



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

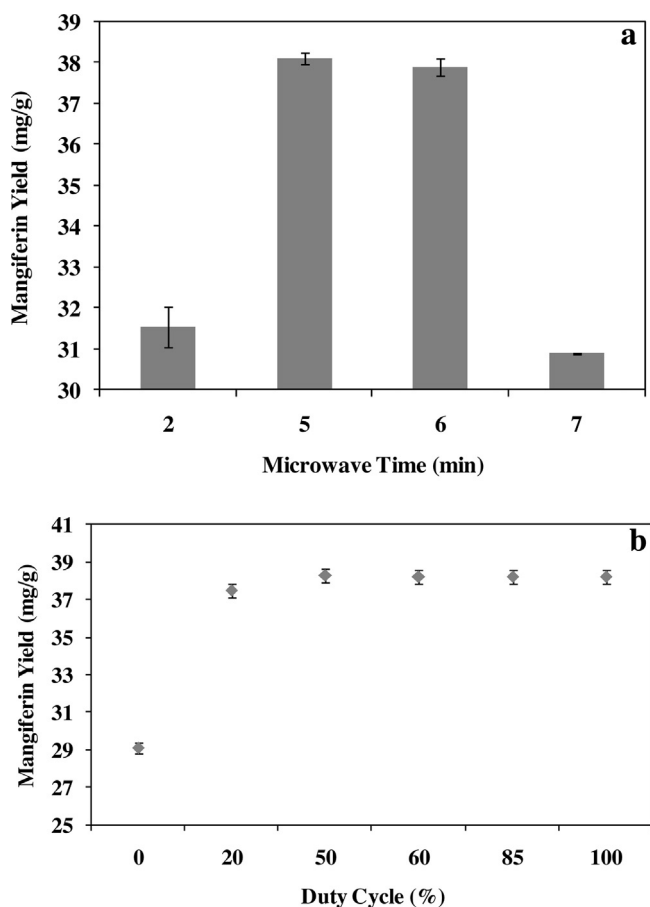


Fig. 1. (a) Effect of microwave irradiation time at power input of 136 W, ammonium sulphate concentration 30% (w/v), slurry to *t*-butanol ratio 1:1, solute to solvent ratio 1:40 and 100% duty cycle. (b) The effect of duty cycle at microwave irradiation time 5 min, power input of 136 W, ammonium sulphate concentration 30% (w/v), slurry to *t*-butanol ratio 1:1, solute to solvent ratio 1:40.

target compound as explained by Veggi et al. [17]. However, as the microwave irradiation time increases beyond 5 min, it leads to an evaporation of solvent along with charring of material resulting in a lower yield. Thus 5 min was considered as the appropriate microwave irradiation time and it was kept constant for next set of experiments.

3.2. Effect of duty cycle on MTPP of mangiferin

Microwave oven can be functioned either in a pulse mode or continuous mode. Operating in a continuous mode is not suggested as it leads to unceasing molecular friction. This increases the temperature persistently, which might degrade the compound. Whereas in a pulse mode, power is supplied in intervals and thus chances of degradation of compound is comparatively less. Also operating in a pulse mode would avoid the unnecessary usage of energy and furthermore evaporation and ebullition of solvent can be avoided. The effect of duty cycle on the extraction yield of mangiferin was evaluated at 25, 50, 65, 80 and 100% duty cycle. Fig. 1b showed that the yield was highest at 50% duty cycle (one minute off and one minute on). The yield remained almost constant for duty cycle above 25%. In order to save the energy it is better to operate the microwave extraction at lower and optimum duty cycle and thus 50% duty cycle was used for further experiments.

3.3. Effect of ammonium sulphate concentration on MTPP of mangiferin

Ammonium sulphate salt is used in TPP to precipitate proteins as at high salt concentrations the surface charges are neutralized leading to the precipitation of the proteins as per Dennison and Lovrein [3]. However, it is important to optimize the concentration of ammonium sulphate as the solubility of proteins or any other biomolecules will vary according to the ionic strength of the solution. Hence, experiments were performed at ammonium sulphate concentrations ranging from 30% to 60% w/v by maintaining other experimental conditions constant such as solute to solvent ratio 1:40, microwave irradiation time 5 min and slurry to *t*-butanol ratio 1:1. The results obtained have been depicted in Fig. 2, which concluded that ammonium sulphate gave the maximum extraction yield at 40% w/v. This is possibly 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 butanol phase. Thus, ammonium sulphate 40% w/v was considered as optimized concentration for next experiments.

