Feasibility of Hydroethanolic Solvent System for Bioactive Compounds Recovery from Aerial Parts of *Silybum marianum* and Kinetics Modeling

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ARTICLE INFO

ABSTRACT

Background: *Silybum marianum* is considered as one of the valuable medicinal plants. This study focused on maximum recovery of total extractable compounds (TEC), anti-radical capacity (AC), and silybin content as a major phenolic compound using hydroethanolic solvent system (HSS).

Methods: The HSS consisted of different ratios of ethanol (EtOH) and water (H_2O). The AC was determined using spectrophotometric analysis. High-performance liquid chromatography was used for analyzing the silybin content. The cells morphology and the mass transfer kinetics were investigated using scanning electron microscope and Peleg model respectively.

Results: Based on the results, the highest TEC (154.60 ± 2.4 mg/g), AC (39.19 ± 0.30 of % $DPPH_{sc}$ and 28.15 ± 0.51 of % OH_{sc}), and silybin (4400 ± 33.00 mg/ kg) were obtained using HSS (80%v/v of EtOH). Moreover, high correlation was found between AC and silybin content. Mass transfer kinetics was successfully explained using Peleg model (R^2 = 0.9970, RMSE = 2.274, and E = 1.22%). Further, intense cellular disruption during HSS caused accelerated release of bioactive compounds.

Conclusion: The HSS composed of 80% v/v of EtOH was found as an efficient system for successful recovery of bioactives which could be introduced as a natural ingredient for food and pharmaceuticals industries.

1. Introduction

Silybum marianum, known as milk thistle, is a medicinal plant belongs to Asteraceae family (Figure 1) [1, 2]. The Asteraceae family is considered as one of the largest flowering plant families, with over 23,000 species and 1,600 genera. Plants in the Asteraceae family are found all over the world and potentially are the sources of cytotoxic, antimicrobial, anti-inflammatory, neurotoxic, phototoxic compounds as well as a wide range of other properties [3, 4].

This plant shows different health-promoting effects and has been used to treat different disorders such as hepatitis, cirrhosis, and jaundice [5]. It is usually found in different provinces of Iran such as Gilan, Mazandaran, East and West Azerbaijan, Khuzestan, Kermanshah, Bushehr, and Fars [1]. Phytochemical researches on plant sources are necessary to maximize the recovery of target solutes for further utilization [6, 7]. Silybin is one of the main phenolic compounds of *S. marianum* with the highest biological activity [1, 8]. Its chemical structure presented in Figure 2.

Figure 1: *Silybum marianum* (2)

Moreover, it is a flavonolignan which belongs to the flavonoid group [8]. Researchers have paid a lot of attention to phenolic compounds due to their radical scavenging activity [9]. The main beneficial activities of silybin include hepato-protective and anticancer [7, 10]. Several researches were conducted to explore the effect of various solvents and techniques to recover the different bioactive compounds successfully [11-13]. However, there is lack of knowledge on successful recovery of bioactive compounds from the aerial parts of *Silybum marianum*.

Extraction process is considered as a crucial phase to recover different solutes from plant materials. This step is very complex due to the wide variations of the chemicals structures and polarities [14]. It has been shown that the applied solvent affects the extract compositions of active biological compounds significantly [12, 14, 15]. In order to perform an effective extraction process, selecting an appropriate solvent is an important parameter that depends on the specific nature of the target solutes [15]. Different extraction solvents such as aqueous and/or organic mediums have been applied to recover bioactive compounds [16]. There are several drawbacks regarding applying organic solvents including environmental pollution, waste disposal, and solvent residue in the product [17]. Ethanol (EtOH) and water (H_2O) are commonly used for extraction due to their different polarity [18].
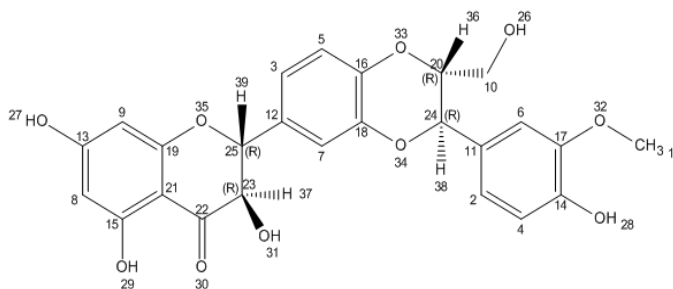

Figure 2: Chemical structure of silybin (7)

Some studies have used hydroethanolic solvent effectively to extract different valuable bioactive compounds [9, 15, 19]. However, there is still the demand to investigate the hydroethanolic solvent system (HSS) to extract valuable bioactive compounds from herbal sources and introduce new functional additives. Such extracts could be valuable source of beneficial bioactives such as phenolics with a good antioxidant capacity. It has been stated that phenolic compounds profile highly affects the anti-radical capacity of extracts. [20].

