

Research Article

Optimization of Aqueous Extraction Conditions of Inulin from the *Arctium lappa* L. Roots Using Ultrasonic Irradiation Frequency

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Ultrasound-assisted extraction is a promising technique to obtain active compounds from plants with high efficiency. The present study was conducted in two sections. In the first phase, the effect of solvent type (methanol, ethanol, water, and water-ethanol (50 : 50, v/v)) on inulin extraction yield from burdock roots (*Arctium lappa* L.) was investigated by the conventional method. The second phase aim was to optimize the condition of inulin and phenolic compounds including sonication time (10–40 min), sonication temperature (40–70°C), and solid/solvent ratio (1 : 20–1:40 g/ml) using response surface methodology (RSM). The results demonstrated that the highest inulin efficiency was extracted by water in the conventional method, which is equal to 10.32%. The optimum conditions of ultrasound-assisted water extraction for independent variables including sonication time and temperature as well as solid/water ratio were 36.65 min, 55.48°C, and 1 : 35 g/ml, respectively, which were determined on the maximization of inulin and total phenol content and minimization of IC₅₀. At this optimum condition, inulin yield, phenolic compounds, and IC₅₀ were found to be 12.46%, 18.85 mg GA/g DW, and 549.85 µg/ml, respectively. Regarding the results of this research, ultrasound-assisted extraction can be used as an alternative to the conventional extraction methods in extracting bioactive compounds from medicinal plants because it may improve the mass transfer, reducing the extraction time and the solvent used.

1. Introduction

Inulin is a fructan that is not only found in many plants as a storage carbohydrate but has also been part of human's daily diet for several centuries. It consists of a long chain made up of 22–60 fructose molecules linked to each other by β (2 \rightarrow 1) bonds, with a terminal glucose molecule [1]. The sequence of fructose monomers in the structure of inulin makes inulin indigestible in the human gastrointestinal tract and does not increase blood sugar levels [2]. Another important reason to pay attention to inulin is the publication of findings that show that inulin has beneficial effects on the composition of the intestinal flora, mineral absorption, blood lipid composition, and prevention of colon cancer [3].

In addition, inulin is a low-calorie fiber and has the potential to be used in the production of fat-reducing foods. It is used as an ingredient in foods with reduced or no sugar and fat, such as chocolates, ice creams, and yogurt. Inulin is in the category of water-soluble dietary fiber and is used as a functional substance due to its probiotic and bifidogenic properties [4]. Many food and pharmaceutical industries have found applications for inulin in the production of functional foods, nutritional composites, and medicines. Inulin is naturally present in 15% of flowering plant species such as onion, garlic, asparagus, banana, Jerusalem artichoke, chicory, dahlia, leek, Salsify, and burdock root and is also produced by some bacteria and fungi. The amount of inulin in these plants is in the range of 1 to 20% of the weight

of the fresh plant [2]. Among these plants, chicory roots and artichoke from the Compositae family are outlined for inulin production on an industrial scale due to its higher content and stability in producing a high fructose/glucose ratio and because of its regular growing, even in moderate climates [1].

Burdock (*Arctium lappa* L.), a perennial herb in the family of Compositae, stores most of its nutrients during the first year. These nutrients are then used for the flower-blooming process afterward. The plant, which can be found worldwide, has been cultivated as a vegetable for a period of a long time in Asia [5]. The extracts from different parts of burdock have long been considered to be good for health. They help enhance the body's immune system and improve metabolic functions [6]. In many countries in the Middle East, Asia, and Europe, this plant has many medicinal uses. Studies have shown that different parts of this plant, including leaves, roots, and stems, have a variety of effects, and different anti-inflammatory, antimicrobial, antidiabetic, anticancer, and antiviral activities are known for it [7]. Burdock is traditionally used to treat diseases such as sore throat and infections such as rashes, boils, and various skin problems. The dried root of 1-year-old burdock is the major part used for different therapeutic purposes [5]. It is suggested that this plant has the ability to reduce oxidative stress, and this ability is possible by increasing the content of glutathione and cytochrome-p450 and reducing the amount of malondialdehyde and leads to scavenging free radicals in the liver and kidney poisoning and shows strong anti-inflammatory effects [8]. It has been reported that the lyophilized extract of the leaves of burdock exhibits antimicrobial activity against oral microorganisms and is most effective against bacteria related to endodontic pathogens such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Candida albicans* [6]. The major active ingredients isolated from this herb are inulin, arctigenin, tannin, trachelogenin 4, caffeic acid, arctiin, beta-ecdysmol, sitosterol-beta-D-glucopyranoside, chlorogenic acid, lappaol, and diartigenin [9]. Apart from these compounds, burdock also contains various common nutrients such as essential amino acids, metallic elements such as potassium, calcium, phosphorus, magnesium, manganese, sodium, zinc, and copper, vitamins B1, B2, C, and A, crude fiber, phosphorus, and carotene [5]. The first basic step in the extraction of active compounds from a plant is the extraction method. Because of the type of chemical compound extracted, its quality and quantity are strongly influenced by plant organs and selected solvent systems. Therefore, it can be said that choosing the appropriate method of extraction can dramatically increase the extraction efficiency of active ingredients in the plant [10]. Since plant extracts are widely used in the pharmaceutical, food, and health industries, extraction technologies have been evaluated to be able to extract these chemical active ingredients from plant sources. Conventional methods such as soaking, which has been used for many years, have disadvantages such as (i) being very time-consuming, (ii) consuming a large amount of solvent, (iii) having low extraction efficiency, and (iv) not being thermally safe and causing the decomposition of a number of existing chemical compounds, because most of the

chemical compounds in plants are thermally unstable and may decompose during thermal extraction and distillation methods [11, 12].

For this reason, there is a great demand for newer extraction methods with a shorter time, lower solvent consumption, and environmental protection. New extraction methods such as microwave-assisted extraction, supercritical fluid extraction, and ultrasound-assisted extraction are very fast and effective in extracting chemical compounds from plants [13]. In recent years, ultrasound-assisted extraction has been used as a new method for extracting chemical compounds from plants, which has advantages compared to conventional methods. The ultrasound extraction method is not only a clean process but also improves the extraction efficiency by increasing the penetration of solvent into the plant cells via cavitation [14]. Another advantage of the ultrasound method process is that it provides the possibility of extracting at lower temperatures, so it prevents the degradation of extracts [15]. Vilkhua et al. [16] in their study showed that the extraction of flavonoids under optimal conditions by ultrasound occurs at much lower temperatures than using a hot water bath at 80°C.