3.4. Effect of power on MTPP of mangiferin

Microwave input power is one of the important parameters as it not only decides yield and time of extraction but also responsible for the cost of process. If the power input is too high then it would lead to the evaporation of the solvent leading to the charring of plant matrices. Thus the effect of power input was investigated at 136, 272 and 400 W. The rest of the experimental conditions were: solute to solvent ratio 1:40, microwave irradiation time 5 min, slurry to *t*-butanol ratio 1:1 and ammonium sulphate concentration used was 40% (w/v). It was confirmed from Fig. 3 that higher extraction was obtained at 272 W and later the yield declined at 400 W as ebullition of water occurred within 2 min. Power dissipation in microwave was found to be 11.7 W and 8.6 W at 272 W and 136 W power input respectively in 5 min. Thus initially, an increase in the power input of microwave results in increase in power dissipation thereby increasing the yield of mangiferin. Also, more electromagnetic energy is transferred and power provides localized heating in the sample. This destroys the plant matrix diffusing the solute in the solvent in a short span of time as illustrated in Veggi et al. [17]. However, the reduction in the yield at higher microwave power of 400 W is attributed to the fact that large power is dissipated in a smaller volume which raised the temperature of mixture in a short time and caused charring of particle. Yan et al. [19] have showed that at 400 W yield declined

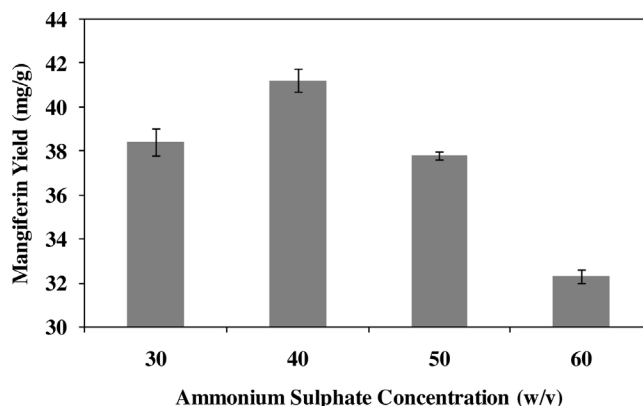


Fig. 2. Effect of ammonium sulphate concentrations at microwave irradiation time 5 min, power input of 136 W, slurry to *t*-butanol ratio 1:1, solute to solvent ratio 1:40 and 50% duty cycle.

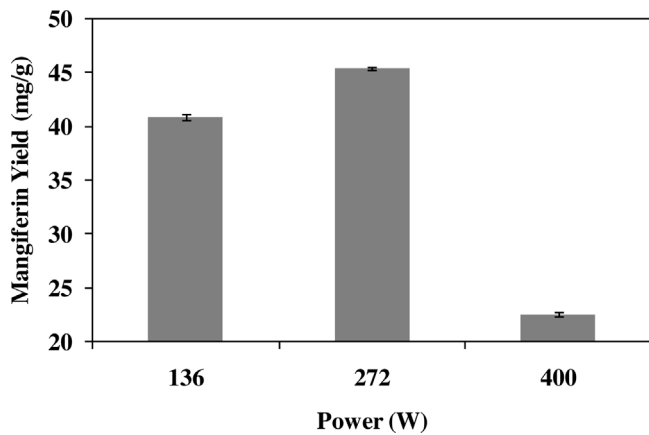


Fig. 3. Effect of power at microwave irradiation time 5 min, ammonium sulphate concentration 40% (w/v), slurry to *t*-butanol ratio 1:1, solute to solvent ratio 1:40 and 50% duty cycle.

because very high microwave irradiation offers superfluous energy to the solvent and matrix, which can disturb molecular interactions thereby affecting the extraction process.

3.5. Effect of slurry to *t*-butanol ratio on MTPP of mangiferin

Effect of slurry to *t*-butanol ratio on extraction of mangiferin was studied by varying it as 1:0.5, 1:1, 1:2 and 1:4. The earlier stated experimental parameters such as solute to solvent ratio 1:40, microwave irradiation time 5 min, power input 272 W and ammonium sulphate concentration 40% (w/v) were kept as constant. From Fig. 4, it was observed that extraction of mangiferin was highest at 1:1 ratio and extraction yield was constant with further decrease in slurry to *t*-butanol ratio. The increase in the amount of *t*-butanol did not increase the yield because as the amount of *t*-butanol increases, viscosity of the slurry increases. As the viscosity increases, the molecular mobility is reduced making it difficult for the molecules to align with the microwave field. This decreases absorption of microwave energy thereby reducing the heat produced by dipole rotation [17]. The extraction is less at slurry to *t*-butanol ratio lower than 1:1 because the amount of *t*-butanol is not sufficient for separation as it does not create effective synergies with ammonium sulphate according to Vidhate and Singhal [18] and Dennison and Lovrein [3]. Thus appropriate slurry to *t*-butanol ratio (1:1) should be maintained to get a proper extraction.