This study aimed to evaluate the effect of HSS composed of different ratios of H_2O and EtOH (50-90% v/v of EtOH) on the total extractable compounds (TEC), anti-radical capacity (AC), and silybin content from the aerial parts extracts of *S. marianum*. The high-performance liquid chromatography (HPLC) analysis was performed to qualify and quantify silybin in the extracts. Moreover, the correlations between the composition of HSS, the AC, and the silybin content were examined. Mass transfer kinetics was investigated to get deep insight regarding the dissolution of valuable bioactive compounds during the different stages of extraction process. Finally, the morphology of the cell structure was analyzed by scanning electron microscope (SEM) to monitor the extraction process effect on the cell structure.

2. Materials and Methods

2.1. Plant materials

Dried aerial parts of the *S. marianum* including stems, leaves, and flowers were purchased from a local market in Zanjan, Iran. The moisture content of plant sample was 6.2 ± 0.2%. Before extraction process, a blender (GOSONIC, Shenzhen, China) ground aerial parts to obtain powder with particle size of around 1.00 mm. Pure ethanol (analytical grade) was purchased from Dr. Mojallali Co., Tehran, Iran. Other chemicals were purchased from Merck, Darmstadt, Germany.

2.2. Preparation of extracts

In order to obtain total extractable compounds from aerial parts of *S. marianum*, hydroethanolic solvent system (HSS) containing different proportions of H_2O and EtOH (Table 1) was used with Soxhlet apparatus (Parmis Teb Azma Co, Tehran, Iran). The ratios of H_2O and EtOH were selected based on our preliminary and previous studies [18]. Briefly, 5 g of the prepared sample was added to 200 mL of proper hydroethanolic solvent at the desired ratio (Figure 3). The extraction process took 6 h in triplicate. After extraction, the extract was filtered and the solvent was removed using vacuum rotary evaporation (IKA, Staufen, Germany). The extract stored in the dark and at -18 °C for further analysis.

Table 1: Different ratios of EtOH: H_2O in the hydroethanolic solvent system

Ratios	EtOH (% *v/v*)	H_2O (% *v/v*)
50:50	50	50
60:40	60	40
80:20	80	20
90:10	90	10

2.3. Measurement of total extractable compounds

To evaluate the efficiency of HSS process, determining the total extractable compounds (TEC) is an important criterion that considered in various studies [21, 22]. In the present study, the TEC was measured using Equation 1:

$$\text{TEC (mg/g)} = \frac{m_e}{m_s} \times 1000 \tag{1}$$

Where m_e is the weight of extract (g) and m_s is the weight of plant feed (g) [13].

2.4. Determining the anti-radical capacity

In order to determine the anti-radical capacity (AC) of the aerial parts extracts of *S. marianum* two different assays were performed based on scavenging of DPPH and ˙OH free radicals.

2.4.1. Determination of dpph free radical scavenging activity

The 2, 2- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of aerial part extracts of *S. marianum* was measured based on the procedure by Chen et al. (2020) with minor changes. First, 0.1 mL of diluted extract with ethanol (1 mg/mL) was mixed with 2.9 mL of 0.1 mM ethanolic solution of DPPH. Then, the mixture was kept at ambient temperature for 30 min. Finally, absorbance of prepared samples was measured at 517 nm using a UV-Vis

Figure 3: Soxhlet extractor for bioactive compounds extraction from *S. marianum* aerial parts

spectrophotometer (SPECORD 250, Analytikjena, Jena, Germany) against the blank sample. Ethanolic DPPH˙ solution was used as a blank sample, and ascorbic acid was used as a positive control activity. The DPPH radical scavenging activity (% $DPPH_{sc}$) was calculated using Equation 2:

$$\%\text{DPPHsc} = (A_b - A_s)/A_b \times 100 \tag{2}$$

Where A_s was the absorbance of the sample and Ab was the absorbance of the blank [23].

