



Effects of Binary Solvent System on Radical Scavenging Activity and Recovery of Verbascoside from *Lemon verbena* Leaves

Rana Roshani Neshat ^a | Mandana Bimakr ^{a*} | Ali Ganjloo ^a

a. Department of Food Science and Engineering, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

*Corresponding author: Department of Food Science and Engineering, Faculty of Agriculture, University of Zanjan, Zanjan, Iran. Postal code: 45371-38791.

E-mail address: Mandana.bimakr@znu.ac.ir

ARTICLE INFO

Article type:
Original article

Article history:
Received: 4 April 2020
Revised: 18 May 2020
Accepted: 27 May 2020

DOI: [10.29252/jhehp.6.2.4](https://doi.org/10.29252/jhehp.6.2.4)

Keywords:
Lemon verbena
Verbascoside
Binary solvent system
RP-HPLC
Kinetic mass transfer

ABSTRACT

Background: Verbascoside is the major biophenolic compound of *Lemon verbena* leaf. This study aimed to investigate the effects of the binary solvent system on the free radical scavenging activity (FRSA) and verbascoside recovery from *L. verbena* leaves, as well as the kinetic mass transfer of verbascoside.

Methods: Classic extraction was performed using various ratios of ethanol (EtOH) and water (H₂O) (50:50-90:10% v/v). The FRSA was analyzed using spectrophotometric methods (2,2-diphenyl-1-picrylhydrazyl [DPPH] and hydroxyl radical [HO·] assays). Reversed-phase high-performance liquid chromatography was used for the qualification and quantification of verbascoside, and the Peleg model described the kinetic mass transfer of verbascoside.

Results: The hydroethanolic solvent was composed of EtOH:H₂O (80:20% v/v) as the optimal medium for the maximum recovery of verbascoside (19.20 ± 0.12 mg/g) and FRSA of the extracts (45.25 ± 0.95% DPPH_{sc}% and 31.17 ± 1.20% HO_{sc}%). The Peleg model had a proper fit for the observed data with the highest coefficient of determination (R² = 0.999), the lowest root mean square error (RMSE = 0.093), and the mean relative percentage deviation modulus (E = 0.968).

Conclusion: The valuable bioactive compounds of *L. verbena* could be successfully extracted using the binary solvent system. The Peleg model is also an efficient non-linear model to describe the verbascoside release rate during extraction.

1. Introduction

The chemotherapeutic applications of medicinal plants are known in the prehistoric records of mankind. Since ancient times, various herbal medicines have been used for the treatment numerous diseases. A wide range of organic compounds as primary and secondary metabolites are produced by plant species. Secondary metabolites possess various chemical structures, which are capable of specific biological effects on the human health. Plants produce these valuable compounds through their defense mechanisms against pathogens, microbes, predators, and abiotic stress [1]. In particular, herbal sources possess diverse valuable

bioactive compounds, such as polyphenols, phenolic acids, flavonols, flavanols, flavonoids, procyanidins, proanthocyanidins, anthocyanins, glycosides, carotenoids, saponins, tannins, alkaloids, sterols, steroids, triterpenes, quinones, peptides, and carbohydrates, which have been reported to have variable biological activities, including antibacterial, antioxidant, antiviral, antifungal, antimicrobial, anticancer, anti-diabetic, anti-diarrheal, anti-mutagenic, anti-inflammatory, antihypertensive, anti-cardiovascular, and anti-cholesterol properties [2]. Finding the alternative natural and safe sources of antioxidant compounds has been the focus of numerous studies



How to cite: Roshani Neshat R, Bimakr M, Ganjloo A. Effects of Binary Solvent System on Radical Scavenging Activity and Recovery of Verbascoside from *Lemon verbena* Leaves. *J Hum Environ Health Promot.* 2020; 6(2): 69-76.

considering the adverse consequences of synthetic antioxidants on the human health and environment.

Lemon verbena, also known as *Lippia citriodora* or *Aloysia citrodora*, is a deciduous flowering perennial shrub from the Verbenaceae family [3]. With its lemon-like aroma, *L. verbena* is used in herbal teas and as a flavoring agent for foods and beverages. The traditional use of *L. verbena* in medicine is to alleviate fever, gastrointestinal disorders, spasms, asthma, and anxiety attacks [3]. The main components of *L. verbena* are flavonoids, iridoid glycosides, and phenylpropanoids with verbascoside as the most abundant constituent [4].

Extraction is considered to be the most critical step in various analytical approaches of chemistry. The extraction process is affected by several factors, such as capital/operating costs, operation simplicity, being environmentally friendly, the amount and type of the liquid phase, and the availability and practicality of the standard procedures [5,6]. Various conventional methods are used to extract natural compounds from plants; such examples are hydrodistillation, Soxhlet, and maceration. The principle of these methods is the selection of a solvent coupled with heat and/or agitation. Conventional Soxhlet extraction (CSE) was developed by Franz von Soxhlet in 1879 and is considered to be the primary reference for evaluating the performance of other solid-liquid extraction methods [5,7]. In the CSE method, the proper choice of the extraction solvent for the recovery of the targeted compounds from plants is essential. The application of various solvents in this method has resulted in different crude extracts with diverse compositions.

The knowledge of kinetics of bioactive extraction processes is of particular technological significance. Kinetic studies could facilitate the estimation of the dynamic extraction time for the complete recovery of the targeted compounds from a natural matrix. The Peleg model is a two-parameter empirical model, which is used to assess the kinetics and predict the equilibrium concentrations of bioactive compounds.

