

## Research Article

# Study of Extraction Kinetics of Total Polyphenols from Curry Leaves

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Solid-liquid batch extraction of total polyphenol content from curry leaves (*Murraya koenigii* L.) was studied in this paper. The effect of different solvent concentrations and temperatures on total polyphenol content was investigated by performing batch experiments. The experimental studies showed that the kinetics of solid-liquid batch extraction was influenced by different solvent concentrations and temperatures. In solid-liquid batch extraction, more recovery of total polyphenols was obtained for 50% (v/v) aqueous methanol and at 333 K temperature. The total polyphenol obtained at optimum conditions was 79.34 mgGAE/L. Mathematical modelling is an important engineering tool used to study the kinetics of extraction as well as in the design of the extraction process to reduce time, energy, and chemical reagents. Peleg and Power law, the two mathematical models, were used to study the kinetics of the batch extraction process. The Peleg model showed the best fit to explain the kinetics of process with  $R^2 > 0.99$ . Further conventional extraction methods are compared with the novel extraction method.

## 1. Introduction

The extraction of bioactive compounds mainly polyphenols has increased in recent years because of their medicinal value. Polyphenols are the plant secondary metabolites which constitute phenolic acids, flavonoids, stilbenes, and lignans. These are the natural antioxidants that replace synthetic antioxidants such as propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ). The consumption of food rich in polyphenols offers better protection against the development of cancers, cardiovascular diseases, diabetes, osteoporosis, and other diseases. These compounds

are found in fruits, vegetables, spices, cereals, and plants with varied concentrations [1–4]. Herbal plants are loaded with bioactive compounds such as lipids, phytochemicals, pharmaceuticals, flavours, fragrances, and pigments [5]. Curry leaves (*Murraya koenigii* L.), a member of the family Rutaceae, is used as a spice and easily available in India. It is mostly used in curry preparation because of its characteristic flavour and aroma [6]. Curry leaves have the properties of anti-inflammatory, antidysenteric, antioxidant, antidiabetic antimicrobial, antibacterial, positive inotropic, and cholesterol-reducing activities [7, 8].

The polyphenols present in plant materials can be extracted by solid-liquid extraction process. Solid-liquid

extraction is a mass transfer phenomenon in which separation and isolation of desired bioactive compounds or group of compounds from the plant matrix take place by using a suitable solvent. The extraction is a two-stage process: the fast washing stage and the slow diffusion stage. To study the extraction process, the physical and chemical properties of solute and solvent are important and solute-solvent interactions must be known [9]. The studies have been carried out by many researchers for the solid-liquid batch extraction of polyphenols from different raw materials under different extraction conditions such as solvent composition, particle size, solid-to-solvent ratio, time, and temperature [2, 10–13]. The kinetics of the extraction process is well predicted using suitable models [14]. Mathematical modelling is an important engineering tool which is used to facilitate simulation, optimisation, design, and control of processes [2]. By applying the experimental data to model equations, the parameters like mass transfer coefficients in washing and diffusion steps as well as diffusivity can be determined. Fick's law of diffusion [15–17], first- and second-order kinetic modelling [4, 18, 19], Peleg model [2, 13, 20], Power law model [21], and two-site kinetic model [14, 19, 22] are used in the extraction study of polyphenols and other bioactive compounds from plant matrix using conventional and advanced extraction methods.

The present paper focuses on the study of the effect of solvent concentration and temperature on the recovery of total polyphenols from curry leaves by solid-liquid batch extraction. Literature data on mathematical modelling of total polyphenols from the curry leaves are scarce. To have insights into the mathematical modelling, an attempt has been made to study the extraction kinetics by the application of Peleg model and Power law model to the obtained experimental data. The conventional extraction methods are compared with the novel method in terms of percentage recovery of polyphenols.