2.4.2. Determining ˙OH free radical scavenging activity

The ability of aerial part extracts of *S. marianum* to scavenge hydrogen peroxide was measured based on the procedure by Ruch *et al.* (1989) with slight changes. A hydrogen peroxide solution (43 mM) was prepared in phosphate buffer (pH 7.4, 0.01 M). One mg/mL of sample extract was mixed with a solution of hydrogen peroxide (43 mM, 0.6 mL). Then, the solution was held for 60 min and finally the absorbance was read at 530 nm using a UV-Vis spectrophotometer (SPECORD 250, Analytikjena, Jena, Germany). The blank test was conducted with ethanol instead of crude extract, and ascorbic acid was used as a positive control activity. The inhibition percentage of scavenged hydroxyl radicals (% OH_{sc}) was measured using Equation 3:

$$\%\text{OHsc} = (A_b - A_s)/A_b \times 100 \tag{3}$$

Where A_s was the absorbance of the sample and Ab was the absorbance of the blank [24].

2.5. Qualification and quantification analysis of silybin

Qualification and quantification analysis of silybin content of extracts from aerial parts of *S. marianum* under different conditions of HSS was performed using a high-performance liquid chromatography device equipped with a Varian 9012 HPLC pump (CA, USA) and a six-port Cheminert HPLC valve from Valco (Houston, USA) with a 20 μL sample loop, and a Varian 9050 UV-Vis detector was used. The separation was performed with a reversed phase column (25 cm × 4.6 mm × 5 μ m, Supelco, USA) at ambient temperature. The eluent composed of water (H2O, solvent A), methanol (MeOH, solvent B), and formic acid (CH2O2) was added to both solvents (0.1% v/v) [4]. The gradient program was performed as follows: 15% A: 85% B (0-10 min), 30% A: 70% B (10-16 min), 50% A: 50% B (16-23 min), 70% A: 30% B (23-30 min), 85% A: 15% B (30-40 min). The injection volume and elution rate was set at 20 μ L and 1 mL/min, respectively. Before separation, the mobile phases were filtered through a filter paper (0.45 μm).

2.6. Scanning electron microscopy

To monitor the cell rupture and microscopic changes during the extraction process, the scanning electron microscopy (SEM) was used. *S. marianum* powder was observed under the SEM (Quanta 450 FEG, FEI Inc., Oregon, USA) for morphological characterization before and after the extraction. The gold-palladium coating (6-11 nm; 40 s; 10 mA) was applied to create a reflective surface for the electron beam (15 kV) after drying the solid residues in a ventilated oven at 60 °C.

2.7. Statistical analysis and mass transfer kinetics study

The present study investigated the effect of HSS on TEC, AC, and silybin content of extracts obtained from aerial parts of *S. marianum*. A significant effect ($P < 0.05$) of ethanol concentration was determined by ANOVA using Minitab software (v.17) (State College, PA, USA). The experiments were conducted in triplicate and data was expressed as mean ± SD (standard deviation). To evaluate the correlation coefficients among means of assessments the Pearson correlation test was performed.

To obtain a deep and comprehensive insight about bioactive compounds release during 6 h of the extraction process, the mass transfer kinetics was studied. Non-linear regression using Levenberg-Marquardt method was run to fit the experimental data based on Peleg model using the STATISTICA software (v.6.0) (StatSoft, Inc., Tulsa, OK, USA) [20]. After determining the best ratio of EtOH and H₂O to obtain the maximum response studied, the extraction process was performed in twelve extraction time interval between 30 min to 6 h. At the end of each interval, the extract was collected and weighted to determine the TEC values (Equation 1).

Peleg model is a commonly-used model to describe the extraction behavior of bioactive compounds [20, 25-27]. Peleg proposed this equation to describe the kinetics of water absorption during the rehydration [28]. The model explaining the extraction kinetics could be written as Equation 4:

$$C_t = C_0 + \frac{t}{K_1 + K_2 t} \tag{4}$$

Where K_1 and K_2 are constants which are related to Peleg's rate constant (min/g /mg) and Peleg's capacity constant (g/mg), respectively. Ct is the content of extract at time t (mg/g). As the initial value of TEC is zero at the beginning of the process, therefore the model is modified according to Equation 5:

$$C_t = \frac{t}{K_1 + K_2 t} \tag{5}$$

The slope and intercept values from the plotted graph between $1/C_t$ and $1/t$ were presented as K_1 and K_2 values, respectively [17, 20]. Ct was obtained from Equation 4 at different times to determine the effectiveness of the proposed model [29]. When t tends to ∞, Equation 6 showed the relations between equilibrium content of extract and K_2 [17].

