using HPLC.

Various parameters which have effects on the extraction of Mangiferin from *Mangifera Indica* leaves in a batch process were studied. Parameters such as temperature, stirring time, stirring speed and solute to solvent extraction were optimized.

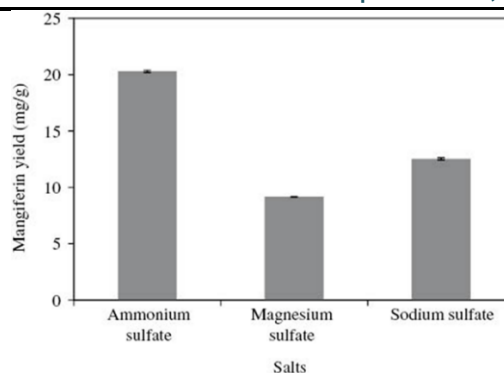
**•Analysis of Mangiferin:**

Analysis of mangiferin was carried out by High Performance Liquid Chromatography which is also known as HPLC (Agilent 1260 infinity high-performance auto sampler) to determine the actual Mangiferin content extracted from the sample solution. All experiments were performed on a C18 column for several times.

The analysis conditions were maintained at a flow rate of 1 ml/min. The mobile phase used was methanol and acidified water in 30:70 v/v ratio. All experiments were performed several times to check the reproducibility, and their average values with a standard deviation were reported.

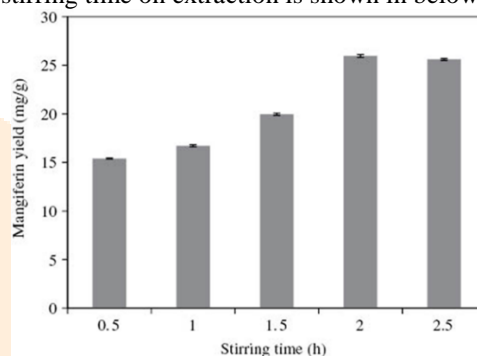
**•Results:****• Factors affecting the TPP extraction of mangiferin:****1. Selection of right salt for extraction:**

We can use various salts such as ammonium sulphate, magnesium sulphate, sodium sulphate, to study its effect on TPP extraction. It was observed that ammonium sulphate gave maximum extraction yield. This might be due to the fact that ammonium sulphate is more water soluble than the other two salts. Also, sulphate ions act as a dehydrating agent which ultimately increases their effective radius. Thereby, large ions crowd together which separates proteins out of water phase. This dehydrating action of salts alters solubility of Mangiferin creating unfavourable conditions for it in aqueous phase and this causes push to the Mangiferin into organic phase. Other salts are also effective for extraction but they give lesser yield of Mangiferin in comparison with ammonium salt as shown in below figure.



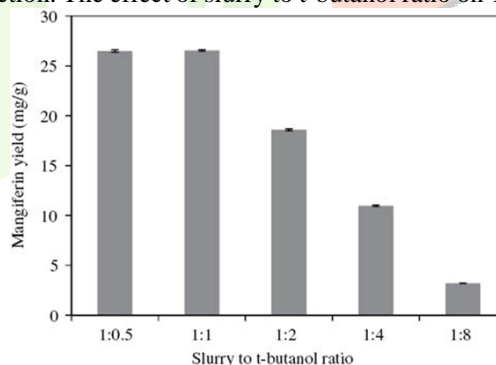
## 2. Effect of stirring time on TPP extraction:

The time required for extraction is critical in determining cost of process and thus it is essential to obtain optimum time for extraction of Mangiferin. Samples were withdrawn at time intervals of 30 minutes such as 30 min, 60 min, 90 min, 120 min and 150 min. It was observed that yield of Mangiferin increases upto a certain amount of time and then it remains constant on further stirring for extraction. So, in terms of considering economic conditions, 120 minutes was decided as optimum time for extraction. Effect of stirring time on extraction is shown in below figure.



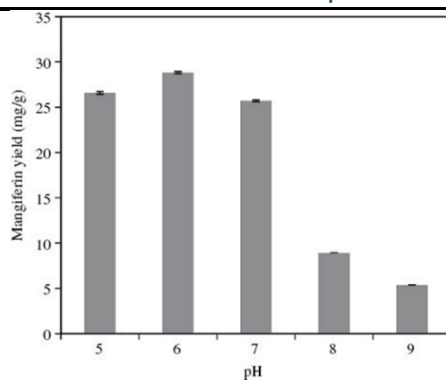
## 3. Effect of slurry to t-butanol ration on TPP extraction:

Tertiary Butanol (t-butanol) have komostrope properties which helps to be in synergism with sulphate. A lesser quantity of t-butanol does not provide adequate synergism with ammonium sulphate. Here the different slurry to t-butanol ratios were tested such as 1:0.5, 1:1, 1:2, 1:4 and 1:8. It was observed that slurry to t-butanol ratio of 1:0.5 gave lesser extraction yield. Further increase in t-butanol volume beyond 1:1 ratio lead to decrease in concentration gradient between both phases, which resulted in decreased mass transfer. This cause reduction in extraction yield of Mangiferin. Hence, slurry to t-butanol ratio of 1:1 was considered optimum for extraction. The effect of slurry to t-butanol ratio on TPP extraction is shown in below figure.