To the best of our knowledge, no prior studies have attempted to produce a verbascoside-rich extract from *L. verbena* leaves using environmentally friendly and non-toxic food grade binary solvent systems, such as H₂O and ethanol (EtOH). The present study aimed to evaluate the effects of various EtOH:H₂O solvents (50-90% of EtOH) on the free radical scavenging capacity and verbascoside content of *L. verbena* leaf extract. Furthermore, the kinetic studies were carried out using the Peleg model to obtain a better understanding of the mass transfer behavior of verbascoside recovery during the extraction process.

2. Materials and Methods

2.1. Materials and Chemicals

L. verbena leaves were collected from a botanical garden in the Modern Biological Techniques Research Center in Zanjan, Iran. Pure EtOH (analytical grade) and methanol (chromatography grade) were purchased from Scharlau Chemical (European Union). Potassium persulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and verbascoside

standard were purchased from Sigma-Aldrich (Kappelweg, Schnelldorf, Germany).

2.2. Binary Solvent Extraction

The leaves were washed with distilled water and dried at the temperature of 40°C for 24 h and processed using a grinder mill (MX-335, Panasonic, KL, Malaysia) before extraction. The approximate size of the samples was 1.5-2.5 mm. The binary solvent extraction (BSE) was performed using the EtOH-H₂O solvent and a Soxhlet apparatus. In brief, 3 g of the *L. verbena* leaf samples was placed in a thimble and inside the main chamber of the Soxhlet extractor. The vessel was attached to a distillation flask and filled with the binary solvent. Approximately 150 ml of a hydroethanolic solvent containing EtOH and H₂O at various ratios (50:50%, 60:40%, 70:30%, 80:20%, and 90:10% v/v, respectively) were added to each container. The range of the hydroethanolic solvent concentration and ratio of the meal to the EtOH:H₂O binary solvent (1:30) were determined based on the primary observations.

Each extraction process was performed in triplicate for six hours. After extraction, the solvent was removed through vacuum rotary evaporation (RV10, IKA, Staufen, Germany), and the samples were placed in an oven (KM-85, Pars Azma Co., Tehran, Iran) at the temperature of 40°C to reach the constant weight. Finally, the samples were preserved at the temperature of -18°C for further chemical analysis. Following that, extraction was performed using the optimal hydroethanolic concentration for the modeling of the mass transfer kinetics of verbascoside recovery. In total, 10 ml of the samples was maintained at 30-min intervals during extraction for the reversed-phase high-performance liquid chromatography (RP-HPLC) analysis.

2.3. Free Radical Scavenging Assays

2.3.1. DPPH Assay

The DPPH radical scavenging ability of the extracts was measured using the procedure suggested by Yen and Chen (1995) [8]. In brief, DPPH (100 μM) was dissolved in 96% EtOH, and fresh radical stock solution was prepared daily. One milliliter of the prepared solution was mixed with one milliliter of the extract and three milliliters of EtOH. After vortexing for one minute, the absorbance of the samples was read at 517 nm in a one-centimeter quartz cell after 1-60 min at 10-min intervals using a UV-Vis spectrophotometer (SPECORD 250, Analytik Jena, Jena Germany). The inhibition rate of the scavenged DPPH free radicals (%DPPH_{sc}) was calculated using Equation 1:

$$\%DPPH_{sc} = (A_b - A_s) / A_b \times 100 \quad (1)$$

where A_b shows the absorbance of the blank, and A_s represents the absorbance of the samples.

2.3.2. Hydrogen Peroxide Assay

The hydroxyl radical (HO·) scavenging activity of the extracts was determined based on the procedure proposed by Poodi *et al.* (2018) with some modifications [9]. Initially, monopotassium phosphate (0.24 g), disodium (1.44 g),

sodium chloride (7.9 g), and potassium chloride (0.2 g) were dissolved in distilled water (1,000 ml) in order to prepare phosphate buffer saline (0.01 M) with the pH of 7.4. Afterwards, 1,10-phenanthroline monohydrate (27.03 g) was dissolved in 200 ml of EtOH to prepare 0.75 phenanthroline. To prepare ferrous sulfate (0.75 mM), ferrous sulfate.2H₂O (0.114 g) was dissolved in 1,000 ml. At the next stage, phenanthroline (2 mL) was added to phosphate buffer (4 ml), ferrous sulfate (2 ml), and the sample solution (2 ml). The prepared solution was shaken, and hydrogen peroxide (2 ml; 0.01% w/v) was mixed with the solution. The sample absorbance was read at 536 nanometers after incubation at the temperature of 37°C for 60 min. Finally, both the sample solution and hydrogen peroxide were replaced by the same volume of distilled water, and the absorbance was obtained. In addition, the inhibition rate of the scavenged hydroxyl radicals (%HO_{sc}) was determined using Equation 2:

$$\%HO_{sc} = (A_b - A_s) / A_b \times 100 \quad (2)$$

where A_b is the absorbance of the blank, and A_s shows the absorbance of the samples.

2.4. Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

The samples were analyzed using the RP-HPLC system, which was composed of a UV-Vis detector (model: Varian 9050; Runcorn, Cheshire, UK) and an HPLC pump (model: Varian 9012; CA, USA). Analytical separation was carried out using an Eclipse reverse phase-C18 column (25 cm × 4.6 mm × 5 μm; Supelco, Bellefonte, PA, USA). The column temperature remained ambient, and the injection volume was set at 20 microliters. The solvents were composed of water (solvent A) and methanol (solvent B), and the elution mode was selected as the gradient for the better separation of verbascoside (Table 1). Standard verbascoside was dissolved in the HPLC-grade methanol, and quantitative analysis was performed based on standard linear calibration curves [10].