## 2. Materials and Methods

**2.1. Plant Material.** The curry leaves (*Murraya koenigii* L.) were collected from local market. In laboratory, the leaves were separated from stem and washed with water and finally with distilled water to remove dust and impurities. Sejali and Anur [23] found that the medicinal quality of plant parts was affected due to thermal degradation of active constituents and investigated the effect of drying process on phenolic compounds present in neem leaves powder. They observed that phenolic contents were higher in shade drying powder than oven drying at 45°C and at 70°C. So, the cleaned curry leaves were shade dried at ~ 30–35°C for 10 days. Periodically, the weight was checked to ensure complete removal of moisture. Cleaned and dried samples were ground using a grinder machine (Jaipan). Ground samples were sieved and separated, and 36-mesh (0.425 mm) particle size material was used for

further experimentation. The sample was stored in air-tight containers at 4°C to avoid the direct contact with air.

**2.2. Chemicals.** Gallic acid (Sigma-Aldrich), Folin–Ciocalteu reagent, methanol, and anhydrous sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) of Merck were used in the study.

## 3. Methods

**3.1. Soxhlet Extraction.** The thimble containing 10 g of 0.425 mm of plant material packed in a white muslin cloth was placed between condenser and round-bottom flask containing 400 mL of 50% (v/v) methanol-water solvent [24]. After boiling the solvent, the extraction chamber progressively furnishes with new solvent from the round bottom flask. When meeting the maximum level in the extraction chamber, the condensed solvent from the extraction chamber was flushed back into the round bottom flask by a siphon. This condensed solvent carries the solutes into the solvent. The extraction was performed for 7 h and sample was analysed for the total polyphenol content. The result obtained from the Soxhlet method is considered as maximum value of polyphenol concentration and the rest of the experimental values from batch and ultrasound-assisted extraction were compared with this.

**3.2. Batch Extraction.** The solid-liquid batch extraction was performed in a glass reactor of 300 mL capacity equipped with a three-blade marine propeller of diameter 4 cm to provide the stirring. The stirring helps in avoiding the settling of the solid particles due to gravity. The experiments were performed to study the effect of methanol-water solvent compositions (0%, 50%, and 100% v/v) and temperatures (313, 323, and 333 K) for the particle size of 0.425 mm and solid-to-solvent ratio of 1:40 (g/mL). The batch extraction was performed for 7 h. The samples were withdrawn at fixed intervals and filtered with Whatman No. 42 filter paper prior to analysis.

**3.3. Ultrasound-Assisted Extraction.** Ultrasound-assisted extraction (UAE) was performed by using ultrasound probe DP 120 model supplied by PCI Analytics, Mumbai, of 20 kHz frequency and 250 W power. The probe was operated in pulse mode (10 s on-off) to avoid heat dissipation and loss of solvent due to heat [25]. The other experimental conditions were kept similar as in batch extraction. The mixture was sonicated for 1 h using 50% (v/v) methanol-water solvent concentration and samples were withdrawn at fixed time interval, filtered, and analysed.

**3.4. Determination of Total Polyphenol Content (TPC) by Folin–Ciocalteu Method.** TPC was determined by the Folin–Ciocalteu method [26]. For all analysis, 1.0 mL of sample was added to at least 60 mL of distilled water in a

100 mL volumetric flask. 5 mL of Folin–Ciocalteu reagent was added to the flask and mixed. After 1 min and before 8 min, 15 mL of 20% sodium carbonate solution was added and the volume was adjusted to 100 mL with distilled water. The samples were kept in water bath at 40°C for 30 min, and the absorbance was measured at 765 nm wavelength in a 3 mL cuvette using UV double beam spectrophotometer (Model: Shimadzu UV1800, Japan). A calibration curve was prepared from gallic acid standard solutions (50, 100, 150, 200, 500, and 1000 mg/L) ( $R^2 = 0.996$ ) and the blank was prepared with distilled water. The total polyphenol content was expressed as mgGAE/L.

**3.5. Mathematical Models.** The kinetics of total polyphenols extraction by batch process was studied by fitting the obtained experimental data to Peleg and Power law model equations.

**3.6. Power Law Model.** The Power law model is one of the empirical equations used in kinetic study of solid-liquid extraction. The model has been used in kinetic study of camptothecin from *Nothapodytes nimmoniana* [21] and vanillin from *Vanilla planifolia* Andrews [27] by microwave-assisted extraction.