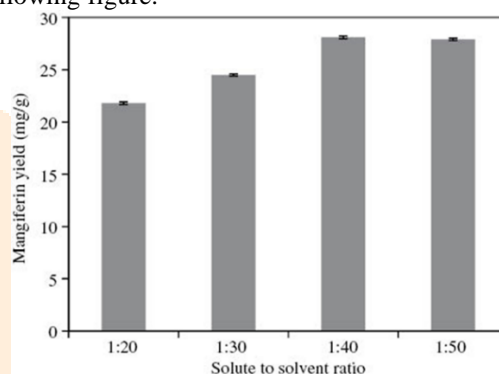
## 4. Effect of pH on TPP extraction:

Mangiferin extraction was observed to be significantly higher in acidic range as compared to basic range. This is due to solubility of ammonium sulphate in water is higher compared to Mangiferin. So, solubility of Mangiferin is altered in aqueous phase as large sulphate ions become crowded in aqueous phase creating unfavourable conditions for Mangiferin which leads to pushing it in t-butanol rich phase and higher extraction in t-butanol is achieved. The effect of pH on TPP extraction is shoen in following figure.



##### 5. Effect of solute to solvent ratio on TPP extraction:

Different solute to solvent ratios such as 1:20, 1:30, 1:40, 1:50 were tested to obtain the optimum yield of Mangiferin. It was reported that maximum yield of Mangiferin of about 28mg/g was obtained at ratio of 1:40. So, it was observed that the extraction yield of Mangiferin increases as solute to solvent ratio increases. But it does not increase the yield further due to equilibrium been achieved. Thus, it is essential to maintain the proper balance of solute to solvent ratio for getting the optimum yield. This is important to not to exceed the decided expense on the project. The changes in yield with respect to changes in solute to solvent ratio are shown in following figure.



##### •Discussion:

Here, we compared the Three Phase Partitioning (TPP) extraction method with Soxhlet extraction and conventional Batch extraction to optimise the best method for extraction of Mangiferin. It was observed that Soxhlet extraction gave Mangiferin yield of 57mg/g in 5 hours of time, whereas the conventional Batch extraction gave the Mangiferin yield of about 23 mg/g in total time of 2 hours and 40 minutes. But the optimum yield of Mangiferin was observed in the Three Phase Partitioning (TPP) extraction which was about 28 mg/g in 2 hours duration. So, in lab scale we can conclude that the best method for extraction of Mangiferin is the TPP extraction.

So, in this report, TPP extraction method required less amount of time as the extraction was done rapidly than the time required in Soxhlet extraction and Batch extraction. Also, the solvent requirement in TPP method is significantly less than in Soxhlet extraction and Batch extraction. This decreases the operating cost of the process. It gives higher recovery yields than compared with Batch extraction and Soxhlet extraction within shorter span of time. It does not require any special apparatus for process whereas Soxhlet extraction method requires a special thimble along with extractor. This method is also considered as green extraction technique due to its environment friendly operation.

But as there are advantages of TPP extraction over the other methods, there are disadvantages too of the TPP extraction in comparison with other two methods. TPP extraction method cannot be used on a large scale for the extraction as it gets difficult in operation and becomes very expensive to produce in large scale. Also, there are now new methods are practiced which are the modified versions of TPP such as Microwave assisted TPP, Ultrasound assisted TPP, Enzyme assisted TPP, etc. which even takes lesser time and amount of solvent than it is required in the traditional TPP method.

##### •Future work:

As we discussed earlier, TPP extraction is one of the best alternatives to those traditional and conventional extraction methods which are in operation in the industries. TPP extraction does have comparatively simple setup than the other methods of extraction. But this extraction method lacks when it comes to scalability and becomes little expensive than the traditional methods. This makes difficult to use the method in large scale such as industry. There are certain alternatives for this method in the form of modification of this same method such as Microwave assisted TPP extraction method also known as 'MTPP' which uses microwave radiation to heat the extraction solvent which improves the dispersing ability of the solvent into the sample. This accelerates the phase partitioning of the compound to be extracted from the liquid or solid sample into the solvent. Due to this, it decreases the time and solvent requirement for the extraction. There is one other method as Ultrasound assisted TPP extraction also known as 'UTPP' which have the influence of the ultrasound waves on the three phase partitioning which creates cavitation of small bubbles in the solvent which allows for a greater penetration of the solvent within the solution increasing the surface area. This helps in accelerating the extraction method making it the one of the rapid methods of extraction. Enzyme assisted TPP extraction also known as 'ETPP' uses the enzymes to disrupt the cell wall of source material to improve its extraction yield. This improves the overall recovery of material to be extracted to have optimum

yield. These all mentioned techniques are also known as green extraction techniques due to their eco-friendly and environment friendly operation.

#### •Conclusion:

This report aimed at study of the extraction process and various alternative extraction methods which can be used to get the optimum yield of a certain material or component to be extracted. In this particular report, the Three Phase Partitioning method was compared with Soxhlet extraction and Batch extraction to see which was the best or optimum method to get the maximum yield of Mangiferin content. Also, the future alternatives for the TPP method such as Microwave assisted TPP method (MTPP), Ultrasound assisted TPP method (UTPP) and Enzyme assisted TPP method (ETPP) were also discussed to make the traditional TPP method as efficient method for industry purpose to be used on large scale. This study may lead to develop new TPP strategies for the extraction of Mangiferin from *Mangifera Indica* (Mango) leaves which would be used by the pharmaceutical industry. Also, it may help in making TPP method more economic and industry efficient.

#### •References:

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