2.5. Statistical Analysis and Modeling of the Mass Transfer Kinetics

In the present study, the effect of hydroethanolic concentration was investigated on the verbascoside recovery and free radical scavenging activity (FRSA) of the products in terms of %HO_{sc} and %DPPH_{sc}. The significance of the effects of the process parameters ($P < 0.05$) was assessed using the one-way analysis of variance (ANOVA) in the Minitab software version 17 (State College, PA, USA). The experimental data were observed by triplicate analysis and expressed as mean and standard deviation (SD). Furthermore, Pearson's correlation-coefficient was used to distinguish the between FRSA and verbascoside content of the extracts. For the modeling of the mass transfer kinetics, non-linear regression was performed using the Levenberg-Marquardt method to fit the database to the Peleg equations in the STATISTICA software version 6.0 (StatSoft, Inc., Tulsa, OK, USA).

Table 1: Gradient Elution Program of RP-HPLC Mobile Phase for Analysis of Verbascoside in *L. verbena* Leaves

Time (min)	Flow rate (mL/min)	A%	B%
0	1.40	100	0
5	1.40	90	10
10	1.40	85	15
15	1.40	80	20
20	1.40	70	30
25	1.40	60	40
30	1.40	50	50
35	1.40	40	60
40	1.40	0	100

The equation of the Peleg model expressing the kinetics of the verbascoside recovery has been presented in Equation 3:

$$C_t = C_0 + \frac{t}{K_1 + K_2 t} \quad (3)$$

where C_t is the verbascoside content at time t (mg of verbascoside/g), K₁ shows the Peleg rate constant (min/g/mg), K₂ is the Peleg capacity constant (g/mg), and C₀ represents the primary verbascoside content, which was equal to zero due to the use of the fresh solvent.

Extraction is often performed in two phases with the first-order behavior in the first phase of the process and zero-order behavior in the second phase. The modified Peleg equation that revealed the content of the target solute in the surrounded medium versus time could be described as Equation 4:

$$C_t = \frac{t}{K_1 + K_2 t} \quad (4)$$

The curve between 1/C_t against (Peleg rate constant) 1/t could be drawn to determine the K₁ and K₂ values from the slope and intercept, respectively. Furthermore, C_t was calculated using Equation 4 at various extraction times in order to determine the fitting of the proposed model [11]. When t tends to ∞, Equation 4 provides the relations between the equilibrium content of the target solute, and K₂ could be determined using Equation 5.

$$\lim_{t \rightarrow \infty} C_t = C_{eq} = \frac{1}{K_2} \quad (5)$$

The developed model was evaluated based on various parameters, including the coefficient of determination (R₂), root mean square error (RMSE), and mean relative percentage deviation modulus (E). These values were calculated using Equations 6-8:

$$R^2 = \frac{\sum_i (V_{exp} - V_{pre})^2}{\sum_i (V_{exp} - \bar{V}_{pre})^2} \quad (6)$$

$$RMSE = \frac{1}{n} \left[\sum_{i=1}^n (V_{exp} - V_{pre})^2 \right]^{0.5} \quad (7)$$

$$E(\%) = \frac{100}{n} \sum_{i=1}^n \frac{|V_{exp} - V_{pre}|}{V_{exp}} \quad (8)$$

where V_{exp} is the experimental/observed data, V_{pre} shows the predicted data, n is the number of the observed data, and \bar{V}_{pre} represents the mean of the observed data. Notably, a model is considered acceptable with the highest R₂ value and the lowest E and RMSE values [12].

3. Results and Discussion

3.1. Effect of the Hydroethanolic Solvent Concentration on the Free Radical Scavenging Activity of the Extracts

In the current research, the binary solvent extraction system composed of EtOH and H₂O was used to obtain the extract of the *L. verbena* leaves, which could be safely introduced into the food, pharmacy, and cosmetic industries. The FRSA of the extracts was assessed using the DPPH and HO assays. Several methods are available to measure the antioxidant capacity of extracts in terms of the FRSA, while no single assay has been recommended for the evaluation of antioxidant capacity due to the variety of the reaction mechanisms. The DPPH and HO radical scavenging activity assays have successfully been employed in numerous studies for the evaluation of the antioxidant activity of natural sources [13-15].

Among reactive oxygen species, hydrogen peroxide (H₂O₂) is stable at physiological temperatures and pH and has the ability to diffuse the membrane and penetrate to long distances, which is indicative of its critical effect in the pathogenesis of various disorders [16]. Furthermore, H₂O₂ is not considered toxic and could change into other more toxic hydroxyl radicals, which interfere with cellular damages. H₂O₂ could also produce singlet oxygen through reaction with hypochlorous acid, superoxide anion or chloramines in living systems [17,18]. Therefore, the measurement of H₂O₂ scavenging ability is an important stage in the evaluation of the antioxidant capacity of bioactive extracts.

DPPH radical scavenging capacity is a rapid, simple, and inexpensive method used for the assessment of the free radical scavenging capacity of numerous natural extracts. DPPH generates stable organic nitrogen radicals, which are detected by a dark purple color within the range of 515-520 nanometers [19]. The DPPH radical is one of the few stable radicals that acts in hydrogen atom transfer and single electron transfer systems, making it possible to detect a substance or a complex mixture and donate the electrons or hydrogen atoms in a homogeneous system [9].