$$\underset{t \to \infty}{C} = C_{eq} = \frac{1}{K_2} \tag{6}$$

Several statistical criteria including the R² (coefficient of determination), RMSE (root mean square error), and E (mean relative percentage deviation modulus) were used to determine the accuracy of the final developed model [17]. The criteria could be obtained from Equations 7-9:

$$R^2 = \frac{\sum_i (V_{exp} - V_{pre})^2}{\sum_i (V_{exp} - \bar{V}_{pre})^2} \tag{7}$$

$$RMSE = \frac{1}{n} \left[\sum_{i=1}^n (V_{exp} - V_{pre})^2 \right]^{0.5} \tag{8}$$

$$E(\%) = \frac{100}{n} \sum_{i=1}^n \frac{|V_{exp} - V_{pre}|}{V_{exp}} \tag{9}$$

Where V_{pre} and V_{exp} were the predicted and experimental values, respectively. Further, n was the number of experimental runs and $\bar{V}_{pre}$ was the mean of the observed data [28].

3. Results and Discussion

3.1. Effect of hydroethanolic solvent system on TEC

Bioactive compounds are phytochemicals with added nutritional value found in limited amounts in various natural sources. Therefore, selecting a proper extraction solvent and procedure are highly demanded and crucial for maximum recovery of these valuable compounds from herbal sources [30]. Choosing the extraction medium carefully is vital to extract bioactive compounds from natural sources using solid- liquid extraction method. In this method, analytes were separated from a solid matrix with the aid of a solvent as an extraction medium [17, 31]. Generally, the solid-liquid extraction mechanism can be summarized as making the surface of solid phase wet by the liquid phase (solvent), solvent penetration, dissolution of the analytes, and transportation of the analytes from the interior to surface of solid matrix along with the release of the analytes to the solvent [13]. The amount of TEC was significantly ($P < 0.05$) affected by the EtOH concentration of HSS. This result could be due to the different solvents effect on solubility, mass transfer, and diffusion kinetics of solutes [32]. Furthermore, the affinity between solvent polarity and target solutes

shows a key role in the separation processes [33]. It has been proven that by increasing the polarity of the solvent applied, solutes with a higher degree of polarity was extracted and vice versa. The effect of EtOH concentration on TEC (mg/g) of aerial parts of *S. marianum* was shown in Figure 4. The TEC was varied between 81.00 ± 2.22 to 154.80 ± 2.00 mg/g using different conditions of HSS, which reflected the potential of proposed system for recovery of the bioactive compounds from the aerial parts of *S. marianum*. Variations in the amounts of TEC were attributed to the polarities of different HSS which led to separating compounds with same polarity from the solid matrix. It can be observed that the TEC values increased gradually by increasing ethanol concentration from 50 to 80% v/v, beyond that no considerable difference was observed. In a similar study by Alara *et al.* (2018) on the extraction of bioactive compounds from Vernonia cinerea leaves using Soxhlet apparatus by different hydroethanolic mediums, similar finding was found [34]. The present study, demonstrated that EtOH as a relatively cheap, nontoxic, and reusable solvent could be applied in the HSS for successful recovery of potentially bioactive extracts from the aerial parts of *S. marianum*. These products can be incorporated into food and nutraceutical industries for further applications. They have been used in various studies to extract the bioactive compounds [8, 17, 35].

3.2. Effect of hydroethanolic solvent system on the anti-radical capacity

The effect of different conditions of HSS on the AC of extracts obtained from the aerial parts of *S. marianum* was analyzed by DPPH and ˙OH free radical scavenging assessments (Figure 5). It is common that free radicals have adverse effect on the normal biological activity. The DPPH assay has been widely used to determine the ability of natural extracts to scavenge free radicals produced by DPPH reagent [22, 36]. The scavenging reaction results revealed that the color of reaction solution turned yellow from purple.
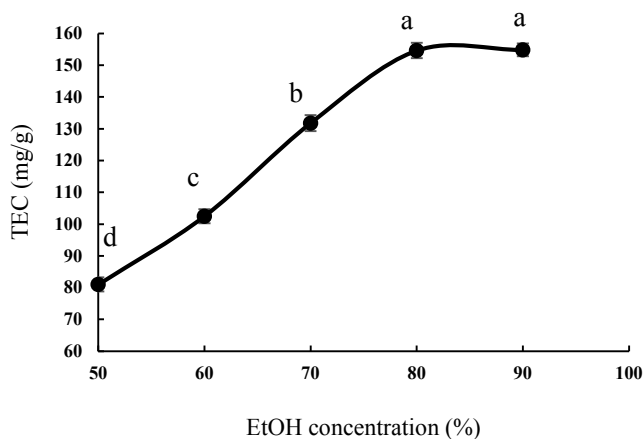