In any extraction method, the extraction variables have considerable effects on the extract yields of chemical compounds. The optimization of extraction variables is generally done with a classical optimization method in which one variable is changed at a time while the other variables are kept constant [14]. This classical optimization approach is time-consuming and requires a large amount of material [13]. Using an experimental design to optimize experimental variables is time-saving and leads to a reduced number of experiments which results in the use of less material and reagent. Also, this method gives information about interactions among the variables [17]. In previous works, response surface methodology with different experimental designs to optimize extraction parameters for the ultrasound extraction method of plants has been widely investigated [13, 14, 18–20].

In this study, the effect of different solvents including water, ethanol, methanol, and water-ethanol on the amount of inulin extraction from burdock roots under constant ultrasound conditions was investigated, and after selecting the desired solvent, in the next step, ultrasound-assisted extraction parameters including temperature, time, and the ratio of solid to solvent were optimized using response surface methodology (RSM) with a central composite design (CCD). The phenolic compounds and scavenging activity of the extracts were also investigated.

2. Materials and Methods

2.1. Materials. The burdock roots were obtained from the local medical plant market, Tehran, Iran. Sulfuric acid 96%, D-nitrosalicylic acid, D-fructose, D-glucose, double sodium, potassium tartrate, and sodium hydroxide were purchased from Sigma-Aldrich (Darmstadt, Germany). Other chemicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, ethanol, and phenol crystal were prepared from Merck Company (Germany).

2.2. Preparation of Burdock Roots Powder. The burdock roots were peeled, sliced, and air-dried at 55–60°C overnight. Then, the dried burdock roots were ground and passed through a 50 µm sieve to achieve the same grain size. The prepared sample with approximately 5.2% moisture content was stored in a dry container for further use.

2.3. Extraction by Maceration (Conventional Method). In the first step, in order to select a suitable solvent for the extraction of burdock root extract, the effect of different solvents including water, ethanol, methanol, and water-ethanol (50:50, v/v) on the extraction efficiency was investigated. For this purpose, the burdock root powder was mixed with each of the mentioned solvents in a ratio of 1:10, and the extraction operation was performed on a hot plate with a magnetic stirrer at 250 rpm for 24 hours at room temperature. The extract was then filtered under vacuum by the Buchner funnel with a paper filter (Whatman No. 40, UK). A rotary evaporator (LABORATA4000) at 40°C was used to remove solvents and concentrate the extract. Finally, the concentrated extracts were dried by a vacuum drying oven (Model DZF-6010, China) at 50°C and were stored at 4°C until use in sealed and impermeable containers [21]. The extract with the highest content of inulin and phenolic compounds was selected as the optimal solvent for the next step.

2.4. Ultrasound-Assisted Extraction (UAE). The process of inulin extraction from burdock root by ultrasound treatment was performed using an ultrasonic bath (ROHS Digitals-DSA100-SK2 South Korea). The ultrasound-assisted extraction experiments were carried out at different solid-to-liquid ratios (1:20–1:40 g/ml), sonication temperatures (40–70°C), and sonication times (10–40 minutes) according to the designated experimental design. Burdock root powder and water in a substance/liquid ratio of 1:10 were placed into an Erlenmeyer flask, and subsequently, the flask was placed into the water bath. After the completion of the ultrasound treatment, the mixture of liquids and solids was centrifuged at 6000 rpm for 10 minutes, and the supernatant was collected by filtering with a paper filter to remove insoluble residues [22].

2.5. Experimental

2.5.1. Inulin Extraction Yield. The extracted liquid was centrifuged at 4500 g for 10 min to remove suspended particles. The supernatant was diluted before determination. Total carbohydrate was determined by the sulfuric acid method of Dubois et al. (1956) using D (-)-glucose as standard. Reducing sugar was determined by the dinitrosalicylic acid method using D (-)-fructose as standard. The pH value was measured with a pH meter. The inulin content was measured with the difference between total carbohydrates and reducing sugars. The percentage inulin yield (%) was evaluated based on the following equation [23, 24]:

$$\text{inulin yield} = \frac{\text{inulin content} \times \text{volume of extraction liquid}}{\text{mass of burdock root powder}} \times 100. \quad (1)$$

2.5.2. Total Phenol Compound Measurement. Total phenol content was measured according to the Folin–Ciocâlteu method. First, 1.6 ml of the extract was mixed with 0.2 mL of the Folin–Ciocâlteu reagent and was placed at rest for 3 minutes to react. Then, 0.2 ml of sodium carbonate 10% was added to it and after one minute was brought to 50 ml with distilled water. The sample was kept in a dark place for 24 hours, and then, its absorbance was read at 760 nm in front of the control. In this assay, gallic acid was used as the standard and its standard curve was drawn. Then, using the resulting line equation, the total phenol content of extracts was determined [25].

2.5.3. Radical Scavenging Activity. The efficacy of the extracts to scavenge DPPH radicals was determined using the spectrophotometry method on the basis of bleaching of the bluish-red or purple color of DPPH solution as a reagent, according to Ersus and Yurdagel [26] with minor changes. 1 mL of methanolic solution of the sample was poured into a test tube, and then, 1 mL of methanolic solution of DPPH with a concentration of 0.004% was added to it. After mixing with the vortex, the test tubes were kept in a dark place for 30 minutes, and then, their absorption was recorded at 517 nm using a UV/Vis spectrophotometer (Model T60 UV, USA). The percentage of DPPH free radical quenching activity was determined using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100, \quad (2)$$

where $\text{Abs}_{\text{control}}$ is the absorbance value at 517 nm of the methanolic solution of DPPH and $\text{Abs}_{\text{sample}}$ is the absorbance value at 517 nm for the sample extracts.

IC50 indicates the concentration of the extract that causes 50% inhibition in the radical capacity and its value is obtained by plotting different values of DPPH scavenging in terms of different concentrations of the extracts and calculating the regression line equation.

