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Microfluidic Extraction of Tannic Acid from Quercus Leaves

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ABSTRACT

In this study, extraction of tannic acid using microchannel was investigated. Affective parameters were optimized. Different solvents including buthanol, ethylacetate, and n-hexane as organic phase, methanol, propanol, ethanol and water as aqueous phase were investigated. Microchannels with different confluence angles and diameters were examined. Microchannels with different confluence angles and diameters were examined. The effects of pH, temperature, and volumetric flow ratio and contact time of the two phases were investigated. The response surface methodology was used to optimize extraction yield of tannin from Quercus leaves in the employed microchannels. Based on this optimization, maximum yield was achieved at pH of 2, temperature=33.1 ℃, volumetric flow ratio=1.2, and contact time of 25.35 s. Results show that extraction-using microchannel has many advantages over traditional methods, including shorter time and higher economic efficiency. Moreover, microchannel provides smaller volume of fluids resulting in lower solvent consumption, lower waste production, shorter analytical times, smaller space requirements, and lower energy consumption.

1. Introduction

Herbal medicines have been used for treatment of various diseases since thousands of years ago in all over the world [1]. Herbal medicines provide a wide spectrum of affective secondary metabolites for treatment and prevention of various chronic diseases [2]. Recently, production of tannin for pharmaceutical, food industries, and nutraceutical applications gained much intention [3]. Tannins are present in bark and fruits of various Quercus types. In chemical terms, tannins are known as tannin acid, gallous tannin, and gallous tannin acid. Tannin acid is very complex, poly-phenol, nitrogen-free, amorphous, and non-toxic compounds of acrid aroma.

Tannin exists in many different parts of plants, such as Banana peel, apples, grapes, coffee beans, and tropical plants [4]. Tannins are dissolvable in water with a molecular weight ranging between 500 and 5000 Da.

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The utilization of tannins in medicine is based on their astringent as well as anti-bacterial and fungicidal action [5]. Tannin derivatives are used in fruit juice purification and are used as antioxidants in food and beverage products [6], In addition, they are used in oil purification, as fish meat and seafood conservator, water based ink [7] production, some of cosmetics products, pharmaceutical applications such as treatment of cholera, bloody diarrhea, and antidote against poisonous mushrooms [8]. Tannin acid has inhibition effect on many different species of food-borne bacteria such as Aeromonas hydrophila, Escherichia coli, Salmonella typhi, and Enterococcus faecalis [9]. In addition, it has an inhibitory effect on many of bacteria and fungi [10]. Many investigations have been conducted on extraction of some effective derivatives of medicinal herbs in order to characterize quantity, qualitative properties, and inhibitory effect on microorganisms [11]. In addition, tannin acid sulfate has an inhibitory role in the cytopathic effect of HIV (human immunodeficiency virus) at 6 $\frac{\mu g}{mL}$ level [12].

Medicinal herbs have been known as the richest bio source of drugs for different types of traditional medicine systems, modern and folk medicines, nutraceuticals, food supplements, and pharmaceutical [13]. The extraction of active components through different approaches is the first step of industrial processing of medicinal and aromatic herbs. The common extraction methods for medicinal herb include maceration, percolation, infusion, decoction, digestion, aqueous-alcoholic extraction via fermentation, hot continuous extraction, ultrasound extraction, microwave-assisted extraction, [14] supercritical fluid extraction [15], subcritical water extraction [16], pressurized liquid extraction [17], and photonic extraction (with hydro fluorocarbon solvents) [18]. However, it should be mentioned that each of these approaches has its own limitations and losses [19]. Substances of interest have been obtained through the extractive process because of oxidation, hydrolysis, and ionization [20]. Extraction with ultrasound is useful in many cases; however, because of high cost, its use on the industrial scale is limited. One of main and known disadvantages of using ultra sound is the effect of its energy on active constituents of medicinal herbs by forming free radicals and undesirable changes on pharmaceutical molecules [21]. New and different techniques are introduced to extract nutriention' effective constituents, leading to a decrease in solvent usage, a decrease in contact time of the two phases, improvement in essence quality, and an increase in efficiency.

Liquid-liquid extraction is a method that is used to separate substances based on their relative reactivity in two immiscible phases [22] and available alternative to distillation [23]. However, conventional liquid-liquid extraction requires the use of substantial volumes of fluids to achieve separation, making this process undesirable for expensive materials. By significantly reducing the scale of liquid-liquid extraction to the micro and millifluidic levels, this separation process can be made suitable due to their small volumes [24], high value materials and their high surface-to-volume ratio [25]. A practical application of microfluidic liquid-liquid extraction is the passive separation and purification of biomolecules. Currently, other separation techniques risk degrading the biomolecule by heating (evaporation) or

mechanical force (centrifugation) or result in the loss of product(filtration) [26].

Furthermore, the microchannel devices are used for particle synthesis, [27] high throughput screening in drug development [28], genetics research, biological applications [29], and fuel cell technology [30]. It has been reported that liquid-liquid extraction process using microfluidic systems extracts vanillin from water into toluene extraction, back extraction of HCl by aqueous ammonia from trioctylamine dissolved in n-octanol [31], solvent extraction and stripping with TBP nitric acid system [32], and extraction of oleuropein from ethyl acetate [33].

The aim of this investigation is to enhance extraction yield of tannin from oak using microfluidic system**.** The effect of different parameters on extraction yield was investigated and optimized. To maximize the recovery of tannins from Quercus leaves, the effect of suitable solvent, microchannel confluence angle and diameter, turbulence in microchannel, temperature, pH, volumetric flow ratio, and contact time was studied. Another aim of this study is to determine the optimum condition to extract tannin acid using response surface methodology.

