



A novel method to augment extraction of mangiferin by application of microwave on three phase partitioning



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ABSTRACT

This work reports a novel approach where three phase partitioning (TPP) was combined with microwave for extraction of mangiferin from leaves of *Mangifera indica*. Soxhlet extraction was used as reference method, which yielded 57 mg/g in 5 h. Under optimal conditions such as microwave irradiation time 5 min, ammonium sulphate concentration 40% w/v, power 272 W, solute to solvent ratio 1:20, slurry to *t*-butanol ratio 1:1, soaking time 5 min and duty cycle 50%, the mangiferin yield obtained was 54 mg/g by microwave assisted three phase partitioning extraction (MTPP). Thus extraction method developed resulted into higher extraction yield in a shorter span, thereby making it an interesting alternative prior to down-stream processing.

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1. Introduction

The interest in traditional medicines is growing substantially since several modern drugs are banned due to their increased side effects apart from being expensive. India has a rich array of registered and widely popular medicinal plants. This necessitates detailed documentation and research related to medicinal uses of plants as well as techniques for separation of essential biomolecules. In recent years, the use of herbal medicines to treat diseases has also evinced interest in western countries.

Mango (*Mangifera indica*), the national fruit of India, is cultivated in several tropical and subtropical regions. Mangiferin is a major component of mango leaves and is an important natural drug with wide applications in pharmaceutical and other related industries as mentioned by Sato et al. [16]. It shows antioxidant, antitumor and antiviral properties [6,15,20–22]. Various studies have been done on its medicinal uses [1,2,9,12]. Thus there exists a huge scope to further study and work on mangiferin. Jutiviboonsuk and Sardsaengjun [10], Padmapriya et al. [13] and Zou et al. [23] have published reports on soxhlet extraction, microwave assisted extraction and subcritical extraction of mangiferin using solvent. The conventional techniques like soxhlet or solvent extraction have a disadvantage of larger requirement of solvent and time.

Thus these techniques would not be economical on a large scale. Zou et al. [23], have obtained 36.10 ± 0.72 mg/g yield of mangiferin from *M. indica* leaves under experimental conditions, such as 45% ethanol, liquid-to-solid ratio of 30:1 (mL/g), and extraction time of 123 s under microwave irradiation of 474 W. While Padmapriya et al. [13] have reported an optimal mangiferin yield of 41 μ g/mL in ethanol with an extraction time of 15.32 s for a microwave power of 500 W using microwave assisted extraction of mangiferin from *Curcuma amada*.

In this study, we report a quick method for simultaneous separation and purification of mangiferin using MTPP. TPP has been widely employed for simultaneous separation and purification of proteins, enzymes and inhibitors from crude suspensions. The method is useful for both upstream with crude samples and downstream where a simple scalable step is needed as stated by Vidhate and Singhal [18]. Dennison and Lovrein [3] have stated that TPP involves addition of *t*-butanol and ammonium sulphate to precipitate enzymes and proteins from aqueous solutions. Kulkarni and Rathod [11] found that the solvent like methanol or ethanol were not able to form a three phase and recommend to use *t*-butanol. Thus *t*-butanol is used as the solvent as it is capable of forming three phases. In TPP, generally lipids, enzyme and pigments are accumulated in upper *t*-butanol phase while polar molecules get concentrated in the aqueous phase. However, in the case of TPP of mangiferin when salt is added in the water phase, the dehydration action of salt takes place due to which large proportion of water molecules are involved in hydrating the

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sulphate ions thereby increasing their effective radius. The large ions crowd together and thus the proteins are segregated after being precipitated out of the water phase [24]. Hence, it can be concluded that due to the dehydrating effect of sulphate ions, mangiferin solubility is altered creating unfavorable conditions for mangiferin in aqueous phase and thus pushing mangiferin into organic phase. Harde and Singhal [8] have shown that TPP requires longer time for separation. Thus efforts were made to reduce the time and increase the yield of forskolin from *Coleus forskohlii* roots by three phase partitioning using ultrasound and enzyme treatment. They reported that enzymatic treatment followed by ultrasonication and TPP gave 79.95% and 83.85% recovery in 4 h. While the time required for Soxhlet extraction was 12 h. In recent times, microwave assisted extraction has been widely accepted in various sectors such as perfumery, pharmaceutical, and nutraceutical industries due to its numerous advantages over the conventional extraction methods [5]. Unlike conventional extraction methods, microwave assisted extraction requires lesser solvent, time and energy. Thus it simplifies the extraction process with a higher yield as demonstrated in earlier studies [13,23]. To the best of our knowledge, there are no reports available on coupling of microwave assisted extraction with TPP. Thus, the objective of this work was to explore the application of microwave in TPP so that the suggested process can have the benefits of both the methods. Our endeavor is to reduce the time required for extraction and to increase the yield of mangiferin.