2.6. Experimental Design and Statistical Analysis. Response surface methodology (RSM) was used to estimate the effect of independent variables on the inulin extraction yield (%), polyphenol content (mg GA/g DW), and IC50 (µg/ml). Three extraction variables considered for this research were X1 (sonication time), X2 (sonication temperature), and X3 (solvent: solid) for the ultrasound-assisted extraction method. A Face Central Composite Design was employed for designing the experimental data. Experimental data were modeled using the Design-Expert software version

6.01 (Stat-Ease Inc., Minneapolis, MN, USA) and three-dimensional representations of the response surface generated by the model. Experimental data were fitted to a quadratic polynomial model. The model proposed for the response (Y) was as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + \varepsilon. \quad (3)$$

The coefficients of the polynomial model were represented by b_0 (constant term), b_1 , b_2 , and b_3 (linear effects), b_{11} , b_{22} , and b_{33} (quadratic effects), and b_{12} , b_{13} , and b_{23} (interaction effects). The significant terms in the model were found by the analysis of variance (ANOVA) for each response. The adequacy of the model was checked accounting to R^2 and adjusted R^2 . The numerical and graphical optimization technique of the Design-Expert software was used for simultaneous optimization of the multiple responses. The desired amounts for each variable and response were chosen. All the independent variables were kept within the available range while the responses were either maximized or minimized [17, 23].

3. Results and Discussion

3.1. Phase 1: Selection of the Extraction Solvent by Maceration Method

3.1.1. Inulin Extraction Yield. Figure 1 shows a comparison of the average extraction yield of inulin by different solvents with the maceration method. The results of ANOVA showed that the solvent type has a significant effect on the efficiency of inulin extraction from the burdock root. According to the results, it was found that the highest inulin extraction efficiency belonged to water solvent, which was equal to 10.32%. The lowest inulin extraction efficiency was observed in methanol solvent which was 2.55%. Inulin and oligofructose are soluble dietary fibers that increase the possibility of extracting these compounds from plant sources by increasing the degree of polarity of the solvent [27]. In this research, among the studied solvents, water has the highest degree of polarity, so it showed the highest inulin extraction efficiency. As can be seen, the polarity of the solvent was reduced by adding ethanol to the water, so the extraction efficiency was significantly reduced to 5.12% ($p < 0.05$).

In a similar study, Predes et al. [28] investigated the antioxidant and antimicrobial effect of burdock root extract and reported that the highest extraction efficiencies were obtained in water solvent and then water-ethanol solvent, respectively, and the lowest efficiency belonged to dichloromethane solvent. Martono et al. [27] and Milani et al. [23] also used water solvent to optimize inulin extraction conditions from Gembili (*Dioscorea esculenta* L.) and burdock root because it showed the highest extraction yield which is consistent with the results of this study. Ferracane et al. [29] also attributed the higher inulin extraction efficiency in the aqueous extract of burdock roots to the higher solubility of inulin in water than ethanol.

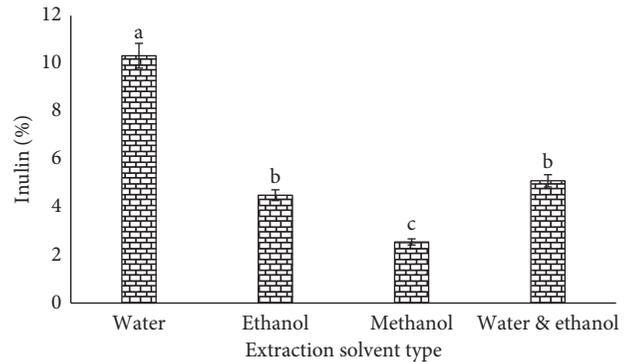


FIGURE 1: Comparison of mean inulin extraction yield by different solvents in maceration method.

3.1.2. Total Polyphenol Content. The results of the effect of the different solvents on the extraction of phenolic compounds from the burdock roots extract are shown in Figure 2. According to the results of ANOVA, the type of solvent had a significant effect on the amount of this parameter ($p < 0.05$). As can be seen, the highest amount of phenolic compounds was measured in the extract of water-ethanol solvent (50:50, v/v) which was equal to 17.22 mg GA/g DW. The amount of phenolic compounds was measured in the aqueous extract with a rate of 16.38 mg GA/g DW, although lower than the water-ethanol solvent, but this difference was not statistically significant ($p > 0.05$). Also, according to the results (Figure 2), the amount of phenolic compounds in the extract of pure methanol and ethanol solvent was measured (14.85 and 13.5 mg GA/g DW, respectively), which was significantly lower than water and water-ethanol solvents.

Phenols are compounds that comprise one or more hydroxyl groups (polar section) attached to an aromatic chain (nonpolar section) and this structure distinguishes phenolic compounds based on their polarity. Therefore, the solubility of phenolic compounds in a solvent can be explained by this spatial structure and the intermolecular force (mainly hydrogen bonding) that occurs between them and the solvent [30]. The use of water as an extraction solvent creates a highly polar environment that is suitable for the extraction of high-polarity bioactive compounds. While the water-ethanol solvent is suitable for the extraction of bioactive compounds with a wide range of polarity, this is due to the relatively polar environment created by the addition of organic solvents to water [31]. On the other hand, water is needed for the swelling of plant tissue cells, so with the presence of appropriate amounts of water in organic solvents, the permeability of the cell wall increases and can be easily broken by processes such as ultrasound, but in a pure organic solvent such as ethanol, there is no water available for cell swelling, so in most cases, the combination of water with ethanol or methanol will be effective. It is reported that ethanol and methanol solvents mixed with water have a greater ability to extract phenolic compounds due to their higher polarity than their pure alcoholic solvent [32].

In this study, the water showed a greater ability to extract phenolic compounds due to its high polarity than ethanol

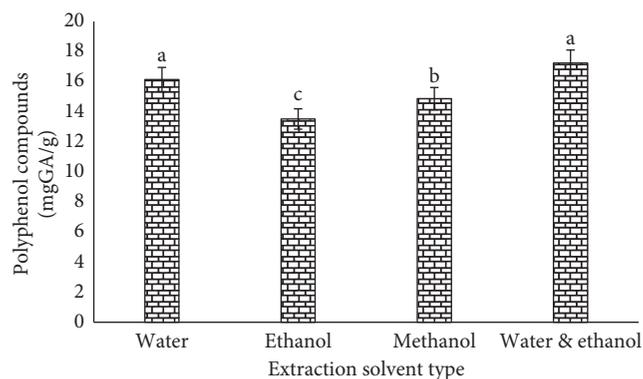


FIGURE 2: Comparison of mean phenolic compounds of extracts obtained from different solvents in the maceration method.

and methanol solvents, and although the addition of ethanol resulted in higher solvent efficiency in the extraction of these compounds, this difference was not considerable. This is probably due to the greater polarity of the phenolic compounds in burdock roots extract, which is more soluble in water and water-ethanol mixtures; therefore, it can be said that the nature of phenolic compounds in the plant determines the effectiveness of the solvent in the extraction of phenolic compounds.