The equation for Power law model is

$$C_t = Bt^n, \quad (1)$$

where  $C_t$  is a concentration of total polyphenols in extraction solvent (mgGAE/L) at time  $t$  (s),  $n$  is Power law exponent (<1), and  $B$  is constant related to extraction rate (L/g s).

**3.7. Peleg Model.** Peleg proposed a nonexponential empirical equation in 1988 for sorption curves, i.e., moisture versus time [28]. Because of the similarity between the sorption curves and concentration curves, Peleg model can also be used to explain the kinetics of solid-liquid extraction [2]. The model has been used to explain the kinetics of solid-liquid extraction of polyphenols from soybeans [13] and grape seeds [2]. The equation for Peleg model is

$$C(t) = C_0 + \frac{t}{K_1 + K_2(t)}, \quad (2)$$

where  $C(t)$  is the concentration of total polyphenols at time  $t$  (mgGAE/L),  $C_0$  is the concentration of total polyphenols (mgGAE/L) at time  $t = 0$ ,  $K_1$  = Peleg's rate constant that relates the initial extraction rate (min.g/mg), and  $K_2$  = Peleg's capacity constant that relates the maximum extraction yield (g/mg). In the solvent, the initial concentration of polyphenols is zero; hence, the term  $C_0$  can be excluded from equation (2). Therefore, equation (2) is modified as

$$C(t) = \frac{t}{K_1 + K_2(t)}. \quad (3)$$

**3.8. Statistical Analysis.** The consistency between the predicted and the experimental data was examined using the

coefficient of correlation ( $R^2$ ) and root mean squared error (RMSE), which are defined as

$$R^2 = 1 - \frac{\sum_{i=1}^n (x_i - \hat{x}_i)^2}{\sum_{i=1}^n (x_i - \bar{x})^2}, \quad (4)$$

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^n (x_i - \hat{x}_i)^2},$$

where  $n$  is the number of samples,  $x_i$  is the experimental value of the  $i^{\text{th}}$  sample,  $\hat{x}_i$  is the predicted value of the  $i^{\text{th}}$  sample, and  $\bar{x}$  is the mean value of all experimental data, with a high  $R^2$  value and low RMSE value indicating high consistency between the experimental and predicted data.

## 4. Results and Discussion

**4.1. Effect of Solvent Concentration on TPC.** The selection of an appropriate solvent or solvent mixture is an important step in extraction of polyphenols from the plant matrix. The polyphenols mainly consist of phenolic acids, flavonoids, stilbenes, and lignans and have a complex structure. Hence, it is difficult to predict the solute-solvent interactions. The choice of a solvent depends on polarity, the solubility of solute in the given solvent, reactivity, safety, and cost. The polar compounds are soluble in polar solvents such as methanol, ethanol, ethyl acetate, and acetone, whereas nonpolar compounds are soluble in nonpolar solvents such as toluene, benzene, hexane, and cyclohexane. It was found that the mixtures of alcohol and water work best in extracting phenolic compounds compared to the mono-component solvent [29]. The effect of methanol-water solvent compositions (0%, 50%, and 100% v/v) on extraction of total polyphenols by batch extraction for particle size of 0.425 mm and solid-to-solvent ratio of 1 : 40 (g/mL) and at room temperature (27°C) was studied. It was observed from Figure 1 that maximum amount of total polyphenol content of 75.42 mgGAE/L was obtained for 50% (v/v) methanol-water composition, while less concentration of total polyphenols was obtained for absolute water and absolute methanol solvents. For all solvent compositions, it was seen that initially the rate of extraction of polyphenols was higher and afterwards it remains constant approaching towards equilibrium. The concentrations obtained at 7 h were considered as equilibrium concentrations attained during extraction. The probable reason for obtaining the higher amount of polyphenols in 50% (v/v) methanol-water composition is that the addition of small amount of water to methanol increases the polarity of the medium and breaking of hydrogen bonding, thus facilitating the extraction [30]. The polarity index of 50% (v/v) methanol-water mixture is 7.05 which is higher than methanol. Also, the addition of water increases the swelling of the plant material which in turn enhances the contact surface area for solvent-solid. Increasing the concentration of methanol in solvent mixture causes protein denaturation and prevents the dissolution of polyphenols in the solvent and hence affects the extraction rate [31]. Our findings are in accordance with Jovanovic et al. [32]. They reported a maximum yield of total polyphenol of