2. Experimental

2.1. Materials

Ethyl acetate, ethanol, methanol, propanol, hexane, sodium hydroxide, butanol, dibasic sodium phosphate, potassium dihydrogen phosphate, phosphoric acid, and Tannic acid (purity≥ 99 % by HPLC) were purchased from Merck Chemical Company. Leaves of Quercus were collected in August 2015 from Zagros Mountains. They were washed by distillated water and dried at room temperature. Wrapped in packaging paper and stored in dark and dry place for next experiments. Before use, dry sample was crushed using a house blender.

2.2. Standard solution and chromatogram of tannic acid

The stock solution of tannin acid was prepared by dissolving 20 mg of tannin acid in 100 mL methanol, creating a 200 $\frac{\mu g}{mL}$ solution. This solution was diluted with the solvent as required to prepare different standard solutions (5, 10, 20, 40, 80, and 100 $\frac{\mu g}{mL}$). These concentrations were used for the calibration curve, and typical chromatogram of standard solution of tannin acid obtained by using a microfludic device is shown in Fig.1 and Fig. 2, respectively.

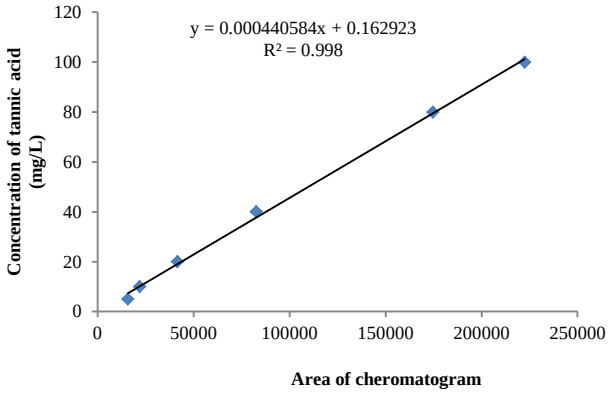


Figure 1. Calibration curve of tannic acid.

High-performance liquid chromatography is generally utilized as a standard expository instrument. HPLC runs were performed at a flow rate of 1 $\frac{mL}{min}$. The wavelength of detection was 280 nm. The HPLC system used consists of dual pumps, UV visible detector, vacuum degasser, and system controller. A manual injector with a 1 µL sample loop was applied for loading the sample. A reversed-phase C18 analytical column (250 mm_ 4.6 mm I.D., 3µm particle size) was used as a stationary phase. The mobile phase consists of deionized water

HPLC grade and methanol. Prior to preparation of the mobile phase, the deionized water and methanol were degassed separately using a Millipore vacuum pump.

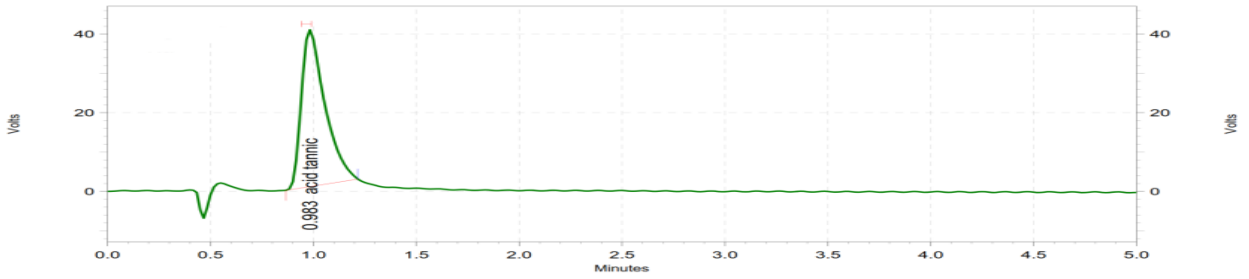


Figure 2. HPLC chromatogram of standard solution of tannic acid.

2.3. Microfluidic extraction

Mixing of samples and fluids in microfluidic systems is highly important, particularly in chemical and biological applications in which the reactants should be mixed with each other. It is clear that macro-scale mixing can be feasibly obtained by creation of turbulence. Since fluids are laminar on a micro scale, mixing would be harder to accomplish. In the case of micro scale, the most convenient method for mixing enhancement is to increase the flow rate. As higher flow rates are disadvantageous due to increasing backpressure and solvent and sample consumption, using mixers at lower flow rates seems useful. Microfluidic mixers are designed to decrease mixing time. These mixers are devised to induce mixing by reducing diffusion distance and increasing contact surface between the mixing fluids or both. A common design is using T-shaped connection as the mixer mixing laminar parallel flows with each other.

A schematic of microfluidic system is shown in Figure 3. The main part of this device is a microchannel mixer. Controlling entry feed stream in aqueous and organic phases is conducted by syringe pump. When syringe pump is used, the equipment should be sealed completely. To fix temperature of microfluidic system in the target range, a water bath is used. At the end of experiment, because of density difference and immiscibility, aqueous and organic phases are separated and injected into HPLC device for analysis and measurement. To find the optimum conditions of experiment, effect of many parameters is investigated including confluence angle in micro channel, pH, and temperature, volumetric flow ratio of aqueous phase to organic phase, solvent type, and contact time; obtained results are reported as efficiency percent of extraction. The extraction yield is calculated according to Equation (1):

$$\text{yield } \% = \frac{\text{Tannin acidcontent in aqueous phase}}{\text{content of Tannin acidin feed}} \times 100 \quad (1)$$

In all diagrams, error bars are evaluated in terms of relative standard deviation (RSD %).