According to the findings of the current research, the *L. verbena* leaf extract with various ratios of the EtOH:H₂O binary solvent system (50:50-90:10% v/v) possessed DPPH[•] (inhibition range: 13.10 ± 1.35-46.26 ± 1.10%) and HO[•] scavenging activity (inhibition range: 10.19 ± 1.55-33.20 ± 1.30%). Table 2 shows the FRSA of the *L. verbena* leaf extract in terms of %DPPH_{sc} and %HO_{sc}.

The results of the present study indicated the ability of the *L. verbena* leaf extract to neutralize free radicals, which have been reported to cause various clinical diseases, such as renal failure, cancer, diabetes mellitus, liver disorders,

and degenerative diseases [20,21]. The obtained results also demonstrated that the highest FRSA values of the extract were achieved using the binary solvent system composed of the 90:20% v/v ratio of EtOH:H₂O (46.26 ± 1.10% DPPH_{sc} and 32.20 ± 1.30 HO_{sc}), while no significant changes were observed with the binary solvent system composed of the 80:20% v/v ratio ($P > 0.05$) (Table 2). In a study in this regard, Waszkowiak and Gliszczynska-Swiglo (2016) reported that the percentage of EtOH in a binary extraction solvent had a significant effect on the antioxidant activity of the extract ($P < 0.05$) [22]. Furthermore, the mentioned study indicated that the highest antioxidant activity of the flaxseed extract could be achieved using the binary system composed of EtOH:H₂O (90:10% v/v). Figure 1-A, B shows the FRSA of the hydroethanolic extract in terms of DPPH[•] and HO[•] free radical scavenging during the reaction time (60 and 10 minutes, respectively). These findings confirmed the accuracy of the FRSA result.

3.2. Effect of the Binary Solvent System on the Verbascoside Recovery

According to the literature, nutraceutical compounds are the important antioxidants that have emerged in the research conducted in recent decades in food science and technology. Verbascoside is a caffeoyl phenylethanoid glycoside, which is found in medicinal plants. In various studies, it has been stated that verbascoside is the main phenolic compound in *L. verbena* leaf [3,4,23]. The chemical structure of this compound is depicted in Figure 2.

Figure 3 depicts a typical RP-HPLC chromatogram of the hydroethanolic extract of *L. verbena*. Figure 4 shows the concentration of verbascoside as quantified directly from the RP-HPLC chromatograms. According to the results of the present study, *L. verbena* leaf was a potential source of valuable verbascoside compounds, which could be extracted using a proper solvent. A solvent is a substance that dissolves a solute to create a solution. The solubility of the solute in the solvent is affected by molecular polarity. In other words, polar molecules are desirably dissolved by polar solvent molecules and vice versa. Therefore, we examined various ratios of EtOH and H₂O to select the optimal extraction solvent. According to the findings of the current research, the verbascoside recovery enhanced with the increased EtOH concentration up to the thresholds value, while no significant changes were observed at the higher concentrations. Our findings also demonstrated the correlations between the verbascoside content and the surrounding medium during the extraction process. As is shown in Figure 4, the verbascoside content had no significant changes with the increased EtOH concentration in the extraction solvent from 80% to 90% ($P > 0.05$). On the other hand, the increased EtOH concentration to 80% could significantly enhance the verbascoside recovery as the highest content was obtained using 80% EtOH with 20% H₂O ($P < 0.05$).

Table 2: Results of Scavenging Activity of DPPH[•] and HO Free Radicals of *L. verbena* Leaf Extract Obtained by Different Ratios of EtOH:H₂O

EtOH:H ₂ O ratio (%v/v)	%DPPH _{sc} ^a	%HO _{sc} ^a
50:50	13.10 ± 1.35 ^D	10.19 ± 1.55 ^D
60:40	24.57 ± 1.20 ^C	17.80 ± 1.10 ^C
70:30	37.24 ± 1.30 ^B	26.60 ± 1.40 ^B
80:20	45.25 ± 0.95 ^A	31.17 ± 1.20 ^A
90:10	46.26 ± 1.10 ^A	32.20 ± 1.30 ^A

^a Data are expressed as the mean ± SD. Different capital letters in each column indicate a significant difference among different treatments ($P < 0.05$).