Figure 4: Effect of ethanol concentration on TEC (mg/g) of *S. marianum* aerial parts. Different lower letters indicate significant difference (*P*<0.05)
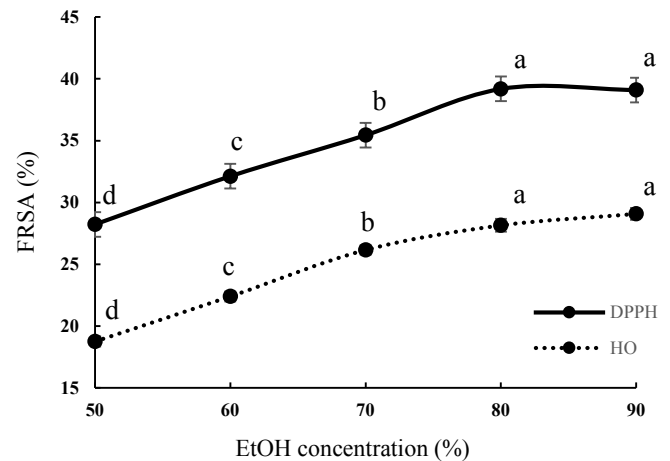

Figure 5: Effect of ethanol concentration on free radical scavenging activity (FRSA, %) of *S. marianum* aerial parts extracts. Different lower letters in each line indicate significant difference (*P*< 0.05)

As shown in Figure 5, extracts showed AC in terms of inhibition of DPPH (from 28.22 ± 0.35 to 39.19 ± 0.35%) and ˙OH free radicals (from 18.75 ± 0.48 to 29.08 ± 0.41%). The AC of extracts was lower compared to ascorbic acid as a positive control (95.10 ± 0.28% $DPPH_{sc}$ and 87.20 ± 0.40% OH_{sc}). In addition, the results showed that the highest level of AC was observed with hydroethanolic solvent composed of 80:20% v/v, beyond that a slight reduction was observed. It has been stated that the hydrogen atom transfer and the single electron transfer are important aspects in measuring anti-radical capacity which are highly influenced by the type and polarity of the solvents. Furthermore, extracts obtained by aqueous-organic solvents could contain bioactive compounds without radical scavenging activity that may interfere in AC assays [37]. Therefore, it is necessary to determine the best HSS concentration for selective separation of bioactive radical scavenger compounds Since the solvent polarity affects the separation of radical scavenger compounds such as phenolic compounds, using a pure solvent may not be effective for the efficient recovery of target constituents from plant matrix as described in Prasad *et al.* (2011). Furthermore, Lang and Wai (2006) stated that water leads to swelling plant cells while solutes disruption and plant matrices bounds could be occurred by the ethanol [38, 39]. In addition, it was observed that the mixture of ethanol and water (aqueous ethanol) as extraction medium showed the best result in seperating the phenolic compounds from various plant matrixes [8, 17, 18, 35, 38, 39].

Moreover, Roshani Neshat *et al.* (2020) investigated the effects of the binary solvent system on the separation of the bioactive compounds with radical scavenging activity from Lemon verbena leaves. They found that the hydroethanolic solvent composed of EtOH: H_2O (80:20% v/v) was the optimal extraction medium for the maximum recovery of bioactive

scavenger constituents which showed 45.25 ± 0.95 of % $DPPH_{sc}$ and 31.17 ± 1.20 of % OHsc [17]. In another study, Waszkowiak and Gliszczynska-Swiglo (2016) reported that binary solvent system composed of EtOH and H_2O influenced on radical scavenging capacity of Flaxseed extracts significantly (P <0.05). Further, they found that the aqueous ethanol (60:40% v/v EtOH: H_2O) is preferable for achieving flaxseed extract with good antioxidant activity [8].