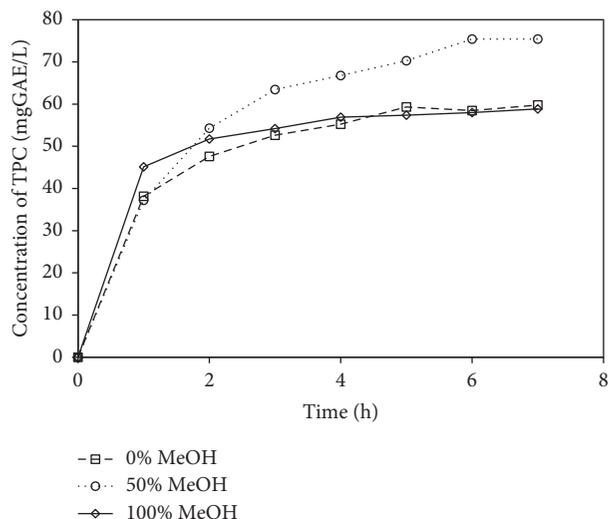


FIGURE 1: Effect of solvent composition on the concentration of TPC by batch extraction.

19.6 mgGAE/L by maceration, 22.6 mgGAE/L by heat-assisted extraction, and 24.9 mgGAE/L by UAE from *Thymus serpyllum* leaves at 50% ethanol-water concentrations. Costa et al. [33] found 50% ethanol-water as the best solvent for the extraction of polyphenols from *Thymus lotocephalus*. Jokic et al. [13] also found 50% ethanol-water as the best solvent for extraction of polyphenols from soybeans by solid-liquid batch extraction. It was found from the literature that 40–60% (v/v) alcohol-water (ethanol or methanol) works best to get the higher yield of polyphenols from the plant material. It was also reported that extraction of polyphenols from curry leaves was carried out by using water, ethanol, ethanol-water (1 : 1), hexane, and chloroform as the solvents [6] and obtained a highest yield of 168 mgGAE/g of extract from 1 : 1 ethanol-water mixture. The specific difference in results exists due to methodologies followed, experimental work, variety, and location of plant material.

**4.2. Effect of Temperature on TPC.** The temperature is an important factor influencing the extraction of polyphenols from the different plant matrices. With increasing temperature, the solubility and diffusivity of phenolic compounds increase and enhance the extraction of polyphenols. Also increasing the temperature reduces the surface tension and viscosity of the extraction medium, thus accelerating the mass transfer rate and hence extraction of polyphenols [4]. The batch extraction was performed at 313, 323, and 333 K for particle size of 0.425 mm, solid-to-liquid ratio 1 : 40 (g/mL), and methanol-water composition 50% (v/v). Figure 2 shows the positive influence of temperature on the concentration of polyphenols. The extraction of polyphenols has increased from 313 to 333 K. This increase in the concentration of TPC might be due to increased diffusivity of polyphenols into solvent. The maximum amount of polyphenols obtained at 333 K was 79.34 mgGAE/L. The rate of extraction was fast in the initial 1.5 hours, i.e., washing stage,

and thereafter, it was slow in the diffusion stage. However, a too high temperature may cause loss of solvent by evaporation and thermal degradation of polyphenols may affect the efficiency of extraction [34]. Ghitescu et al. [35] observed similar results for the temperatures of 313–333 K for extraction of polyphenols from spruce wood bark by UAE. Wang et al. [36] also achieved similar results of extraction of phenolic compounds from wheat bran by increasing temperature from 298 K to 348 K.