In comparison with our results, Horng et al. [33] indicated that the content of total polyphenols in burdock extract obtained by reflux extraction for three hours reached 48.4 mg GA/g. However, our results were higher compared to the results of Ferracane et al. [29] who used a mixture of methanol/water (70 : 30, v/v) at room temperature to extract phenolic compounds from the burdock seeds, roots, and leaves and showed that the highest and lowest contents of these compounds were measured in the seeds and roots of the plant, which were equal to 45.76 and 2.87 g GA/100 g, respectively. These researchers expressed that the amount of total phenolic compounds in the burdock seed extracts was very high when compared to that found in other medicinal plants. For example, Cai et al. [34] reported that phenolic contents on methanol extracts of 112 traditional Chinese medicinal plants (whole plants, seeds, leaves, roots, stems, and flowers) ranged from 0.22 to 50.3 g GA equivalent/100 g DW.

It was demonstrated that the content of total phenols in the burdock roots was lower in comparison with the seeds and leaves content, but the amount of inulin in the roots of the plant is considerably higher than seeds and leaves, and since the main purpose of this study was inulin extraction, the roots were used. However, according to the inulin extraction yield as well as phenolic compounds, water was selected as the extraction solvent among the studied solvents, and in the next stage, the inulin ultrasound-assisted water extraction conditions were optimized.

3.2. Phase 2: Optimization of Ultrasound-Assisted Extraction Condition

3.2.1. Inulin Yield. The results of ANOVA of the effect of the independent variables on the inulin extraction efficiency of burdock roots extract are given in Table 1. As can be seen,

the effect of all three variables includes sonication time and temperature as well as solvent/solid ratio on inulin extraction efficiency was significant ($p < 0.05$). Figure 3(a) shows the simultaneous effect of sonication time and temperature on inulin efficiency. The amount of inulin extracted by the treatments ranged from 4.65 to 13.48%. According to the results, time had a significant effect on extraction efficiency so that with increasing time from 10 to 40 minutes, extraction efficiency was upward and the maximum value was obtained in 40 minutes. As regards the fact that the linear effect of time was greater than its quadratic effect (Table 1), less curvature can be seen in the diagram. However, in the case of sonication temperature, the quadratic effect was much higher than linear, so more curvature was expected. The results were that with increasing temperature from 40 to 55°C, the extraction efficiency increased, but with increasing temperature up to 70°C, the amount of inulin extracted was decreasing (Figure 3(a)). It can be deduced from Figure 3(b) that the relationship between inulin extraction efficiency and water/solid ratio is linear, which is confirmed by the significant linear effect and the nonsignificance of the quadratic effect of this independent variable (Table 1).

The results showed that, with increasing the water/solid ratio, especially at higher times of the ultrasound process, inulin extraction increased due to the increase of solvent penetration into the root cell walls. On the other hand, with increasing the amount of water consumed, the extraction efficiency increases due to the increase of diffusion phenomenon and more movement of inulin from the root cell to the solvent [23].

Ultrasonic waves pass through the solvent and produce cavitation bubbles to increase the rate of mass transfer. As a result, inulin can be easily released through cell walls and increase extraction performance. It has also been proven that high shear forces increase the mass transfer of the extractive material [35]. In addition, the cavitation process near the solvent-solid interface sends a fast-moving stream of solvent through the cavity at the surface [36]. The increase in yield with increasing sonication temperature is also due to the improved mass transfer caused by the increase in inulin solubility and the decrease in solvent viscosity. It is also stated that the increase in extraction efficiency of polysaccharides is due to the strong effect of temperature during extraction on the mass transfer of water-soluble polysaccharides in the cell walls [37].

However, the results of this study showed that as the temperature approaches 70°C, the efficiency decreases considerably, which may be attributed to the degradation of inulin into free sugar (monosaccharides). Furthermore, increasing the extraction temperature may lead to increased solvent volatility, energy loss, and increased extraction of impurities with inulin [23]. The amount of inulin obtained from aqueous extraction of burdock roots by ultrasound in this study is consistent with the findings of Cao et al. [38] who reported that the inulin content of Korean origin plants was 9% dw. It was also demonstrated that the efficiency of the inulin extraction process from the burdock roots of Iranian origin using high-intensity ultrasound irradiation

TABLE 1: Table of ANOVA for the experimental variables as linear, quadratic, and interaction terms of each response variable.

| Source | DF | Inulin yield | | Total phenols | | IC50 | |
|--------------------------------|----|--------------|-------------------|---------------|----------------|--------------------|-------------------|
| | | Mean square | <i>p</i> value | Mean square | <i>p</i> value | Mean square | <i>p</i> value |
| Model | 9 | 9.20 | <i>p</i> < 0.0001 | 17.83 | 0.0025 | 2.78×10^5 | <i>p</i> < 0.0001 |
| <i>Linear</i> | | | | | | | |
| <i>b</i> ₁ | 1 | 59.19 | <i>p</i> < 0.0001 | 43.90 | 0.0011 | 4.51×10^5 | <i>p</i> < 0.0001 |
| <i>b</i> ₂ | 1 | 2.68 | 0.0001 | 14.91 | 0.012 | 29804.6 | 0.0139 |
| <i>b</i> ₃ | 1 | 4.55 | <i>p</i> < 0.0001 | 25.49 | 0.0037 | 4.21×10^5 | <i>p</i> < 0.0001 |
| <i>Quadratic</i> | | | | | | | |
| <i>b</i> ₁₁ | 1 | 4.40 | 0.008 | 46.07 | 0.001 | 6.72×10^5 | <i>p</i> < 0.0001 |
| <i>b</i> ₂₂ | 1 | 9.77 | <i>p</i> < 0.0001 | 30.89 | 0.0024 | 1.03×10^6 | <i>p</i> < 0.0001 |
| <i>b</i> ₃₃ | 1 | 0.064 | 0.092 | 0.55 | 0.483 | 459.38 | 0.664 |
| <i>Interaction</i> | | | | | | | |
| <i>b</i> ₁₂ | 1 | 0.64 | 0.001 | 0.30 | 0.60 | 2751 | 0.311 |
| <i>b</i> ₁₃ | 1 | 0.15 | 0.0029 | 14.54 | 0.019 | 36 | 0.902 |
| <i>b</i> ₂₃ | 1 | 0.34 | 0.0041 | 12.36 | 0.038 | 9506.25 | 0.041 |
| Lack of fit | 3 | 0.022 | 0.086 | 1.60 | 0.061 | 3661 | 0.092 |
| Pure error | 2 | 0.0026 | | 0.018 | | 226.23 | |
| <i>R</i> ² | — | 0.997 | | 0.971 | | 0.995 | |
| <i>R</i> ² adjusted | — | 0.992 | | 0.918 | | 0.988 | |