3.3. Effect of hydroethanolic solvent system on the silybin content

Silybin, the main bioactive compound of *S. marianum*, has been consumed for centuries to treat liver diseases [1, 7]. This compound belongs to flavonolignans and its chemical structure consists of two main units (Figure 2). Since the 1970s, silybin has been considered as a substance possesses hepato-protective property [9]. Recently, silybin has been studied as a potential chemo-preventive and anticancer compound [40]. Lucini *et al.* (2016) reported that silybin was the major compound among the target analyzed antioxidants in milk thistle which was in line with the study by Valenzuela *et al.* (1989) [4, 41]. Effect of ethanol concentration on silybin content (mg/kg) of the aerial parts of *S. marianum* extracts was presented in Figure 6. In the current study, silybin showed values ranging from 3215 to 4400 mg/kg depending on the type of HSS. These values were compared to those obtained (4300-5200 mg/kg) previously from milk thistle achenes in Iran [42]. It should be noted that these differences could be due to the different plants genotypes and the climatic conditions in which plants grew along with the variations in the extraction and analysis procedures [4]. In addition, the results of the current study showed that there is a correlation between the EtOH concentration in HSS and amount of extracted silybin. Moreover, it was observed that the amount of target compound was raised by increasing the EtOH concentration in HSS up to a certain level (80% v/v) and beyond that no considerable change was found. The similarity between polarity of solute and solvent is considered important to achieve the highest recovery of the target compound [17]. As the findings of the current study revealed, the highest recovery of silybin from *S. marianum* was obtained using HSS composed of 80% v/v ethanol. Lucini *et al.* (2016) used 80% v/v hydroethanolic solvent as the most efficient extraction solvent for separating silybin from different cultivars of milk thistle. In another study, Roshani Neshat *et al.* (2020) investigated the effect of binary solvent composed of different ratios of water and ethanol for the maximum verbascoside recovery from Lemon verbena leaves. Verbascoside is the major phenolic compound of Lemon verbena leaf. They found that the hydroethanolic solvent composed of EtOH: H_2O (80:20% v/v) was the optimal medium for the maximum verbascoside recovery (19.20 ± 0.12 mg/g) [17].
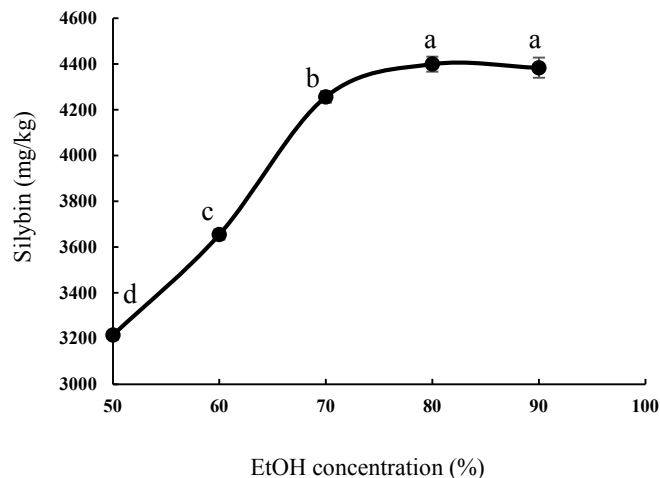

Figure 6: Effect of ethanol concentration on silybin content (mg/kg) of *S. marianum* aerial parts extracts. Different lower letters indicate significant difference (P < 0.05).

3.4. Correlation between anti-radical capacity and silybin content

Generally, different methods of the anti-radical capacity measurements are based on different reaction mechanism which leads to different results. Therefore, determining correlation coefficients is necessary to decide whether anti-radical capacities can be predicted in different assays. As shown in Figure 7 (A), two different assays including DPPH and ˙OH free radical scavenging assessments were positively correlated with R^2 = 0.98, which confirmed the accuracy of the results. Based on the results, the applied anti-radical assays could provide reliable information on the radical scavenging capacity of bioactive compounds obtained from the aerial parts of *S. marianum*. The correlation between antioxidant activity measurements methods were observed in different studies [17, 43]. Figure 7 (B) depicts the correlation between free radical scavenging activity assays and silybin content from the aerial parts of *S. marianum* extracts. Positive correlations were observed between silybin content and DPPH (R^2 = 0.95) and ˙OH (R^2 = 0.97) radical scavenging assays. These results confirmed the antioxidant activity of silybin. Further, Valenzuela (1989) was also reported the antioxidant activity of silybin which confirmed our findings [41].

3.5. Mass transfer kinetics of bioactive compounds release

As indicated in Figure 8, the extraction curve obtained based on the observed data and Peleg model constants. The results showed a good agreement between observed data and those predicted by the developed Peleg model. The calculated Peleg model constants (K_1 and K_2) and the checking accuracy parameters (R^2, RMSE and E) were presented in Figure 8. K_1, K_2 and C_{eq} were found as 0.085 min g/mg, 0.006 g/mg, and 156.93 mg/g, respectively.