**4.3. Comparison of Kinetic Models.** The experimental data obtained by performing batch extraction were analysed by using two kinetic models: the Peleg model and the Power law model. The mathematical modelling of the process helps to understand the complex diffusion and mass transfer phenomena. Evaluation of best fit among the models was done based on the value of correlation coefficient ( $R^2$ ) and root mean squared error (RMSE).

**4.4. Peleg Model.** The graph of  $1/C_t$  versus  $1/t$  yields the values of Peleg model constants  $K_1$  and  $K_2$ . The lower values of  $K_1$  and  $K_2$  imply the faster rate of extraction and the maximum concentration at equilibrium, respectively. The calculated Peleg's model constants  $K_1$  and  $K_2$ , experimental and predicted equilibrium concentrations, and values of  $R^2$  and RMSE are shown in Table 1. The values of  $R^2$  are in the range of 0.997–0.998 and RMSE are in the range of 0.343–1.267 (Table 1). The higher values of correlation coefficients and lower values of RMSE signify that the model fits the experimental data well compared to the Power law model. With higher values of  $R^2$ , Figure 3 shows good correlation between all experimental and predicted values of total polyphenol concentration for Peleg model at different temperatures and methanol-water solvent compositions.

**4.5. Power Law Model.** In this model, a plot of  $\ln C_t$  versus  $\ln t$  yields the values of Power law constants  $n$  and  $B$ . The values of the Power law model constants,  $R^2$ , and RMSE are shown in Table 2. The values of  $R^2$  are in the range of 0.954–0.991, while RMSE are in the range of 0.543–4.0 (Table 2). Figure 4 shows experimental and predicted concentration values of total polyphenols for Power law model at different temperatures and methanol-water solvent compositions.

**4.6. Comparison of Extraction Methods.** UAE is a novel, clean, and green extraction method. UAE requires less time, solvent, and energy compared to conventional extraction methods. The Soxhlet extraction and batch extraction methods were compared with the UAE method. The comparison of methods in terms of percent extraction of total polyphenol content is shown in Figure 5. In all three methods, a particle size of 0.425 mm, solid-to-solvent ratio of 1 : 40 g/mL, and solvent concentration of 50% (v/v) methanol-water were maintained. Soxhlet extraction that was performed for 7 h results in total polyphenols concentration of 100.66 mgGAE/L and was considered as 100% extraction and the remaining values were compared with

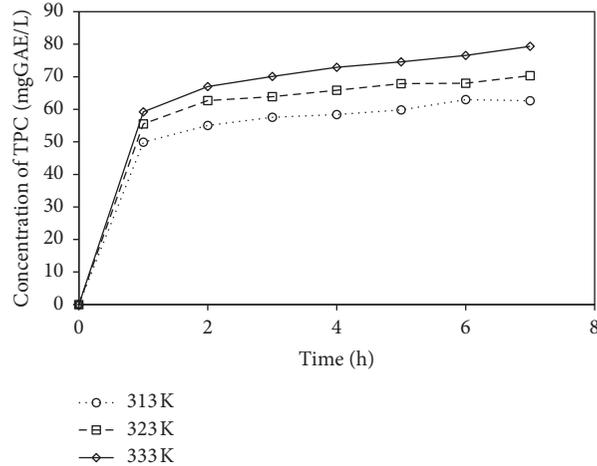


FIGURE 2: Effect of temperature on the concentration of TPC by batch extraction.

TABLE 1: Kinetic parameters for Peleg model and experimental and predicted equilibrium concentration values at different extraction conditions.

Parameter	$K_1$ (g min/mg)	$K_2$ (g/mg)	Experimental $C_e$ (mgGAE/L)	Predicted $C_e$ (mgGAE/L)	$R^2$	RMSE
<i>Temperature (K)</i>						
313	0.0046	0.0156	62.62	61.51	0.997	1.031
323	0.0041	0.0140	70.34	68.56	0.998	0.827
333	0.0047	0.0124	79.34	76.41	0.997	1.267
<i>Methanol-water conc. (%v/v)</i>						
0	0.0112	0.0151	59.78	59.88	0.998	0.686
50	0.0156	0.0108	75.42	76.50	0.998	0.954
100	0.0006	0.0162	58.87	58.62	0.997	0.343