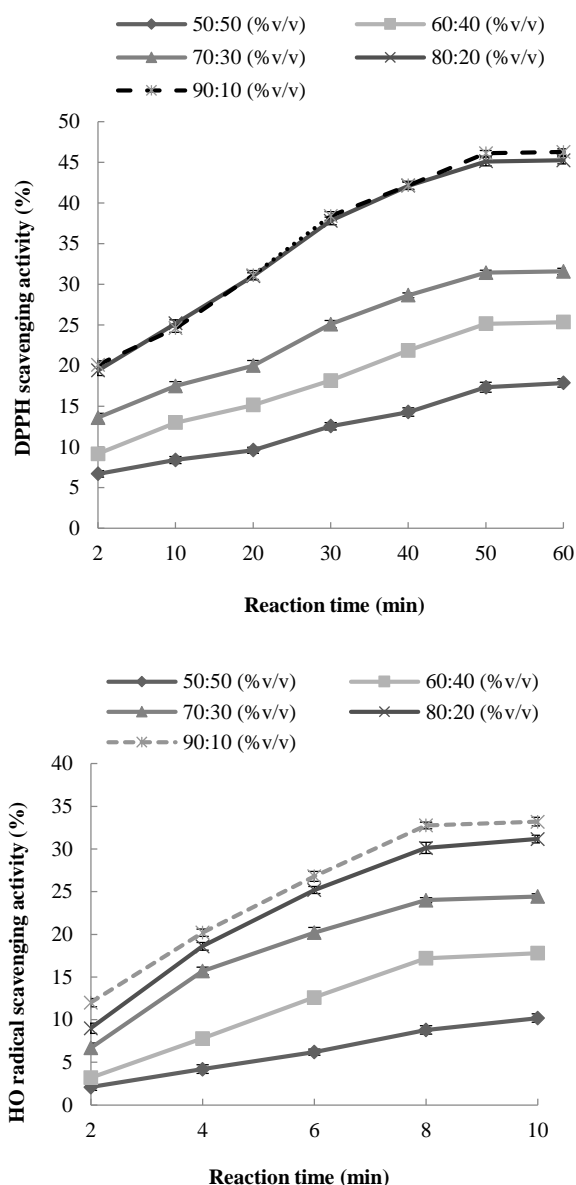


Figure 1: Effect of Reaction Time on Radical Scavenging Activity of *L. verbena* Leaf Extract Obtained by Hydroethanolic Solvent (EtOH:H₂O) at Various Ratios; a) DPPH Free Radicals and B) HO Free Radicals

The yield of bioactive compounds recovery largely depends on the solvent type. According to the literature, less polar solvent is less efficient in verbascoside recovery. Similar findings have been reported by Waszkowiak and Gliszczynska-Swiglo (2016) in the assessment of the effects of binary EtOH-H₂O solvents on the antioxidant activity and phenolic composition of flaxseed extract [22]. Accordingly, the total phenolic content (TPC) of the extract in terms of phenolic acids, secoisolariciresinol diglucoside, and their glucosides reduced with the increased EtOH concentration within the range of 60-90%.

In a study in this regard, Taha *et al.* (2011) investigated the effect of various EtOH concentrations on the TPC of sunflower meal extract [24], and the obtained results revealed that 60% EtOH was the most effective concentration. In another study, the EtOH concentration range of 50-60% was reported to be most efficient in the preparation of the extracts from defatted borage meal [25].

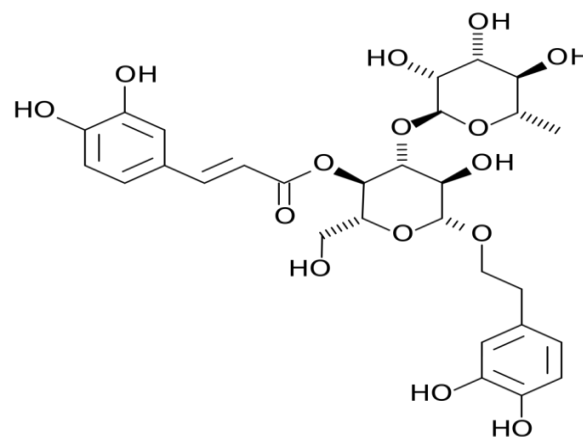


Figure 2: Chemical Structure of Verbascoside (Source: adapted from [36])

Furthermore, Zhang *et al.* (2007) investigated the effects of extraction parameters, including temperature (20-60°C), EtOH concentration (50-100%), and time on yield [26], observing that the EtOH concentration range of 56-80% had the highest efficacy.

In another study, Lau *et al.* (2014) evaluated the effect of EtOH concentration on the recovery of rosmarinic acid from *Orthosiphon stamineus* [12], reporting that the content of rosmarinic acid increased with the EtOH concentration range of 0-70% v/v. Afterwards, the rosmarinic acid content reduced with the further increment of the EtOH concentration from 70% to 100% v/v.

3.3. Correlation of FRSA and Verbascoside Recovery

The results of the present study showed a significant correlation between %DPPHsc and %HOsc (Pearson's correlation-coefficient=0.928), which reflected the reliability of the FRSA results. Several studies have also demonstrated the correlation between FRSA results [27-29].

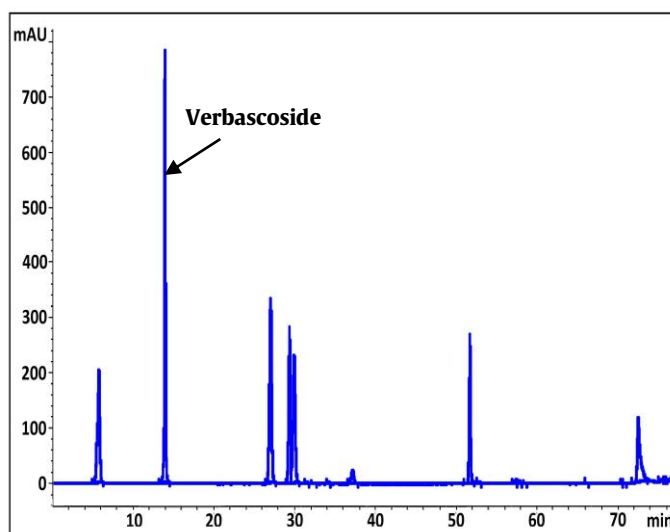


Figure 3: Typical RP-HPLC Chromatogram of Extract Obtained by Binary Solvent System for Verbascoside Analysis