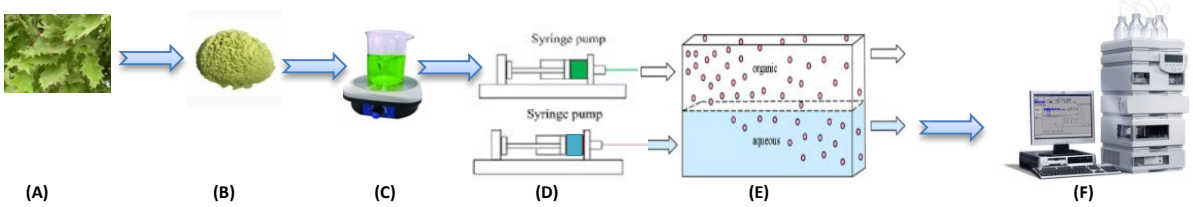


Figure 3. Schematic diagram of the experimental setup: (A) leaves of Persian oak, (B) leaves powdered, (C) stirrer, (D) syringe pump, (E) microchannel, and (F) HPLC system.

3. Results and discussion

Medicinal plants have been traditionally used in folk medicine for their natural healing and therapeutic effects. Method of isolating active components of medicinal plants is important. The separation process chosen is microfluidic system. Continuous processing in microfluidic devices plays a crucial role in the pursuit of more efficient, compact, safe, and environmentally-friendly processes. Liquid-liquid extraction finds numerous applications in industry. In this paper, the extraction of tannic acid from Quercus leaves is discussed along with the effects of extraction factors. The appropriate solvent was selected as organic and aqueous phase to study the effect of microchannel confluence angle; four different angle sizes were used for the experiments. The suitable pH, volumetric flow ratio, and contact time of the two phases and temperature were selected to analyze the product using HPLC. In these extracts, tannic acid was determined by HPLC analysis, and quantitative analyses were performed by calibration curve. The data collected were processed using a statistical package. Design-Expert software was used for analysis of variance (ANOVA), mathematical modelling, regression analysis, predicted output, and optimization. The optimization of the processing conditions was tailored to assess maximum yield of extraction of tannic acid. ANOVA was used to study the effect of independent variables on the response variable. Response Surface Methodology (RSM) is widely employed to construct and explore the estimated functional relationship between a response variable and design variables.

3.1. Solvent selection

Although the choice of extraction method may have a significant effect on the extract quality, the solvent used provides the most obvious means of influencing the extract qualitative composition. The preliminary experiments were conducted to select a suitable solvent. To investigate the effect of solvent extraction, 20 g of air-dried and pulverized Quercus leaves extracted by mechanical were stirred for 24 h using 100 mL of organic solvent. A filter (Whatman filter paper) separated the supernatant phase. Consequently, this phase was used as a feed for microfluidic extraction. Samples were extracted with buthanol, ethyl acetate, and n-hexane as organic phase, Methanol (10 %, 30 %, 50 %) , propanol(10 %, 30 %, 50 %), ethanol (10 %, 30 %, 50 %), and water (100 %) as aqueous phase, respectively. The best solvent was selected according to the values of responses. The results are illustrated in Figure 4. Based on the obtained results, ethyl acetate and ethanol 10 % were chosen. It appears that by increasing the number of carbons, the polarity and solubility of alcohols would be decreased. Therefore, the lowest extraction is obtained in the aqueous phase by a combination of water and propanol. By increasing ethanol, methanol, and propanol percentage in the aqueous phase, the rate of extraction was reduced due to an increase in the immiscibility rate in both phases. Differences observed in the extraction rates by using different extracts are associated to difference between the employed solvent polarities. Solvents with low polarity such as hexane and butanol in proportion to polar solvents have less ability to extract these combinations.

In this study, aqueous phase solvents (methanol, ethanol, propanol, and pure water) were investigated in percentage rates of 30, 10, and 50; in 50 % state, they could not be

used due to the high miscibility of aqueous and organic phases. Hence, nothing is mentioned in results. Organic phase (butane, hexane, and ethyl acetate) was selected.

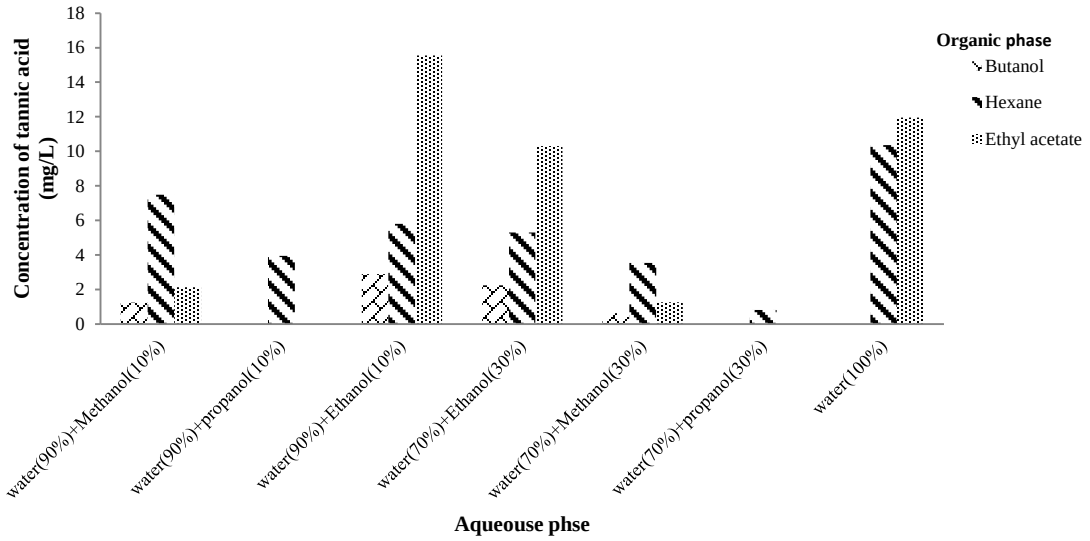


Figure 4. Effect of solvent on amount of extraction.