2. Materials and methods

2.1. Material

Mature leaves of *M. indica* were obtained from the Institute of Chemical Technology garden at Matunga, Mumbai, India. Moisture content of the powder was around 9% with particle size in the range of 0.5–1 mm. Initially the leaves were cleaned and sun dried for 48 h. The dried leaves were then powdered and stored in an airtight container in a refrigerator. Mangiferin, reference standard (Sigma M3547-100 mg \geq 98% HPLC grade), was procured from Sigma-Aldrich, USA. Solvents were of analytical grade, purchased from Hi Media Ltd., Mumbai, India. Distilled water used as a solvent was obtained from Millipore Milli Q 50HPLC grade. Methanol and acidified water (0.1% acetic acid) were used as mobile phase. Ammonium sulphate was obtained from S.D. Fine-Chem Limited, Mumbai, India. Methanol and *t*-butanol of HPLC grade were purchased from Hi Media Ltd., Mumbai, India. All the experiments were performed at a temperature of $30 \pm 2^\circ\text{C}$.

2.2. Analysis of mangiferin

Analysis of mangiferin was carried out by HPLC (Agilent 1260 infinity high performance auto sampler) using C18 column ($5 \mu \times 4.6 \text{ mm} \times 250 \text{ mm}$). The column was equilibrated with methanol and acidified water in 30:70 v/v ratio. The analysis conditions were performed by isocratic elution with a flow rate of 1 mL/min. Extracts of $5 \mu\text{L}$ were prepared from each sample and injected into the HPLC. The amount of mangiferin in the sample was quantified from the standard curve at 254 nm.

2.3. Microwave assisted three phase partitioning (MTPP)

A microwave oven (Morphyrichards, India) used for experiments was modified in a laboratory with the provision of a water condenser and sampling probe. It was provided with maximum power of 800 W with time controller. Special microwave extraction glass vessel of 150 mL capacity was designed for carrying out

extraction. MTPP experiments were performed in a glass vessel by initially making slurry of *M. indica* leaves powder (0.5 g) and water (20 mL). 3.4 g (30% w/v) of ammonium sulphate was added in the slurry part by part with an intermediate stirring, followed by the addition of 20 mL of *t*-butanol. This mixture was further exposed to microwave for a desired time in a microwave for the formation of three layers. After microwave irradiation, the mixture was kept in separating funnel to separate organic and aqueous layers. Organic phase containing *t*-butanol was analyzed to determine the mangiferin content. Similar experiments were performed in order to study effect of various parameters using MTPP. Various parameters studied were microwave irradiation time, ammonium sulphate concentration (30–60% w/v), microwave power input (136, 272, 440 W), slurry to *t*-butanol ratio (1:0.5–1:4), solute to solvent ratio (1:10–1:60), soaking time (2–20 min) and number of duty cycles (6) on extraction of mangiferin.

2.4. Soxhlet extraction

Soxhlet extraction was used as a reference method for comparison with MTPP. In Soxhlet extraction 5 g powdered plant material is placed in a thimble-holder and filled with water as a solvent from a distillation flask. When the water reaches the overflow level, the siphoning action extracts the solution of the thimble-holder and unloads it back into the distillation flask. It carries extracted solutes into the bulk liquid. In the solvent flask, solute is separated from the solvent using distillation. Solute remains in the flask and fresh solvent passes back in the solid bed. Samples were withdrawn from the distillation flask and analyzed for mangiferin content. Soxhlet extraction was carried out for 5 h.

2.5. Statistical design

All experiments were performed thrice to check the reproducibility and their average values have been reported. Statistical analysis was done using one-way ANOVA and *p*-values were obtained. The values were considered statistically significant if the *p*-values were less than 0.05.

3. Results and discussions

3.1. Effect of microwave time on MTPP of mangiferin

It is essential to optimize microwave irradiation time in the beginning of any process as it is one of the important parameters affecting the process in terms of cost, quality and yield. For optimizing time, slurry containing 0.5 g of mango leaves powder in 20 mL water was irradiated with microwave for 7 min at power input of 136 W, ammonium sulphate concentration 30% (w/v), slurry to *t*-butanol ratio 1:1, solute to solvent ratio 1:40. Fig. 1a shows that an increase in microwave irradiation time from 0 to 7 min leads to rapid increase in the extraction yield of mangiferin. The yield was highest at microwave irradiation time of 5 min. The observed increase in extraction yield with time can be attributed to the heat generation due to ionic conduction and dipole rotation which enhances molecular movement. As the microwave irradiation time increases, microwaves cause the polar molecules to rotate at the same frequency for longer time creating a molecular friction. This friction releases heat which further raises the temperature. As temperature increases, solvent penetrates into the matrix more effectively facilitating the extraction. However, as temperature increases beyond a certain threshold, it can either lead to the charring of the plant material or lead to an evaporation of solvent. In some cases it can also increase the diffusivity of the target compound in the matrix depending on the characteristics of

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