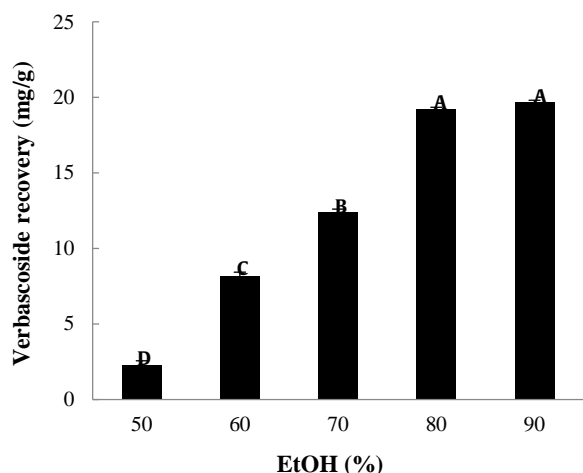


Figure 4: Verbascoside Recovery Using Binary Solvent System (EtOH:H₂O) Mean (n=3) ± SD; error bars representing 95% confidence interval for means; different letters indicate significant difference in various treatments ($P < 0.05$)

Furthermore, the association between the FRSA and the verbascoside content of *L. verbena* leaf extract has been evaluated at various EtOH:H₂O solvent ratios, indicating that the FRSA changes were parallel to the variations in the verbascoside recovery.

According to the results of the present study, the Pearson's correlation-coefficients of the verbascoside content with %DPPHsc and %HOsc were 0.988 and 0.984, respectively. Therefore, it could be inferred that verbascoside possessed potent radical scavenging abilities, which rendered it a valuable bioactive compound. Therefore, *L. verbena* leaf is a valuable agricultural product as a potential and natural source of verbascoside. In this regard, Goulas (2012) reported a close correlation between phenolic compounds (verbascoside and dimethoxy-quercetin) and radical scavenging activity [30]. Furthermore, significant, positive correlations have been observed between phenolic compounds and antioxidant capacity [31] and catechins and antioxidant activity [32].

3.4. Modeling of the Release Kinetics of Verbascoside from *L. verbena* Leaves

To gain further knowledge regarding the mass transfer mechanism of verbascoside from *L. verbena* leaves into the surrounded binary solvent during extraction, the kinetic curves were analyzed using the Peleg model (Figure 5). The kinetic parameters of K_1 (Peleg rate constant; min/g/mg) and K_2 (Peleg capacity constant; g/mg) were determined through the non-linear regression in Equation 3. In addition, the equilibrium concentration of verbascoside (C_{eq}) during the extraction process was determined using Equation 5. According to the findings, the values of K_1 , K_2 , and C_{eq} were estimated at 0.027 min/g/mg, 0.047 g/mg, and 20.936 mg/g, respectively. The experimental results were also plotted using the non-linear Peleg model versus the extraction time (Figure 5). According to the findings, the proposed mathematical Peleg model was consistent with the experimental data. Figure 5 shows that the extraction rate of verbascoside was rapid at the initial stages of the process, while they slowed-down until the completion of the extraction process.

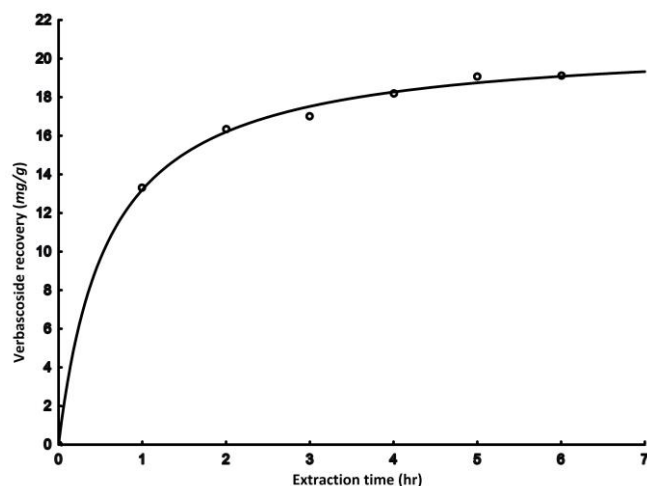


Figure 5: Kinetic Study Verbascoside Recovery from *L. verbena* Leaves Using Peleg Model

According to the results of the present study, the changes in the solute content in the surrounding binary solvent phase influenced the mass transfer of the extraction process [33, 34]. Therefore, it could be inferred that the verbascoside recovery from the *L. verbena* leaves occurred in two stages. At the first stage (from the beginning until approximately two hours), the solute concentration in the surrounding binary solvent was relatively low, causing the verbascoside to diffuse rapidly from the solid phase (*L. verbena* leaves) to the liquid phase. Furthermore, the verbascoside on the surface layers of the *L. verbena* leaves was solubilized as the sample was exposed to the fresh binary solvent [35].

During the second phase of extraction, the diffusion rate of the target compound reduced with the increased extraction time; this phenomenon might have been caused by the high concentration of the target solute in the liquid phase. After a period of time extraction, the solute concentration in the bulk solution reached equilibrium with that of the herbal matrix [12]. However, the extraction time in the present study was prolonged after the extraction of the maximum verbascoside. Moreover, no significant changes were observed in the amount of the extracted verbascoside. Therefore, it could be concluded that the verbascoside content tended to be in equilibrium or saturated after approximately 120 minutes of the extraction process. As a result, the time of the extraction process for thermolabile compounds should be reduced. The statistical results also revealed that the observed data could fit successfully to the Peleg model with the R^2 value of 0.999 and RMSE and E values of 0.093 and 0.968, respectively. It has been documented that higher R^2 values are associated with the better fit of the actual data with the model data. RMSE and E values are also frequently applied to assess the accuracy of the prediction of models, and lower RMSE and E values are associated with the higher accuracy of the model data [12]. In the present study, the values of R^2 , RMSE, and E showed that the kinetics of the verbascoside recovery from the *L. verbena* leaves could be successfully described by the Peleg model. In the study conducted by Lau *et al.* (2014), the Peleg model could be successfully investigated to describe the kinetics of rosmarinic acid extraction from *Orthosiphon stamineus* [12].