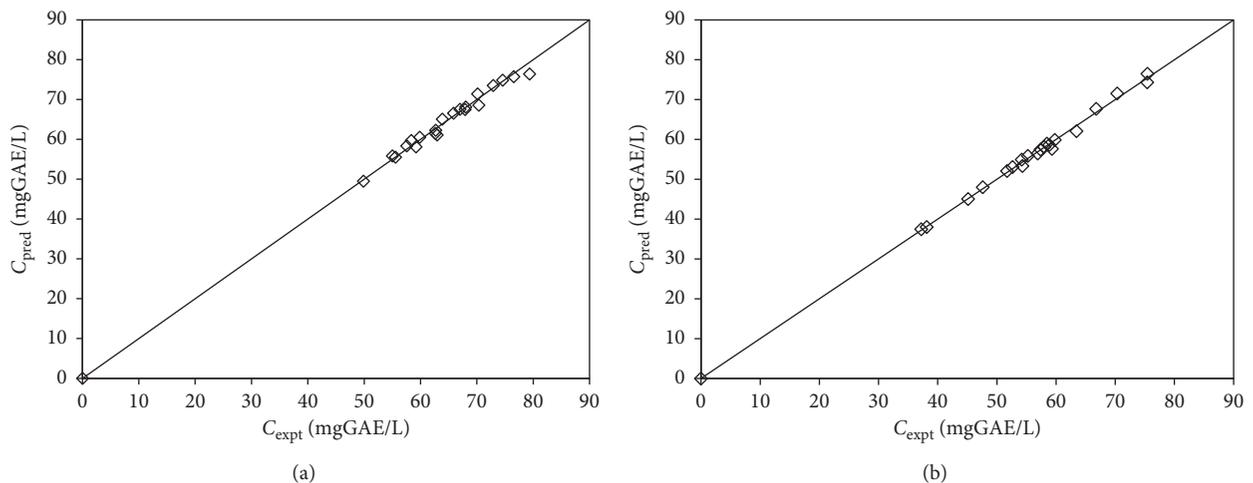


FIGURE 3: Correlation between experimental versus predicted values for concentrations TPC obtained using Peleg model for different temperatures (a) and solvent compositions (b).

this base value [25]. The percentage extraction obtained by batch extraction for 7 h was 74.92% whereas percentage extraction by ultrasound assistance was 65.68% in 1 h of duration. Though higher recovery of polyphenols was obtained in Soxhlet extraction, it requires more time and

energy. UAE results in better recovery of polyphenols than the conventional methods. Since in UAE due to acoustic cavitation, the rupture of plant cells occurs, facilitating the diffusion of solute into the solvent and thus enhancing the efficiency.

TABLE 2: Kinetic parameters for Power law model and experimental and predicted equilibrium concentration values at different extraction conditions.

Parameter	$B$ (L/g s)	$n$ (g/mg)	Experimental $C_e$ (mgGAE/L)	Predicted $C_e$ (mgGAE/L)	$R^2$	RMSE
<i>Temperature (K)</i>						
313	31.14	0.116	62.62	63.01	0.978	0.613
323	35.67	0.112	70.35	70.25	0.966	0.770
333	33.36	0.142	79.34	78.85	0.991	0.543
<i>Methanol-water conc. (%v/v)</i>						
0	15.30	0.232	59.78	62.11	0.961	1.481
50	9.252	0.357	75.42	70.25	0.954	4.00
100	26.68	0.134	58.87	59.97	0.959	0.865