3.2. Microchannel confluence angle

To achieve contact angel of microchannel, $\theta = 45^{\circ}, 90^{\circ}, 135^{\circ}, 180^{\circ}$ angels were tested. Figure 5 shows that confluence angle has significant effect on efficiency. Owing to an increase in applied force, increment mechanism leads to a decrease in length of penetration between the two phases and improves the mixing quality between them. As can be seen, the mixing performance steeply enhances with increasing the angle of confluence, confirming the desirable effect of a large confluence angle for streams of two adjoining microchannels. The mechanism is that an increase in the angle of confluence causes an increase in the exerted force to the fluid at the confluence, which has been previously reported by other authors [34]. In fact, increasing the applied force results in reducing the diffusion length between the fluids and therefore can improve the mixing quality.

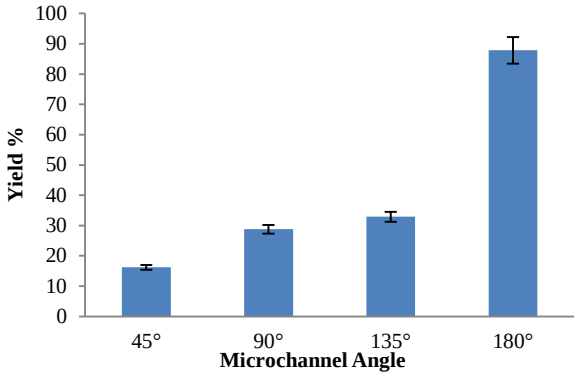


Figure 5. Effect of microchannel confluence angle in extraction.

3.3. Microchannel diameter effect

In pressure flow known as hydrodynamic flow, flow rate Q can be calculated based on $\mathrm{Q} = \frac{\Delta \mathrm{P}}{\mathrm{R}}$, where ΔP is pressure loss in the channel in *Pa*, and R is resistance of the channel in $\frac{\mathrm{pa.s}}{\mathrm{m}^3}$. In flows that are driven by pressure force, there is a constant parameter called resistance of channel *R*. Resistance can be calculated as $\mathrm{R} = \frac{8\mu \mathrm{L}}{\pi \mathrm{r}^4}$ for channels with circular cross-sections. In these equations, μ (*Pa.s*) is viscosity, L is length, and r refers to

radius of the channel. Based on these equations, a long and narrow channel is more resistant against flows, while a short and wide channel exhibits lower resistance against flows. Pressure methods demand for an external pump or a resource for vacuum generation. With respect to $R \propto \frac{L}{r^4}$, a considerable pressure loss is required to derive fluid flow in narrow microchannels. Due to the pressure loss problem, the diameter of 600 µm was selected. Geometrical dimensions of microfluidic system are 60 mm in length and 600 µm in diameter. Figure 6 shows the effect of diameter on extraction efficiency.

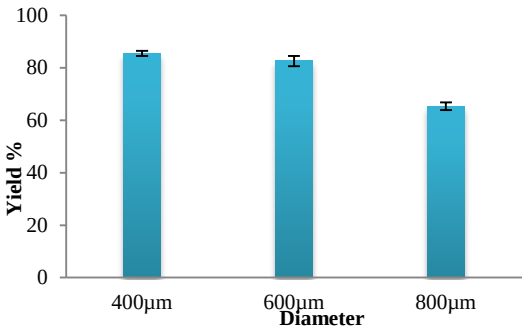


Figure 6. Microchannel diameter effect on extraction efficiency.

3.4. Select of suitable pH

To evaluate the effect of pH on extraction efficiency, the aqueous phase with different pHs in the range between 1 and 10 was used as an extracting phase. In this step, fixed values of temperature, volumetric flow ratio, and contact time of the two phases were used. As Figure 7 shows, an increase in pH value from 2 to 10 leads to a decrease in the yield of extraction, because tannic acid is a polar relatively acidic species. Therefore, the pH value of two phases was selected as the optimum value.

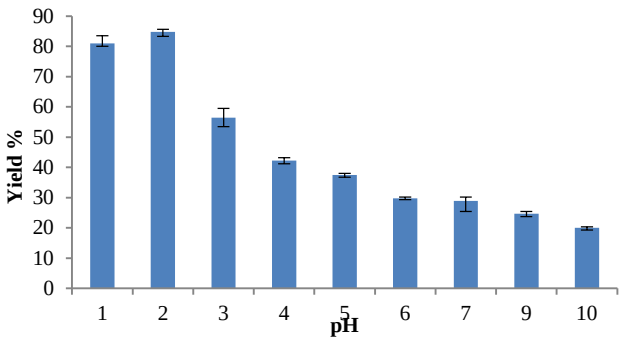


Figure 7. pH effect on extraction efficiency.