4. Conclusion

In this study, binary solvents composed of various ratios of EtOH and H₂O were applied for verbascoside recovery from *L. verbena* leaves. According to the results, the increased EtOH concentration in the binary solvent system significantly changed the yield of the verbascoside recovery and FRSA of the extract obtained from the *L. verbena* leaves. Therefore, the combination of EtOH and H₂O could be recommended as a proper binary solvent owing to their different polarities, possibility of mixing at any proportion, and acceptability for human consumption. Our findings also indicated that the plant extract composition and its FRSA largely depended on the type of the extraction solvent. On the other hand, the combination of miscible polar solvents has been observed to improve the extraction yield of the target compound. The results of this study confirmed that *L. verbena* leaf is a potential source of verbascoside and antioxidants. Furthermore, it could be stated that the application of the binary solvent extraction composed of 80% EtOH and 20% H₂O was a successful and safe procedure for verbascoside recovery from *L. verbena* leaves. The results of the kinetic studies implied that the proposed kinetics model was valid with the R², RMSE, and E values of 0.999, 0.093, and 0.968, respectively. As such, the proposed model could be used for the up-scaling and process design of verbascoside extraction from *L. verbena* leaves.

Authors' Contributions

M.B., and A.G., developed the study concept, performed the statistical analysis, and drafted and revised the manuscript; R.R.N., managed the literature review and data collection. All the authors read and approved the final manuscript.

Conflict of Interest

The Authors declare that there is no conflict of interest.

Acknowledgments

Hereby, we extend our gratitude to the Vice-Chancellor of Research of the University of Zanjan for the financial support of this research project (Project No. 1474771)

References

- Gonzalez S. Dietary Bioactive Compounds and Human Health and Disease. *Nutrients*. 2020; 12: 348-50.
- Dabeek WM, Marra MV. Dietary Quercetin and Kaempferol: Bioavailability and Potential Cardiovascular Related Bioactivity in Humans. *Nutrients*. 2019; 11: 2288-307.
- Soltani B, Rostami Asrabadi R, Shahpiri P, Marques Z, Rahimi AM, Farzaei MH. Aloysia Citrodora Palau (*Lemon verbena*): A Review of Phytochemistry and Pharmacology. *J Ethnopharmacol*. 2018; 222: 34-51.
- Leyva Jimenez FJ, Lozano Sanchez J, Borrás Linares I, Arraez Roman D, Segura Carretero A. Comparative Study of Conventional and Pressurized Liquid Extraction for Recovering Bioactive Compounds from Lippia Citriodora Leaves. *Food Res Int*. 2018; 109: 213-22.
- Altemimi A, Lightfoot DA, Kinse M, Watson DG. Employing Response Surface Methodology for the Optimization of Ultrasound Assisted Extraction of Lutein and β -Carotene from Spinach. *Molecules*. 2015; 20: 6611-25.
- Kronholm J, Revilla Ruiz P, Porras SP, Hartonen K, Carabias Martinez R, Riekkola ML. Comparison of Gas Chromatography Mass Spectrometry and Capillary Electrophoresis in Analysis of Phenolic Compounds Extracted from Solid Matrices with Pressurized Hot Water. *J Chromatogr A*. 2004; 1022: 9-16.
- Luque de Castro MD, Priego Capote F. Soxhlet Extraction: Past and Present Panacea. *J Chromatogr A*. 2010; 1217: 2383-89.
- Yen GC, Chen HY. Antioxidant Activity of Various Tea Extracts in Relation to their Antimutagenicity. *J Agr Food Chem*. 1995; 43: 27-32.
- Poodi Y, Bimakr M, Ganjloo A, Zarringhalami S. Intensification of Bioactive Compounds Extraction from Feijoa (Feijoa Sellowiana) Leaves using Ultrasonic Waves. *Food Bioprod Process*. 2018; 108: 37-50.
- Fernandez L, de Santos J, Diaz Lanza AM, Perez E, Villaescusa L. Quantitative Determination of Verbascoside in Scrophularia Scorodonia by High-Performance Liquid Chromatography. *Pharm Biol*. 2005; 43: 226-29.
- Shirsath SR, Sable SS, Gaikwad SG, Sonawane SH, Saini DR, Gogate PR. Intensification of Extraction of Curcumin from Curcuma Amada using Ultrasound Assisted Approach: Effect of Different Operating Parameters. *Ultrason Sonochem*. 2017; 38: 437-45.
- Lau CH, Chua LS, Lee CT, Aziz R. Optimization and Kinetic Modeling of Rosmarinic Acid Extraction from Orthosiphon Stamineus. *Curr Bioact Compd*. 2014; 10: 271-85.
- Bajpai VK, Baek KH, Kang SC. Antioxidant and Free Radical Scavenging Activities of Taxoquinone, A Diterpenoid Isolated from Metasequoia Glyptostroboidea. *S Afr J Bot*. 2017; 111: 93-8.
- Boulekbache Makhlof L, Medouni L, Medouni Adrar S, Arkoub L, Madana K. Effect of Solvents Extraction on Phenolic Content and Antioxidant Activity of the Byproduct of Eggplant. *Ind Crops Prod*. 2013; 49: 668-74.
- Ma JS, Liu H, Han CR, Zeng SJ, Xu XJ, Lu DJ, et al. Extraction, Characterization and Antioxidant Activity of Polysaccharide from Pouteria Campechiana Seed. *Carbohydr Polym*. 2020; 229: 115409.
- Bandyopadhyay U, Das D, Banerjee RK. Reactive Oxygen Species: Oxidative Damage and Pathogenesis. *Curr Sci*. 1999; 10(77): 658-66.
- Kardag A, Ozcelik B, Saner S. Review of Methods to Determine Antioxidant Capacities. *Food Anal Methods*. 2009; 13: 41-60.
- Mukhopadhyay D, Dasgupta P, Sinha Roy D, Shauroseni A. Sensitive In Vitro Spectrophotometric Hydrogen Peroxide. *Free Radicals and Antioxidants*. 2016; 6: 124-32.
- Locatelli M, Gindro R, Travaglia F, Coisson JD, Rinaldi M. Study of the DPPH-Scavenging Activity: Development of a Free Software for the Correct Interpretation of Data. *Food Chem*. 2009; 114: 889-97.
- Carocho M, Morales P, Ferreira ICFR. Antioxidants: Reviewing the Chemistry, Food Applications, Legislation and Role as Preservatives. *Trends Food Sci Technol*. 2018; 71: 107-20.
- Maqsood S, Adiamo O, Ahmad M, Mudgi P. Bioactive Compounds from Date Fruit and Seed as Potential Nutraceutical and Functional Food Ingredients. *Food Chem*. 2020; 38: 125522.
- Waszkowiak K, Gliszczynska Swiglo A. Binary Ethanol-Water Solvents Affect Phenolic Profile and Antioxidant Capacity of Flaxseed Extracts. *Eur Food Res Technol*. 2016; 242: 777-86.
- Cadiz Gurrera ML, Olivares Vicente M, Herranz Lopez M, Arraez Roman D, Fernandez Arroyo S, Micol V, et al. Bioassay-Guided Purification of Lippia Citriodora Polyphenols with AMPK Modulatory Activity. *J Funct Foods*. 2018; 46: 514-20.
- Taha FS, Mohamed GF, Mohamed SH, Mohamed SS, Kamil MM. Optimization of the Extraction of Total Phenolic Compounds from Sunflower Meal and Evaluation of the Bioactivities of Chosen Extracts. *Am J Food Technol*. 2011; 6:1002-20.