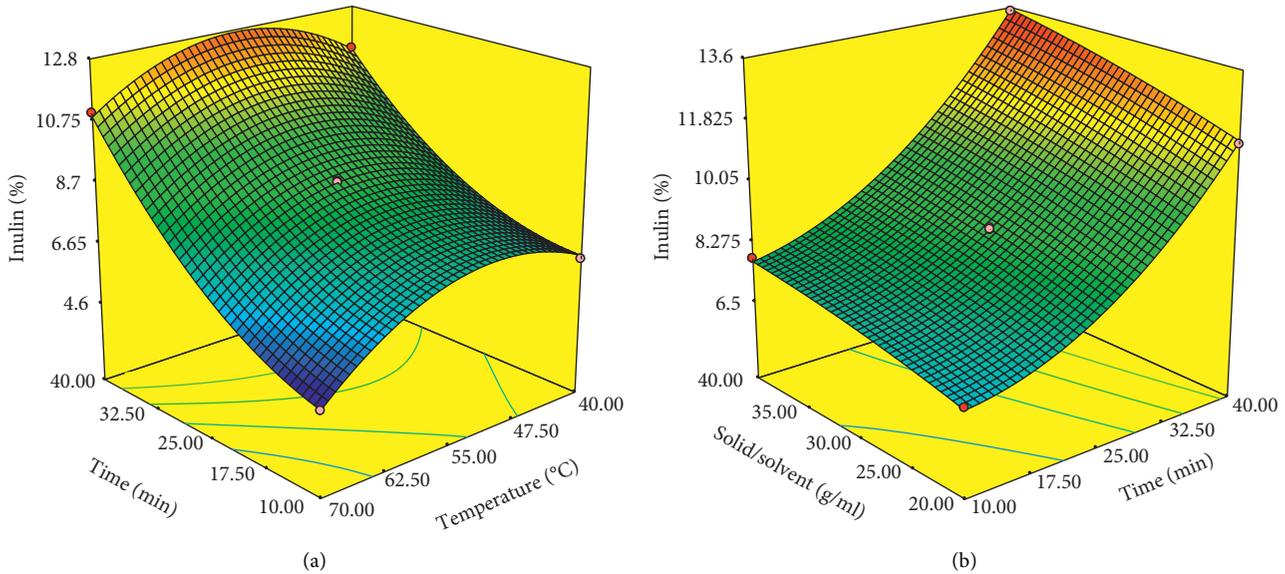


FIGURE 3: Response surface for the effect of (a) sonication time and sonication temperature (water/solid = 1 : 30 g/ml) and (b) sonication time and water/solid ratio (sonication temperature = 55°C) on the yield of inulin.

were equal to 12% [39]. However, there are also some contradictory reports about the quantity of inulin in burdock roots. For example, Bagaoutdinova et al. [40] reported that the amount of inulin extracted from burdock roots of Russian origin was 30%, and according to Petkova et al.'s [41] findings, the highest amount of inulin measured in methanolic and aqueous extracts of burdock roots collected from Bulgaria was 4%. Olennikov and Tankhaeva [42] also measured the inulin content of the burdock root extracts equal to 4.7–5.2% DW.

The researchers also compared the ultrasound and microwave techniques to extract dietary fiber with prebiotic properties (including inulin and fructooligosaccharide) from the burdock roots with two solvents of water and

ethanol 70% concluding that ultrasound-assisted water extraction is a more efficient technique [41]. The diversity of inulin content can be explained by the geographical origin of *A. lappa*, cold weather conditions, and storage conditions [43]. Research has shown that the carbohydrate metabolism stored in the *A. lappa* depends approximately on temperature and other physiological factors [41].

The predicted model to estimate the amount of inulin extracted with respect to regression coefficients was obtained as equation (4), which due to the significance of the model ($p < 0.0001$) and $R^2 = 0.997$ and $Adj-R^2 = 0.992$ and non-significance lack of fit of the model ($p = 0.086$), it can be concluded that the model has the adequacy to estimate the desired response. The high value of the polynomial model

determinant coefficient indicates that 99.7% of the variability in the response could be explained by the second-order polynomial prediction equation.

$$\begin{aligned} \text{inulin} = & -15.574 - 0.197x_1 + 0.77x_2 + 0.228x_3 \\ & + 0.00177x_1x_2 + 0.0013x_1x_3 - 0.00195x_2x_3 \quad (4) \\ & + 0.00485x_1^2 - 0.00722x_2^2 - 0.00131x_3^2. \end{aligned}$$

3.2.2. Total Polyphenol Content. According to the ANOVA results (Table 1), the linear effect of sonication time (x_1) and temperature (x_2) as well as solid/solvent ratio (x_3) on the extraction rate of polyphenol compounds was significant ($p < 0.05$). Also, quadratic effects (except the effect of solid/solvent ratio, x_3^2) and interactions (except the effect of time and temperature, x_{12}) on the amount of this variable were significant ($p < 0.05$). Figure 4 shows the response surface plots of the effects of water/solid and sonication time and sonication temperature on the polyphenol content. Among the studied samples, the total phenolic content ranged between 8.5 and 20.25 mg GA/g for the ultrasound-assisted extract obtained with water. As can be observed, the sonication temperature and time play a quadratic effect on the response, which means that by applying ultrasound waves for 10–25 minutes at a temperature of 40 to 55°C, the extraction rate of these compounds increased. However, with increasing sonication temperature from 55 to 70°C, especially for more than 30 minutes, the amount of phenolic compounds measured decreased (Figure 4(a)). While the effect of the water/solid ratio was linear, and with increasing solvent content, the amount of phenolic compounds extracted showed a significant increase (Figure 4(b)).