3.5. Selection of a suitable volumetric flow ratio

The volumetric flow ratio is defined as the ratio of organic to aqueous phase. To investigate the effect of the volumetric flow ratio (VR) on extraction yield in the three designed microchannels, the volumetric flow ratio of organic phase was changed at a fixed flow rate of the aqueous phase. In this study, the VR of organic phase/aqueous phase varied from 0.5 to 3. Volumetric flow ratio is one of the controlling parameters in the extraction rate[35]. To check this parameter, different VRs of input streams were used. In the macro-scale extraction, reducing the feed to extractant phase ratio leads to an increase in the extraction efficiency. Therefore, it is expected to see an increment response showing maximum extraction efficiency at minimal proportion of feed to extractant phase. Figure 8 shows the result of the experiments in which the effect of VR on the extraction efficiency was investigated. Increasing the VR in the range from 0.5 to 1 positively influences the achieved extraction efficiency. Volumetric flow ratio higher than one does not insure higher values of extraction efficiency.

This could be related to the effective confluence angle between organic phase and aqueous phase in the microchannel. Furthermore, increasing the probability of

mass transfer at the VR of 1 due to the presence of adequate volume of the extractant is likely. According to these results, the VR of one is selected.

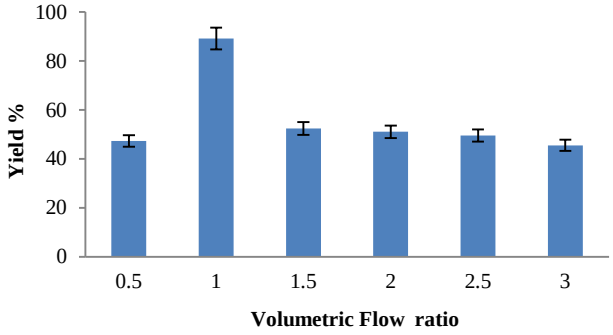


Figure 8. Effect of volumetric flow ratio on efficiency.

3.6. Contact time of the two phases together

One of the main parameters having a significant effect on mass transfer and separation efficiency is contact time of the two phases. To investigate effect of time on contact time between the two phases, a coil was used in the output of microchannel, and result was compared to coils condition. Figure 9 shows the low-efficiency and short contact time in the case without coil. In any case, increasing contact time leads to an increase in contact surface between the two phases, an increase in molecular diffusion, and an increase in mass transfer, finally leading to improved efficiency. However, by increasing the coil length, a more acceptable yield up to 74.86 % is obtained in only 30 s. This demonstrates that increasing the contact time of the two phases culminates in an increase in the extraction yield. It is explicit that longer contact time leads to higher extraction yield. However, it directly increases the pressure drop across the channel. With respect to the limitations in syringe pump and an increase in pressure drop along with an increase in contact time of the two phases, effect of longer contact time was not considered.

The length significantly increases, and it is not possible to examine the higher lengths. Higher lengths are provided; however, it is not possible to conduct the experiment. If it is possible to examine the higher lengths, in view of the micro-channel mechanism, ascending trend may not continue and remain fixed.

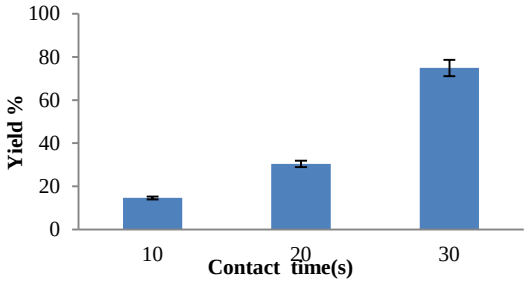


Figure 9. Effect of contact time on yield.

3.7. Selection of suitable temperature

Higher temperatures promote the solute solubility in the solvent and increase the solute diffusion rate into the solvent bulk, leading to a higher mass transfer rate. Various experimental factors, particularly the temperature of the extraction procedure, regulate the effectiveness of phenolic compounds extraction procedure. Increasing the rate of diffusion and the extracted substances solubility is the main reason of enhancing the temperature [36]. To examine the effect of temperature, the microchannel was placed in a water bath at different temperatures, including 15, 30, 45, 60, and 75 ℃. In this step, the experiments were conducted under the following conditions: microchannel confluence angle 180°, organic phase solvent ethyl acetate, aqueous phase solvent Ethanol 10 %, volumetric flow ratio=1, pH of 2, and contact time of the two phases of 30 s. The obtained values of extraction yield were 70.32 %, 85.69 %, 72.51 %, 64.28 %, and 56.71 % at 15, 30, 45,

60, and 75 ℃, respectively. Therefore, the results show that extraction of tannin is optimal when temperature is set to 30 ˚C (Figure 10) due to the presence of the high contact area between the two phases of microchannels where molecular diffusion is responsible for the mass transfer. Therefore, temperature 30 ˚C was selected for further studies, which can be important in terms of energy consumption.