25. Wettasinghe M, Shahidi F. Antioxidant and Free Radical Scavenging Properties of Ethanolic Extracts of Defatted Borage (*Borago Officinalis* L.) Seeds. *Food Chem.* 1999; 67: 399-414.
26. Zhang ZS, Li D, Wang LJ, Ozkan N, Chen XD, Mao ZH, *et al.* Optimization of Ethanol-Water Extraction of Lignans from Flaxseed. *Sep Purif Technol.* 2007; 57: 17-24.
27. Abramovic H, Grobin B, Ulrich NP, Cigic B. Relevance and Standardization of in Vitro Antioxidant Assays: ABTS, DPPH, and Folin-Ciocalteu. *J Chem.* 2018; 1-9.
28. Bimakr M, AR Russly, Ganjloo A. Food Processing and Impact on Active Components in Foods. *CRC Press.* 2015: 407-12.
29. Thaipong K, Boonprakob U, Crosby K, Cisneros Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC Assays for Estimating Antioxidant Activity from Guava Fruit Extracts. *J Food Compost Anal.* 2006; 19: 669-75.
30. Goulas V, Gomez Caravaca AM, Exarchou V, Gerathanassis IP, Segura Carretero A, Fernández Gutiérrez A. Exploring the Antioxidant Potential of *Teucrium polium* Extracts by HPLC-SPE-NMR and On-line Radical-Scavenging Activity Detection. *LWT Food Sci and Technol.* 2012; 46: 104-9.
31. Saifullah Md, McCullum R, McCluskey A, Vuong Q. Comparison of Conventional Extraction Technique with Ultrasound Assisted Extraction on Recovery of Phenolic Compounds from Lemon Scented Tea Tree (*Leptospermum petersonii*) Leaves. *Heliyon.* 2020; 6: e03666.
32. Zhao C, Li C, Liu S, Yang L. The Galloyl Catechins Contributing to Main Antioxidant Capacity of Tea Made from *Camellia sinensis* in China. *Sci World J.* 2014; 863984.
33. Hazwan MH, Hasfalina CM, Hishamuddin J, Zurina ZA. Optimization and Kinetics of Essential Oil Extraction from Citronella Grass by Ohmic Heated Hydrodistillation. *Int J Chem Eng Appl.* 2012; 3(3): 1-6.
34. Pin KY, Chuah AL, Rashih AA, Rasadah MA. Solid Liquid Extraction of Betel Leaves (*Piper betel* L.). *J Food Process Eng.* 2011; 34: 549-65.
35. Sayyar S, Abidin ZZ, Yunus R, Muhammad A. Extraction of Oil from *Jathropa* Seeds Optimization and Kinetics. *Am J Appl Sci.* 2009; 6: 1390-95.
36. Salehi B, Armstrong L, Rescigno A, Yeskalyeva B, Seitimova G, Beyatli A, *et al.* *Lamium* Plants A Comprehensive Review on Health Benefits and Biological Activities. *Molecules.* 2019; 24(10): 1913.