With regard to the calculated regression coefficients, the predicted model was obtained as in equation (5) to estimate the phenolic compounds. According to the explanation coefficient ($R^2 = 0.971$) presented in Table 1, it is clear that the model with 97.1% efficiency and error less than 3% has the ability to estimate the response. As previously explained, a suitable model must have a high explanation coefficient (R^2 & Adj- $R^2 > 0.80$) and nonsignificance lack of fit of the model ($p = 0.061$), which is true as

$$\begin{aligned} \text{polyphenol} = & -39.11 + 0.884x_1 + 1.516x_2 - 0.192x_3 \\ & - 0.00122x_1x_2 \\ & + 0.00413x_1x_3 + 0.000633x_2x_3 - 0.0157x_1^2 \quad (5) \\ & - 0.0128x_2^2 + 0.00387x_3^2. \end{aligned}$$

In general, increasing the temperature can accelerate the swelling of raw materials, softening, and rupture of plant tissue, weaken the phenol-protein and phenol-polysaccharide interactions, increase the solubility of the extracted compounds and the solvent diffusion rate, and reduce the viscosity and surface tension. As a result, with a gradual increase in temperature, the mass transfer of phenolic compounds from the burdock roots improved [44]. The increasing trend of phenolic compounds in the early times of extraction is due to the high concentration gradient of

phenolic compounds between the solvent and the cell walls, which accelerates the extraction process. The most release of bioactive compounds occurs at a specific time, and then at higher times, due to the presence of these compounds in less accessible parts of the plant cell and also decrease in the concentration gradient of these substances, the rate of phenolic compound release decreases [45]. Therefore, with increase in the solvent ratio, the concentration gradient increases, which leads to an increase in the amount of phenolic compounds extracted. Some previous studies have reported this initial upward trend and then a decrease in the antioxidant activity of plant extracts [45, 46].

Generally, the cavitation produced in the solvent during the extraction process by ultrasound can increase the extraction of phenolic compounds compared to the submerged method, and the properties of the solvent such as vapor pressure, surface tension, and viscosity can affect the intensity of cavitation [47]. The effect of solvent vapor pressure on the ultrasound process can be related to the production of cavitation bubbles. Solvents with lower vapor pressures produce a small number of cavitation bubbles that require higher energy to decompose, so plant tissue is destroyed more severely during the extraction process. Low viscosity solvents are also more effective because ultrasonic energy overcomes the intermolecular force of the liquid more easily. Also, the low viscosity solvent easily penetrates into plant tissues due to its low density and high diffusion coefficient [46, 47].

In comparison with our results, Petkova et al. [41] by investigating the effect of pressure liquid extraction and ultrasonic irradiation frequency on the extraction rate of phenolic content and inulin from burdock roots reported that the use of water as extraction solvent and 45 kHz frequency showed the highest extraction efficiency of polyphenol compounds which was equal to 18.16 mg GA/g dw. The use of ethanol/water (70:30, v/v) with a frequency of 35 kHz led to the extraction of phenolic compounds with a rate of 17.74 mg GA/g dw, which is not statistically significant ($p > 0.05$). In another study, by evaluation, the phenolic compound extraction process from citrus peel indicated that the use of ultrasound has significantly increased the yield extraction of phenolic compounds compared to the maceration method. They also stated that, with increasing ultrasound temperature and time, there was an increase in the amount of phenolic compounds extracted from citrus peel; however, a decreasing trend was observed in the amount of phenolic compounds extracted at higher times and temperatures (temperatures above 40°C). These researchers attributed this to the thermal decomposition and polymerization reactions of phenolic compounds with each other [44]. Heidari Majd et al. [47] revealed that, with increasing ultrasound temperature and time and decreasing pH, the rate of extraction of phenolic compounds increases, and the best efficiency was obtained in 35–55 minutes, 35–45°C, and pH = 6–6.5. A study was performed on the effect of using high-power ultrasound on oil extraction from ground olive seeds. It was found that, in the presence of these waves, cell walls and plant tissues were destroyed and more antioxidant compounds (polyphenols and tocopherols) and

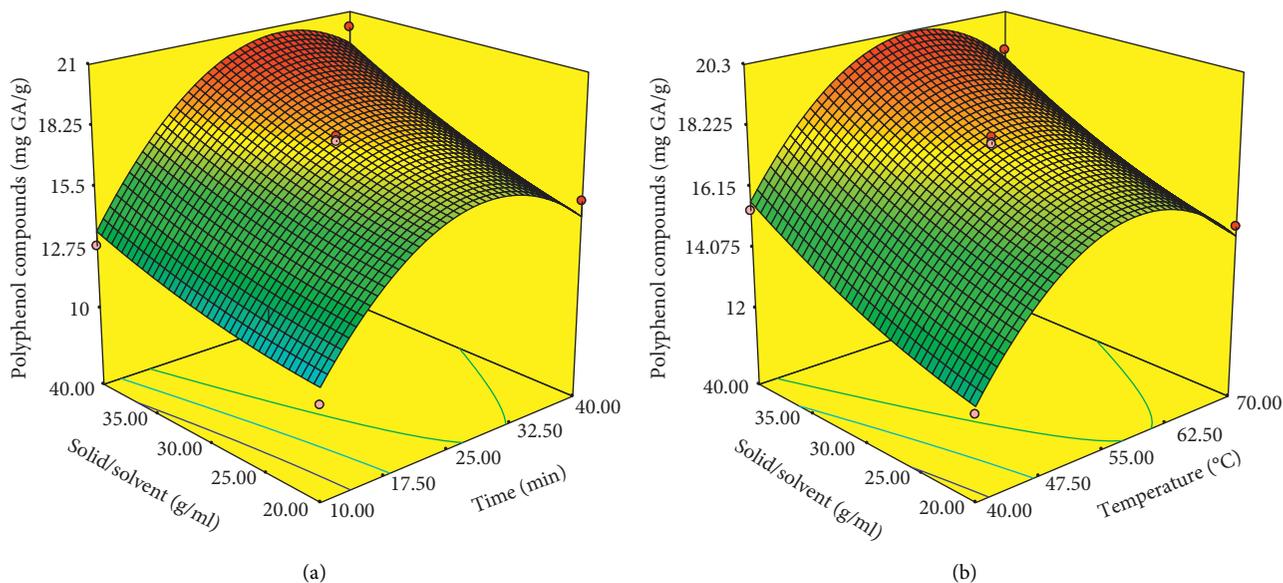


FIGURE 4: Response surface for the effect of (a) water/solid ratio and sonication time (sonication temperature = 55°C) and (b) sonication temperature and water/solid ratio (sonication time = 25°C) on the amount of phenolic compounds.

pigments (chlorophyll and carotenoids) entered the oil and increased the nutritional value [48].