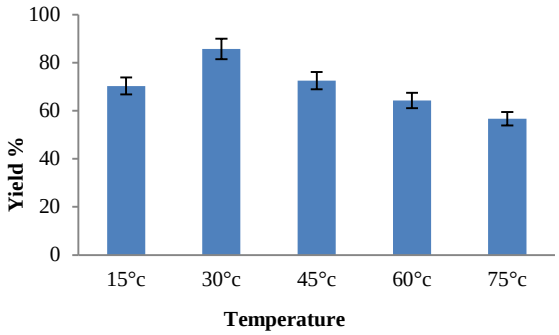
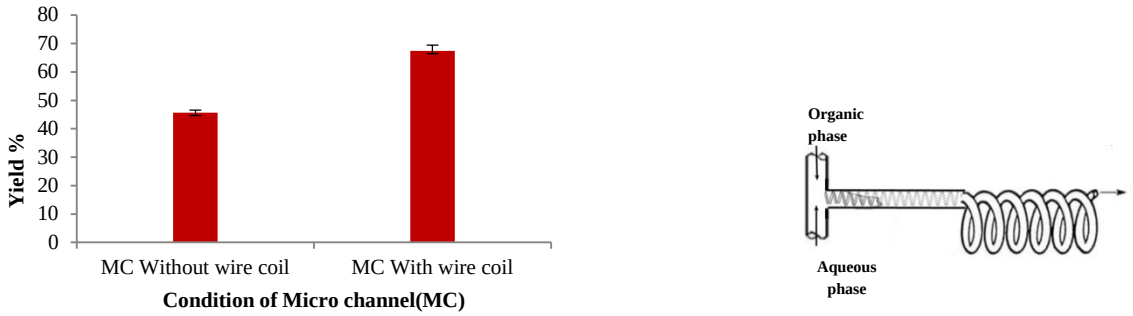


Figure 10. Effect of temperature on yield.

3.8. Turbulence effect on extraction efficiency

One of the simplest tools for increasing mass transport is wire coil. Wire coil can be easily connected and, after analysis, they can be removed, cleaned, and reused. Coil generates rotational and radial velocities in the fluid, increases mixing of the solution specifically around interior walls of the microchannel, and, finally, reduces precipitation of particles and prevents production of a boundary layer, which is an important barrier against mass transfer. Prevention of boundary layer formation is one of the main reasons of enhanced mass transport using wire coil. In this study, a very fine spring was used to create turbulence and investigate the effect of wire coil presence inside the microchannel on efficiency of extraction (Figure 11).



(a) (b)

Figure 11. Effect of turbulence on (a) extraction yield and (b) wire coil inside the microchannel.

4. Experimental design

4.1. Statistical analysis

The quantity and quality of the tannin extracts are affected by several factors. Experiment design software was used for experimental results [37]. RSM method was used for optimizing parameters. RSM is accepted as a powerful tool for optimizing experimental conditions to maximize various responses.

The central composite design is a response surface methodology design employed to find the optimum experimental condition with definite values of key experimental determinants for the maximum yield of tannin in Quercus leaves extract. All the experiments were designed to study the combined and individual effects of four influential experimental parameters on the extraction of tannin. These variables include volumetric flow ratio (0.5, 1.5, and 2.5), contact time of the two phases together (10, 20, and 30s), pH (2, 6, and 10), and temperature (15, 45, and 75 ℃). As Table 1 shows, each parameter had three levels of -1, 0 and +1.

Table 1
Independent variables and their coded levels used in RSM studies.

Factors	Units	Low Level (-1)	High Level (+ 1)
Volumetric flow ratio (VR)	---	0.5	2.5
Contact time(CT)	Second	10	30
pH	---	2	10
Temperature(T)	℃	15	75

Thirty sets of experiments were performed to determine significant factors to extract tannin (Table 2). ANOVA was performed for the independent and dependent values to obtain regression equations that could predict the responses within a given range. Considering two parameters and a response, experimental data were fitted to obtain a second-degree regression equation of the form:

$$y = \beta_{\circ} + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \sum \beta_{ij} X_i X_j \qquad (2)$$

where y is the predicted response, k is the number of factor variables, $\beta_{\circ}$ is the model constant, β_i is the linear coefficient, X_i is the factor variable in its coded form, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient.

Table 3 shows the results of ANOVA for the selected model of quadratic polynomial for tannin acid extraction. The ANOVA of quadratic regression model demonstrated that model was highly significant, evident from Fisher's F-test with high F-value and low p-value.

Table 2
Experimental design recommended by Design-Expert.

Std Order	Run Order	Pt Type	Blocks	VR	CT	pH	T	Yield %
3	1	1	1	1.125	25	4	35	91.98
19	2	-1	1	1.750	10	6	50	72.94
18	3	-1	1	3.000	20	6	50	27.52
4	4	1	1	2.375	25	4	35	44.61
10	5	1	1	2.375	15	4	65	49.61
25	6	0	1	1.750	20	6	50	62.72
11	7	1	1	1.125	25	4	65	86.07
9	8	1	1	1.125	15	4	65	66.50
5	9	1	1	1.125	15	8	35	66.73
22	10	-1	1	1.750	20	10	50	59.97
24	11	-1	1	1.750	20	6	80	57.75
1	12	1	1	1.125	15	4	35	82.67
2	13	1	1	2.375	15	4	35	51.97
16	14	1	1	2.375	25	8	65	54.74
23	15	-1	1	1.750	20	6	20	68.24
17	16	-1	1	0.500	20	6	50	72.94
21	17	-1	1	1.750	20	2	50	67.90
7	18	1	1	1.125	25	8	35	79.15
8	19	1	1	2.375	25	8	35	51.24
6	20	1	1	2.375	15	8	35	63.98
26	21	0	1	1.750	20	6	50	64.58
29	22	0	1	1.750	20	6	50	62.45
14	23	1	1	2.375	15	8	65	57.92
28	24	0	1	1.750	20	6	50	63.98
31	25	0	1	1.750	20	6	50	64.77
13	26	1	1	1.125	15	8	65	48.43
20	27	-1	1	1.750	30	6	50	89.56
12	28	1	1	2.375	25	4	65	46.06
27	29	0	1	1.750	20	6	50	62.41
15	30	1	1	1.125	25	8	65	72.12

Table 3
ANOVA for Response Surface Reduced Quadratic Model.