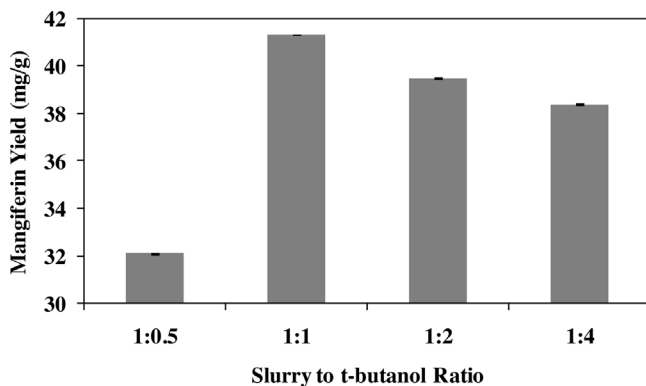


Fig. 4. Effect of slurry to *t*-butanol ratio at power input of 272 W, microwave irradiation time 5 min, ammonium sulphate concentration 40% (w/v), solute to solvent ratio 1:40 and 50% duty cycle.

3.6. Effect of solute to solvent ratio on MTPP of mangiferin

Solute to solvent ratio is one of the important parameters in any type of extraction as it decides the solvent requirement and downstream processing cost. Further, Eskilsson et al. [4] showed that lower recoveries were obtained at large solvent volumes because of non-uniform distribution and exposure to microwaves. Thus, a balance should be maintained to get an optimum yield and to avoid unnecessary use of solvent. Solute to solvent ratio was varied from 1:10 to 1:50 while rest of the extraction conditions were microwave irradiation time 5 min, ammonium sulphate concentration 40% w/v, power input 272 W and slurry to *t*-butanol 1:1. As seen from Fig. 5, mangiferin yield obtained was highest in solute to solvent ratio 1:20 and decreased subsequently. This is because significant absorption of microwave radiation by polar solvents only leads to smaller penetration depth. Thus when large volume of solvent is added, an excessive swelling of the materials occurs. Therefore, comparatively less microwave energy is absorbed directly by the materials as microwaves are completely absorbed at the boundary [7,14].

3.7. Effect of soaking time on MTPP of mangiferin

The effect of soaking time on the extraction of mangiferin was investigated with 0.5 gm powder soaked in 20 mL water for 0, 2, 5, 10 and 20 min. In the power soaked for known time, 40% w/v ammonium sulphate was added followed by addition of 1:1 *t*-butanol. It was then subjected to microwave for 5 min at power input of 272 W. From Fig. 6, it was seen that higher extraction was obtained when the soaking time was 5 min. There was around 12.5% increase in the yield of mangiferin when dried powder was soaked for 5 min. Soaking gives an increase in the moisture content which results in higher microwave penetration as water is the best absorbing microwave material. The increased microwave penetration ruptures the cell more efficiently to release the solutes, thereby improving the extraction yield [14]. Hence optimizing the soaking time would be useful in achieving the optimal extraction of the biomolecules.

3.8. Comparison of UTPP and MTPP

Similar experiments were performed with ultrasound assisted extraction on three phase partitioning of mangiferin (UTPP) where effect of various parameters such as time, frequency, solute to solvent ratio, power, ammonium salt concentration, duty cycle and

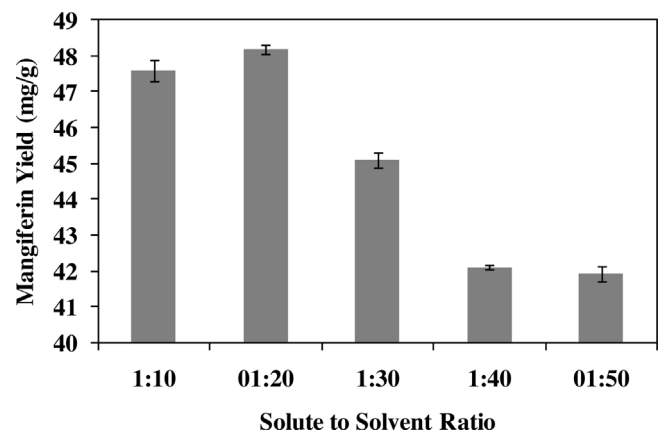


Fig. 5. Effect of solute to solvent ratio at power input of 272 W microwave irradiation time 5 min, ammonium sulphate concentration 40% (w/v), slurry to *t*-butanol ratio 1:1 and 50% duty cycle.