(A)

$y = 1.0886x + 7.7002$
$R^2 = 0.9851$

%DPPHsc (y-axis, 20–45)
%HOsc (x-axis, 15–31)

(B)

$y = 121.04x + 967.37$
$R^2 = 0.9751$

$y = 109.02x + 186.71$
$R^2 = 0.9517$

■ %DPPHsc
● %HOsc

Silybin content (mg/kg) (y-axis, 3000–4600)
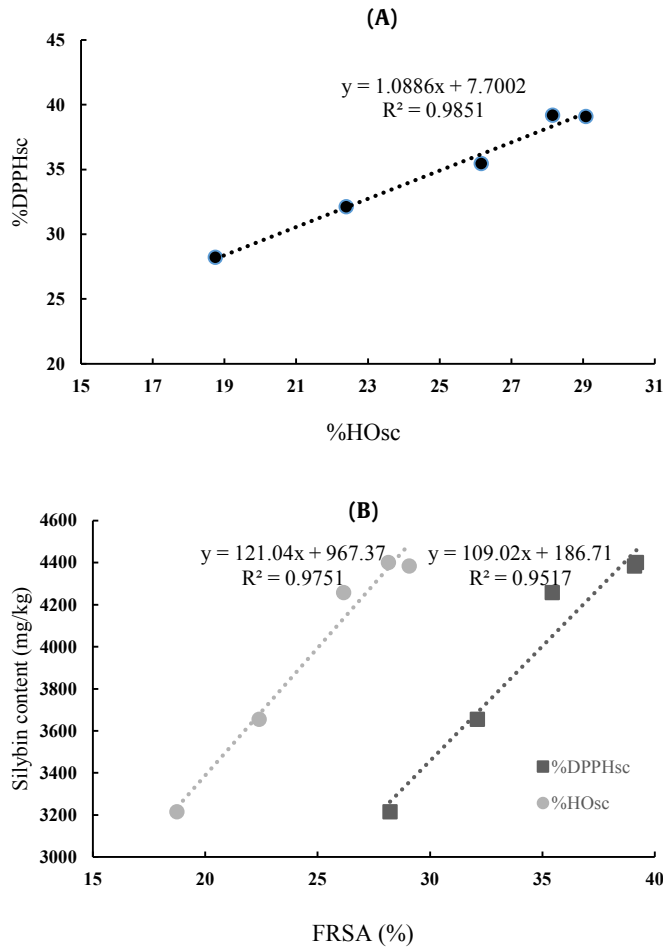FRSA (%) (x-axis, 15–40)

Figure 7: Correlation between (A) anti-radical capacity assays (%) and (B) anti-radical capacities (%) and silybin content (mg/kg)

A model is considered acceptable with the highest value of R^2 and the least values of E and RMSE which was found in the current study. Based on Figure 8, the extraction process happened in two phases. During the first phase the extraction rate was higher due to the lower content of target solutes in the surrounded solvent which accelerated the release of target compounds from the cell matrix into the surrounded medium. This phase can be observed from the beginning of the extraction process until around 100 min. Then, during the second phase, the extraction process rate was decreased to zero order which reflected the mass transfer equilibrium state.

These findings were consistent with those available in the literature. It has been stated that the solid-liquid extraction process occurred in two stages. The major amount of the solutes quickly released during the first stage of process as a
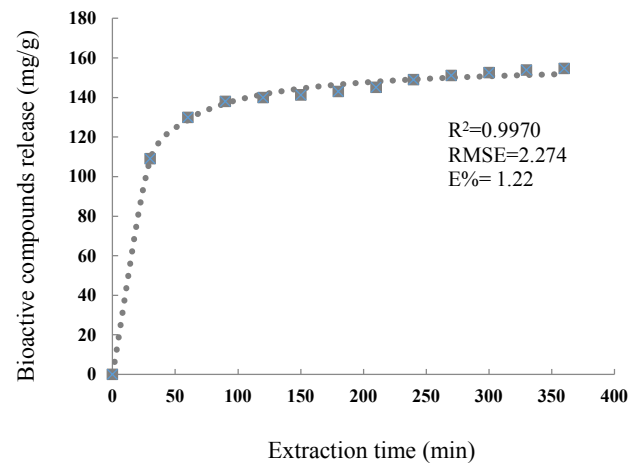

$R^2 = 0.9970$
RMSE = 2.274
E% = 1.22

Bioactive compounds release (mg/g) (y-axis, 0–180)
Extraction time (min) (x-axis, 0–400)

Figure 8: Mass transfer kinetics of bioactive release using HSS composed of 80:20% v/v of EtOH: H_2O

result of higher driving force between the solid and liquid phases. In the subsequent stage, the extraction rate will be decreased and reach to an equilibrium stage which reflected the end of the process [17, 44]. In the current study, the plateau state of extraction process was obtained after around 100 min of extraction process. This finding is a crucial point for scaling up the extraction process to inhibit the thermo-degradation of bioactive compounds and also reducing the energy costs.