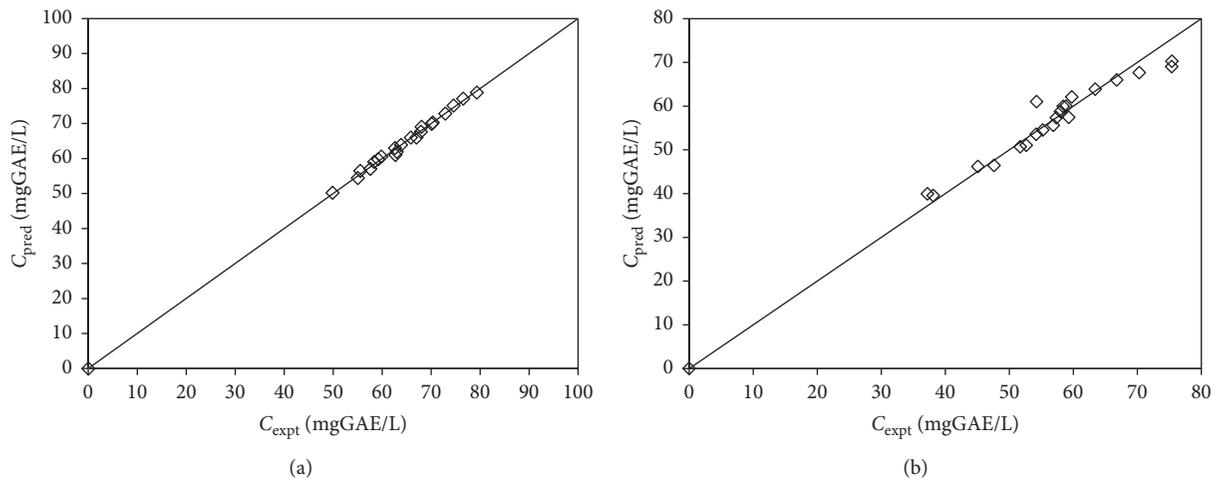


FIGURE 4: Correlation between experimental versus predicted values for concentrations of TPC obtained using Power law model for different temperatures (a) and solvent compositions (b).

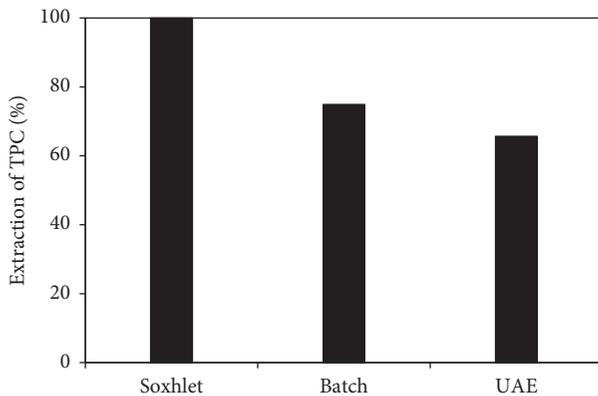


FIGURE 5: Comparison of extraction methods for particle size 0.425 mm, solid-to-solvent ratio 1:40 (g/mL), and solvent concentration of 50% (v/v) MeOH-water.

## 5. Conclusion

The kinetics of solid-liquid batch extraction of total polyphenols from curry leaves was investigated. The results showed that the temperature and solvent concentration have a great impact on extraction efficiency. The optimal batch extraction conditions found are temperature of 333 K and methanol-water concentration of 50% (v/v). Under these

optimal conditions, the maximum concentration of total polyphenol content of 79.34 mgGAE/L was obtained. Mathematical modelling study showed that Peleg model fits the experimental data well. The positive correlation was found between all experimental and predicted values of total polyphenol concentration. The comparison of conventional Soxhlet and batch extraction methods with novel UAE method showed that less time and energy were required for UAE to get higher recovery of polyphenols than conventional methods.

## Abbreviations

- TPC: Total polyphenol content
- GAE/L: Gallic acid equivalent/litre
- UAE: Ultrasound-assisted extraction
- RMSE: Root mean squared error
- $C(t)$ : Concentration of total polyphenols at time  $t$
- $C_e$ : Equilibrium concentration
- $C_S$ : Concentration of total polyphenols at saturation
- $k$ : Rate constant of extraction
- $K_1$ : Peleg's rate constant
- $K_2$ : Peleg's capacity constant
- $n, B$ : Model fitting parameters (Power law model)
- $t$ : Time
- $R^2$ : Coefficient of correlation.

## Data Availability

The results reported in the article are available from the corresponding author to other researchers upon request. Codes cannot be shared with other researchers.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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