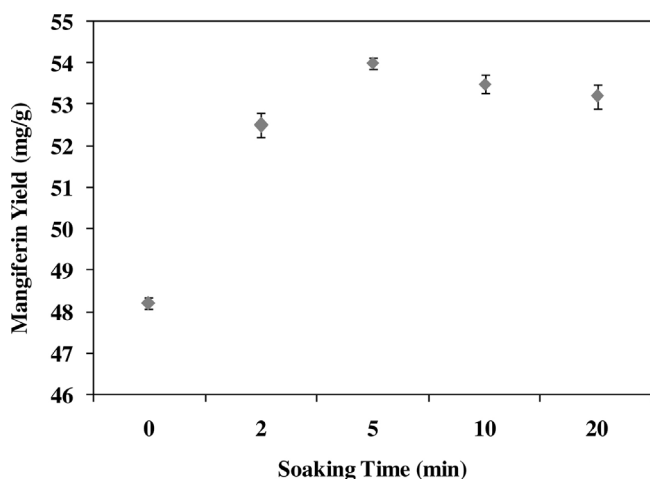


Fig. 6. Effect of soaking time at power input of 272 W microwave irradiation time 5 min, solute to solvent ratio 1:20, ammonium sulphate concentration 40% (w/v), slurry to *t*-butanol ratio 1:1 and 50% duty cycle.

soaking time on mangiferin yield were studied by Kulkarni and Rathod [11]. Mangiferin yield obtained was 41 mg/g in 25 min in UTPP while MTPP yielded 54 mg/g. The yield obtained in MTPP is higher than UTPP because of multiple reasons. Firstly, *M. indica* powder contains some amount of moisture in the cell. Thus when slurry is irradiated with microwave, the moisture present in the particle absorb the energy. As a result, cell wall swells and ruptures releasing the components into the solvent. As soon as the compound is extracted into the solvent, the synergetic action of microwave and dehydrating action of ammonium sulphate pushes mangiferin into the *t*-butanol phase rapidly resulting into simultaneous extraction and purification of mangiferin. Secondly, in our experiments it was observed that power dissipation in microwave is less compared to ultrasound. Hence higher energy in microwave pushes mangiferin into butanol phase rapidly compared to ultrasound treatment. As per Kulkarni and Rathod [11], it is seen that microwave overcomes the limitation of mass transfer with respect to TPP, thereby aiding extraction and separation.

4. Conclusion

It was observed that mangiferin yield obtained by MTPP was about 54 mg/g in 5 min at 272 W power input. The yield in MTPP was 95% of that in soxhlet extraction (57 mg/g) in considerably less time (5 min in MTPP as against 5 h in soxhlet extraction). Also MTPP results in simultaneous extraction and separation by overcoming the limitation of mass transfer with respect to TPP. Hence it can be concluded that MTPP for the extraction of mangiferin proved to be a very efficient and quick technique, which can be used as a prior step before any downstream processing and also for extracting/isolating compounds.