3.6. SEM analysis

In the current study, SEM was used to monitor the cell structure changes before and after the extraction process. Based on Figure 9, the cell structure of dried sample was intact and wrinkled before the extraction process. However, after the process destruction on sample microstructure was obvious which indicated the intense effect of extraction process on cell disruption. It is well-known that high temperatures during the extraction process resulted in proteins denaturation, leading to the cell structures collapse. This cell changes may cause considerable improvements in solutes mass transfer and increases the extractable compounds release into the solvents [45]. In another study, Yu *et al.* (2018) analyzed the effect of conventional Soxhlet extraction on cell structure of ginseng powder. They stated that there were serious structure changes on the surfaces of the samples compared with the untreated sample. These observations led to efficient ginsenosides release and other intracellular compounds into the surrounded solvent [46].
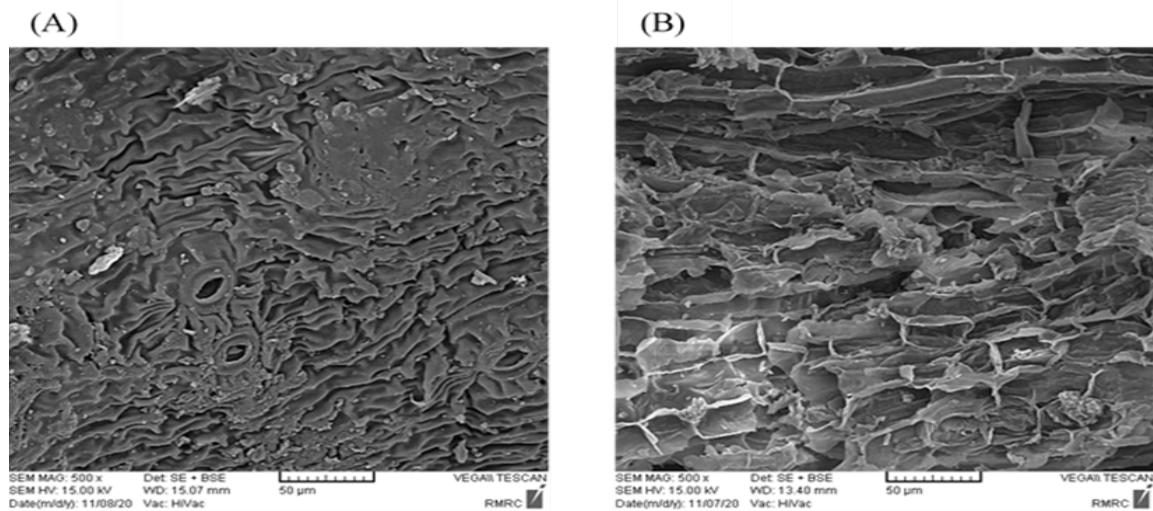
Figure 9: SEM images of the aerial parts of the *S. marianum* (A) Non-treated sample; (B) after Soxhlet extraction process using HSS composed of 80:20% v/v of EtOH: H$_2$O

4. Conclusion

An ethanol-based extraction process was performed for selective extraction of bioactive compounds from the aerial parts of *Silybum marianum*. The results demonstrated the selective extraction of bioactive compounds from the aerial parts of *Silybum marianum* using an appropriate solvent. It was revealed that higher values of TEC and radical scavenging capacity could be obtained by increasing the ethanol concentration up to a certain level (80% v/v). The highest value of silybin content was found in those extracts obtained using HSS composed of 80% v/v hydroethanolic solvent. High values of correlation between radical scavenging assays and silybin content of extracts demonstrated the accuracy of the results and antiradical activity of silybin as the main bioactive compounds existed in the extracts. Furthermore, SEM images confirmed the facilitated release of intracellular compounds from cell matrix into the surrounded solvent. Overall, this study offered a practical and effective protocol for selective extraction of valuable bioactive compounds from the aerial parts of *S. marianum* especially silybin. These bioactive compounds could be considered for potential applications in food industry.

Authors' Contributions

M.B., and A.G., developed the idea for research, performed the statistical analysis, and revised the manuscript. F.M., managed the literature review and data collection. All the authors read and approved the final manuscript.

Conflicts of Interest

The Authors declare that there is no conflict of interes.

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