Kongkiatpaiboon and Gritsanapan [49] also studied the extraction of alkaloid pesticides as didehydrostemofoline from the extract of *Stemona collinsiae* roots by five different extraction methods (high-power ultrasound, reflux, Soxhlet, soaking, and infiltration) with 70% ethanol. The results showed that ultrasound and reflux have the highest efficiency in the extraction of this pesticide. They also concluded that increasing heat or ultrasonic energy during the extraction process can reduce extraction time and help increase efficiency. Therefore, based on the results of the present study and the findings of other researchers, it can be concluded that increasing the temperature increases the diffusion coefficient of the solvent, and increasing the time also increases the mass transfer time. On the other hand, in a constant amount of solvent, the reason for the decrease in the amount of phenolic compounds extraction is due to the saturation of the solvent at higher temperatures and times, as well as the thermal decomposition of phenolic compounds at higher temperatures over time.

3.2.3. Antioxidant Activity (IC₅₀). The antioxidant activity of the burdock roots extract was determined as the IC₅₀. The IC₅₀ is a concentration of the extract that inhibits 50% of the DPPH free radicals in the reaction medium. It is known that the smaller the value of this index, the higher the antiradical and antioxidant activity of extracts [50]. The influence of independent variables on the IC₅₀ has been shown in the response surface (Figure 5). With regard to the coefficient of the independent variables in Table 1, sonication time and following water/solid ratio have a main effect on the antioxidant's changes of burdock root extract ($p < 0.05$). The results demonstrated that the phenolic compound content and thus free radical scavenging activity of DPPH at all

temperatures increased to a maximum with increasing process time up to 25 minutes, which means that IC₅₀ is reduced. In the period between 25 and 30 minutes, the amount of IC₅₀ decreased slightly or remained constant. However, with increasing the extraction time in the range of 30 to 40 minutes, the extraction efficiency of phenolic compounds and thus the antioxidant activity of extract had an increasing trend at temperatures of 40 and 55°C, but at 70°C, a significant decrease in the antioxidant activity of the extract was observed; as a result, the IC₅₀ index increased. However, the ratio of solvent to solid at all temperatures and times had a positive effect on the extraction of phenolic compounds and increased antioxidant activity of the extract, which confirmed the significance of the linear coefficients (Table 1) and the noncurvature of the response surface plots (Figure 5). In fact, by increasing the amount of solvent due to the effect of the concentration gradient of polyphenols between the solvent and the burdock root, a higher phenolic content is extracted, which leads to enhanced scavenging activity of the extract and decreased IC₅₀ index [30]. Moreover, at the beginning of the extraction process, the high phenolic content at the surface of the particles is in contact with the solvent and accelerates the release of these phenolic compounds and thus reduces IC₅₀ [47]. Furthermore, the reason for the higher antioxidant activity in the first 20 minutes of the extraction process may be the fact that in the early times, simple phenolics with more hydroxyl groups and consequently more antioxidant activity were extracted [41].

Two important and effective phenomena in ultrasound are the effect of cavitation and temperature. Cavitation is affected by the bursting of bubbles and heat by swelling and destruction of the cellular structure, thereby increasing the mass transfer of intracellular material to the solvent [51]. In this study, at the beginning of the extraction process at lower temperatures, the effect of heat was negligible, and as a result, the extraction performance was lower. Gradually,

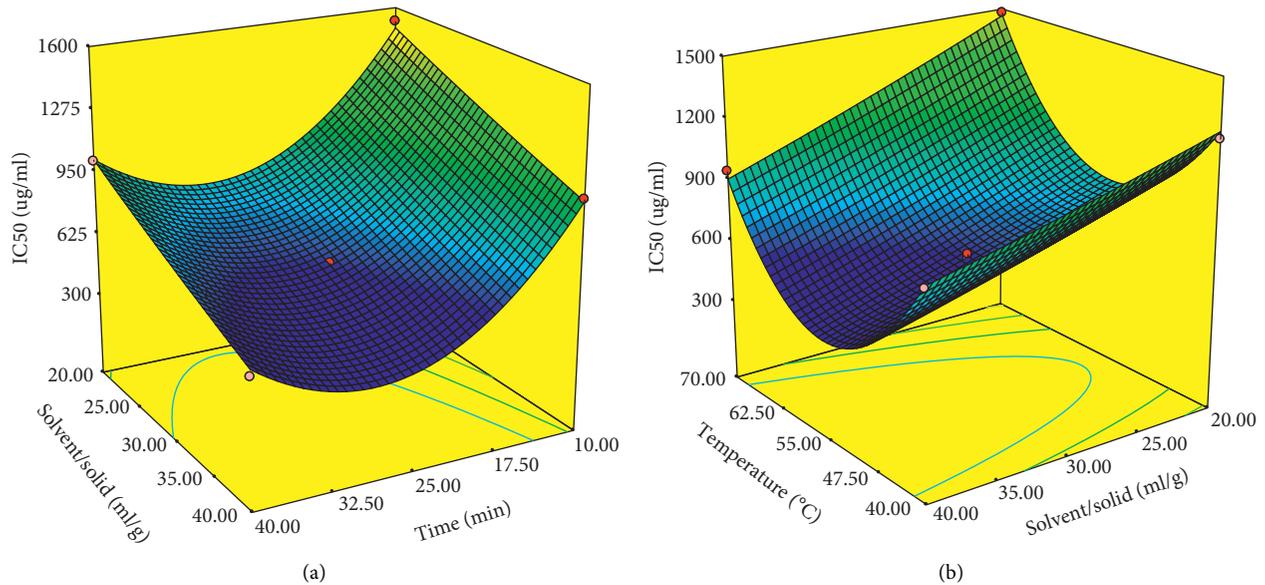


FIGURE 5: Response surface for the effect of (a) sonication time and water/solid ratio (sonication temperature = 55°C) and (b) water/solid ratio and sonication temperature (sonication time = 25°C) on the antioxidant activity of the extracts.