References

- [1] A.O. Aderibigbe, T.S. Emudianughe, B.A. Lowal, Antihyperglycemic effect of *Mangifera indica* in rat, *Phytother. Res.* 13 (1999) 504–507.
- [2] A.O. Aderibigbe, T.S. Emudianughe, B.A. Lowal, Evaluation of antidiabetic action of *Mangifera indica* in mice, *Phytother. Res.* 15 (2001) 456–458.
- [3] C. Dennison, R. Lovrein, Three phase partitioning: concentration and purification of proteins, *Protein Expr. Purif.* 11 (1997) 149–161.
- [4] C.S. Eskilsson, E. Björklund, L. Karlsson, A. Torstensson, Microwave-assisted extraction of felodipine tablets, *J. Chromatogr. A* 1 (1999) 59–70 840.
- [5] F. Chemat, G. Cravotto, *Microwave-assisted Extraction for Bioactive Compounds: Theory and Practice*, Springer Science & Business Media, 2013.
- [6] S. Guha, S. Ghosal, U. Chattopadhyay, Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone, *Chemotherapy* 42 (1996) 443–451.
- [7] Z.K. Guo, Q.H. Jin, Microwave assisted extraction of effective constituents from a Chinese herbal medicine *Radix puerariae*, *Anal. Chim. Acta* 436 (2001) 41–47.
- [8] M. Harde Shirish, S. Singhal Rekha, Extraction of forskolin from *Coleus forskohlii* roots using three phase partitioning, *Sep. Purif. Technol.* 96 (2012) 20–25.
- [9] H. Ichiki, T. Miura, E. Ishihara, Y. Komatsu, K. Tanigawa, M. Okada, New antidiabetic compounds, mangiferin and its glucoside, *Biol. Pharm. Bull.* 21 (1998) 1389–1390.
- [10] A. Jutiviboonsuk, C. Sardsaengjun, Mangiferin in leaves of three Thai mango (*Mangifera indica* L.) varieties, *IJPS* 6 (3) (2010) 122–129.
- [11] V.M. Kulkarni, V.K. Rathod, Extraction of mangiferin from *Mangifera indica* leaves using three phase partitioning coupled with ultrasound, *Ind. Crops Prod.* 52 (2014) 292–297.
- [12] T. Miura, N. Iwamoto, M. Kato, H. Ichiki, M. Kubo, Y. Komatsu, N. Ishida, M. Okada, K. Tanigawa, The suppressive effect of mangiferin with exercise on blood lipids in type 2 diabetes, *Biol. Pharm. Bull.* 24 (2001) 1091–1092.
- [13] K. Padmapriya, A. Dutta, S. Chaudhuri, D. Dutta, Microwave assisted extraction of mangiferin from *Curcuma amada*, *3 Biotech.* 2 (1) (2012) 27–30.
- [14] G. Raman, V.G. Gaikar, Microwave-assisted extraction of piperine from *Piper nigrum*, *Ind. Eng. Chem. Res.* 41 (2002) 2521–2528.419.
- [15] G.M. Sanchez, L. Re, A. Giuliani, A.J. Nunez-Selles, G.P. Davison, O.S. Leon-Fernandez, Protective effects of *Mangifera indica* L. extract: mangiferin and selected antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice, *Pharmacol. Res.* 42 (2000) 565–573.
- [16] T. Sato, A. Kawamoto, A. Tamura, Y. Tatsumi, T. Fujii, Mechanism of antioxidant action of pueraria glycoside (PG)-1 (an isoflavonoid) and mangiferin (a xanthonoid), *Chem. Pharm. Bull.* 40 (1992) 721–724.
- [17] P.C. Veggi, J. Martinez, M. Meireles, A.A. Meireles, Microwave-assisted extraction for bioactive compounds theory and practice, *Fundamentals of Microwave Extraction*, in: F. Chemat, G. Cravotto (Eds.), *Food Engineering Series 4, 2*, Springer, New York, 2013, pp. 15–53 (Chapter).
- [18] S. Vidhate Ganesh, S. Singhal Rekha, Extraction of cocoa butter alternative from kokum (*Garcinia indica*) kernel by three phase partitioning SI: extraction and encapsulation, *J. Food Eng.* 117 (4) (2013) 464–466.
- [19] M.-M. Yan, W. Liu, Y.-J. Fu, Y.-G. Zu, C.-Y. Chen, M. Luo, Optimisation of the microwave-assisted extraction process for four main astragalosides in radix astragali, *Food Chem.* 4 (2010) 1663–1670.
- [20] C. Yoosook, N. Bunyapraphatsara, Y. Boonyakiat, C. Kantasuk, Anti-herpes simplex virus activities of crude water extracts of Thai medicinal plants, *Phytomedicine* 6 (2000) 411–419.
- [21] M.S. Zheng, Z.Y. Lu, Antiviral effect of mangiferin and isomangiferin on herpes simplex virus, *Chin. Med. J.* 103 (1990) 160–165.
- [22] X.M. Zhu, J.X. Song, Z.Z. Huang, Y.M. Wu, M.J. Yu, Antiviral activity of mangiferin against herpes simplex virus type 2 in vitro, *Chung Kuo Yao Li Hsueh Pao* 4 (1993) 452–454.
- [23] T. Zou, H. Wu, H. Li, Q. Jia, G. Song, Comparison of microwave-assisted and conventional extraction of mangiferin from mango (*Mangifera indica* L.) leaves, *J. Sep. Sci.* 36 (20) (2013) 3457–3462.
- [24] C.A. Dennison, *Guide to Protein Isolation (Focus on Structural Biology)*, 2nd edition, (2003) .