Source	Sum of Squares	df	Mean Square	F-values	P-values Prob > F
Model	5690.08	14	406.43	115.64	< 0.0001 significant
A-Volumetric flow ratio	1154.55	1	1154.55	328.49	< 0.0001
B-Contact time	352.2	1	352.20	100.21	< 0.0001
C-pH	275.39	1	275.39	78.35	< 0.0001
D-Temperature	221.02	1	221.02	62.89	< 0.0001
AB	526.93	1	526.93	149.92	< 0.0001
AC	581.05	1	581.05	165.32	< 0.0001
AD	120.67	1	120.67	34.33	< 0.0001
BC	0.31	1	0.31	0.088	0.7713
BD	76.13	1	76.13	21.66	0.0003
CD	1.50	1	1.50	0.43	0.5234
A^2	339.79	1	339.97	96.73	< 0.0001
B^2	491.79	1	491.79	139.93	< 0.0001
C^2	0.24	1	0.24	0.07	0.7956
D^2	2.98	1	2.98	0.85	0.3721
Residual	52.72	15	3.51		
Lack of Fit	46.81	10	4.68	3.96	0.0709 not significant
Pure Error	5.91	5	1.18		
Cor Total	5742.08	29			

$\mathbf{R^2 = 99.08}$ % R^2(Pred) = 95.16 % R^2(adj)98.23 % Std. Dev=1.87

$\mathbf{R^2}$ =R-Squared $\mathbf{R^2(adj)}$ =Adjusted R-Squared $\mathbf{R^2(Pred) = Predict\ R - Squared}$

4.2. Regression analysis

The predictive model equation for extraction yield is given below:

$$\text{yield} = 68.66 - 14.58\text{A} + 9.09\text{B} - 8.04\text{C} - 7.75\text{D} - 18.36\text{AB} + 19.28\text{AC} + 8.79\text{AD} + 0.56\text{BC} + 8.72\text{BD} - 1.23\text{CD} - 9.01\text{A}^2 + 16.94\text{B}^2 - 0.38\text{C}^2 - 1.32\text{D}^2 \quad (3)$$

where: $\text{A} = \text{VR}, \text{B} = \text{CT}, \text{C} = \text{pH}, \text{D} = \text{T}$.

The effects p-values higher than 0.05 are not significant at the 95 % confidence level and are discarded. The sign of the effect marks the response performance. In this way, when a factor has a positive effect, the response is higher at the high level, and when a factor has a negative effect, the response is lower at the high level. The significant second-order polynomial model equation at the 5 % level to optimize efficiency in extraction of tannin acid using microfluidic system is the same as Equation (3). By referring to Table 3, it was found that linear factors, such as volumetric flow ratio (A), pH(C), and temperature (D), showed negative coefficients, respectively, while contact time of the two phases (B) showed a positive coefficient. Square factors such as B^2 showed positive coefficients, respectively, while A^2, C^2, and D^2 showed negative coefficient. Quadratic or interaction factors, such as AC, AD, BC, and BD, showed positive coefficients, respectively, while AB and CD showed negative coefficients. P-value for A, B, C, D and square effect factors, namely $A^2$and B^2, and quadratic or interaction factors AB, AC, AD, and BD were < 0.0001 respectively. This means that linear, square and quadratic or interaction factors play important roles in separation efficiency. P-value was used as an important tool for evaluating the importance and contribution of each factor, and the statistical polynomial model equation reported that the larger the magnitude of the F-value and the smaller the p-value, the more significant the corresponding coefficient. Based on the results of this study, square factors of C^2 , $D^2$and quadratic or interaction factors BC and CD terms do not affect efficiency (p >

0.05). Determination of the goodness of fit of the regression model was performed by calculating R^2, which offers a measure to determine the amount of variability that can be explained by the experimental parameters and their interactions for the obtained response values. The results in Table 3 showed that R^2 value was 99.08 %, indicating the good fitness of the model. A high value of predicted R^2 (0.9516) is an indication of precision of the fitted model. The higher the value of R^2 toward unity is, the better the model fits the experimental data. The absence of non-significant terms in the model caused the high value of adjusted R^2. The high adjusted R^2 and R^2 values showed that there is a strong dependence and relation between the experimental and predicted response values. The second-order polynomial model was tested using ANOVA in terms of adequacy and significance. The results are summarised in Table 4**.** For quadratic versus 2FI, the p-value obtained was less than 0.0001, showing the strength of significance. The significance of regression was evaluated by F and p-values using Fischer's and null-hypothesis tests. The F-value predicts the quality of the entire model considering all design factors at a time. The p-value is the probability of the factors with very little or insignificant effect on the response. Larger F-value signifies better fit of the RSM model to the experimental data; F-value with low p-value indicates the high significance of the regression model.

Table 4
Adequacy of model tested.

Source	Sum of squares	df	Mean square	F-values	P-values	
Mean versus total	1.218E+005	1	1.218E+005			
Linear versus mean	3409.61	4	852.40	9.13	0.0001	
2FI versus linear	1306.59	6	217.76	4.03	0.0090	
Quadratic versus 2FI	973.88	4	243.47	69.27	< 0.0001	Suggested Aliased
Cubic versus quadratic	37.19	8	4.65	2.10	0.1728	
Residual	15.53	7	2.22			
Total	1.275E+005	30	4251.28			

In general, the lack of fit test for the model describes the variation in the data around the fitted model. If the model does not fit the data well, the value of lack of fit will be significant; then, proceeding with investigation and optimization of the fitted response surface is likely to give misleading results.