with increasing temperature, both effects were equally dominant and their beneficial combined effect caused relatively higher extraction performance of phenolic compounds and increased antioxidant activity. However, with increasing extraction time, especially at higher temperatures, some of the extracted heat-sensitive compounds were destroyed, which led to a decrease in the antioxidant activity of the extract. In addition, high ultrasound temperatures have a negative effect on cavitation intensity. At higher ultrasonic temperatures, due to the lower surface tension, the vapor pressure of the solvent increases and causes more solvent vapors to enter a large number of cavitation bubbles; as a result, these bubbles disintegrate with less intensity and the cavitation intensity decreases [51].

In a recent study, Fan et al. [50] investigated the antioxidant activity (IC₅₀) of burdock root extracts obtained by a process of ultrahigh-pressure extraction, and the result was 0.309 mg/ml. These researchers attributed the increase of free radical scavenging of the extracts to the increase in the amount of phenolic compounds in them and stated that there is a linear relationship between the amount of phenolic compounds and the free radical scavenging activity, which is consistent with the results of this study. Rodriguez et al. [52] also performed the extraction of burdock roots using supercritical CO₂ (scCO₂) with methanol as cosolvent and indicated that the extract with the highest antioxidant activity (IC₅₀ = 0.13 mg/mL) was obtained when the extraction was carried out at 15 MPa and 333.15 K with a solvent/solid ratio of 4 : 1, which was the highest value compared to other extraction conditions ($p < 0.05$). In this regard, Ferracane et al. [29] indicated that burdock roots contain quinic acid, chlorogenic acids, and ester of caffeic acid, which are responsible for the antioxidant properties of root extracts. Liu et al. [53] also demonstrated that caffeoylquinic acids and lignans are responsible for the antioxidant activity of

burdock roots. Therefore, phenolic compounds inflated the antioxidant activity of obtained burdock extracts.

The predicted model to estimate the amount of IC₅₀ with respect to regression coefficients was obtained as in equation (6). Based on the results presented in Table 1, the predicted model is statistically significant ($p < 0.0001$), and its coefficient includes the R² and Adj-R² with values of 0.995% and 0.988%, respectively, which were appropriate and confirm the adequacy of the model in predicting the response. As mentioned before, in a suitable model, the lack of fit should not be significant, where in the case of this model, this coefficient was equal to 0.092, which is not significant ($p < 0.05$).

$$\begin{aligned} \text{IC}_{50} = & 9115.05 - 103.63x_1 - 241.12x_2 - 11.28x_3 - 0.116x_1x_2 \\ & - 0.020x_1x_3 - 0.325x_2x_3 + 1.89x_1^2 + 2.34x_2^2 + 0.112x_3^2. \end{aligned} \quad (6)$$

3.3. Optimization. The optimum condition for ultrasound-assisted extraction of inulin from burdock root is determined to obtain maximum inulin content and polyphenolic compounds with minimum IC₅₀ using numerical optimization of Design-Expert software. This optimum condition with the desirability of 90% is tabulated in Table 2 which provides the highest value of inulin content = 12.46% and total phenol = 18.85 mg GA/g DW with the lowest IC₅₀ = 549.85 μg/ml. In the aqueous extraction method of inulin by maceration method, the extraction efficiency was 10.32% after 24 hours at ambient temperature, and the maximum extraction rate of phenolic compounds was 16.38 mg GA/g DW, which is lower than the maximum measured amount of these compounds in the ultrasonic method. Therefore, by comparing the results of the

TABLE 2: Predicted optimum condition for ultrasound-assisted extraction of inulin from the burdock root.

| Factor | Low | High | Optimum |
|-----------------------------|--------|--------|---------|
| Sonication time (min) | 10 | 40 | 36.65 |
| Sonication temperature (°C) | 40 | 70 | 55.48 |
| Water/solid ratio (g/ml) | 1 : 20 | 1 : 40 | 1 : 35 |
| Desirability | 90% | | |

optimization phase (ultrasound-assisted extraction) with the conventional method, we can understand the positive role of ultrasound frequency in increasing the extraction efficiency of inulin and other bioactive substances from burdock roots and reducing the extraction time as well as saving energy consumption.

4. Conclusion

Burdock root contains significant amounts of inulin and phenolic compounds with antioxidant properties that can be extracted by efficient methods and used in food and pharmaceutical industries. The results of this study showed that the polarity of the solvent has a significant effect on the extraction efficiency of bioactive compounds from the burdock root so that, among the solvents studied by the maceration extraction method, water had the highest extraction efficiency in terms of inulin content. Regarding the extraction of polyphenolic compounds, although water showed lower efficiency than water-ethanol solvent, this difference was not statistically significant. Therefore, water was selected as an effective solvent, and following that, a central composite design was used to optimize extraction parameters in an ultrasound bath for the extraction of inulin and polyphenol compounds. According to the results, the highest extraction of inulin and phenolic compounds and the highest antioxidant activity were obtained in the first 25 minutes of the ultrasound extraction. The amount of these compounds remained unchanged in a short period, and then with increasing extraction time, they were exposed to thermal degradation and reduced, especially at higher sonication temperatures. The optimum conditions for ultrasound-assisted extraction of inulin were sonication time = 36.65 min, sonication temperature = 55.48°C, and water/solid = 1 : 35 g/ml. Regarding the optimization results, among the studied factors, sonication time and water/solid ratio variables are the most effective factors on inulin extraction efficiency, and the effect of the sonication temperature factor was less than the other two factors. Generally, the results of this study confirm the high efficiency and shortening of the extraction time of the ultrasound-assisted extraction compared to the conventional method for extracting inulin as a prebiotic compound and other bioactive compounds from the burdock root.

Data Availability

The accuracy of the data used in the article is confirmed, and all the necessary data are provided in the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest with respect to the authorship and/or publication of this article.

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