Table 3 shows the result of the lack of fit, and it was found that F- and p-values for the lack of fit were 3.96 and 0.0709, respectively. The insignificant p-value, thus, indicates that the model is good and fits the experimental data well.

4.3. Determination and experimental validation of the optimal conditions

The numerical optimization technique based on desirability function was performed to determine the optimum conditions for the extraction yield. To provide an ideal case for tannic acid extraction, the goal for volumetric flow ratio, contact time, temperature, and pH was set in the range, and extraction yield was set to maximum. The “importance” of goals (Options 1–5) for all variables was considered equally in a setting of 3. For response, the “importance” was set at 5 to meet the objective of obtaining maximum extraction

yield. Table 5 presents the optimal level of different factors achieved using the desirability function approach.

Table 5.
Optimum conditions and the predicted and experimental value of responses at the optimum conditions.

Optimum conditions				Predicted value Yield (%)	Observed value Yield (%)
Volumetric flow ratio	Contact time (s)	pH	Temperature (°C)		
1.2	25.35	2	33.1	98.72	96.76±1.97

To verify the prediction of the model, the optimal reaction conditions were applied to three independent replicates for biodiesel synthesis, and the mean ± standard deviation was determined. As Table 5 shows, the obtained and predicted values of extraction efficiency are consistent with each other. Relative error between predicted and practical data is 1.97 % for efficiency of extraction. The verification value of the obtained efficiency is approximately 98.72 % of predicted values that obviously indicates that the model performed very well in fitting the practical data; therefore, the optimal extraction yield of tannic acid was obtained in the specified range of process parameters. Figure 12 exhibits the correlation between the experimental and predicted data of calculated extraction yield of tannin acid by the microfluidic system. It can be observed that the predicted data calculated from the model are in good agreement with the experimental data in the range of operating conditions.

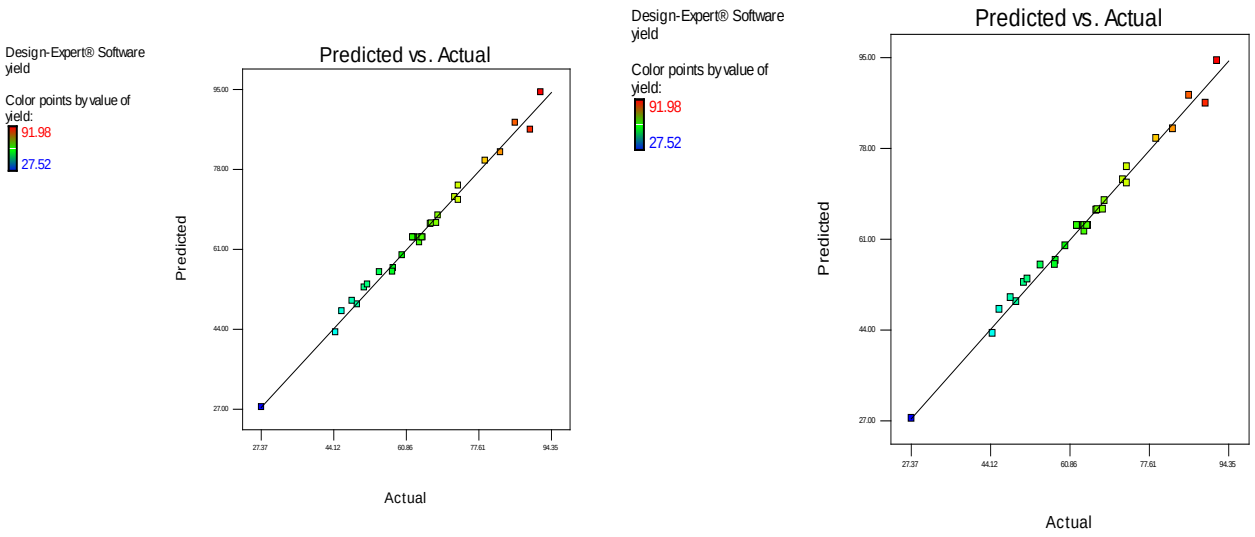


Figure 12. Correlation between the experimental values versus the calculated values using the model equation.

A normal probability plot for residuals can be used to examine the normality of population. The plot will seem a straight line when the residuals are normally distributed (Figure13-A). Residuals versus fits plot can be used to examine the assumption of constant variance. In this plot, the residuals should fall in a random pattern on both sides of 0, and no recognizable patterns should be observed. In a common pattern, the residuals are increased by increasing the fitted values. The plot of residuals versus order (time order

of data collection) can be used to examine the independence, particularly of effects relating to time. A positive relation or a negative relation is observed if the independence assumption is rejected. If no pattern is observed in the plot, the assumption is confirmed. Fig.13 presents the outcomes. In this figure, it is observed that data are scattered randomly around the residual line, y=0, without a certain pattern. In conclusion, it can be said that the regression model sufficiently fits the data. Testing hypotheses for the individual regression coefficients were performed to decide on keeping or excluding variables. The first step of a simple analysis is a main effects plot. The main effects plot is defined as a plot of the response variable means at each level of a factor. This plot can help a researcher to determine the important key effects.

The total average of the parameter is subtracted from the average of each level to compute the main effect. Fig. 14 shows the locations of the main effects for extraction yield. Analysis indicates that factors VR, pH, and T decrease yield when they move from the low level to the high level. Each level of the factors affects the response differently. Factors pH and T appear to have slight effect on the responses, with a low slope. If the slope was close to zero, the magnitude of the main effect would be small. The VR has shown negative effect on extraction yield, and the CT has shown a positive effect on extraction yield.

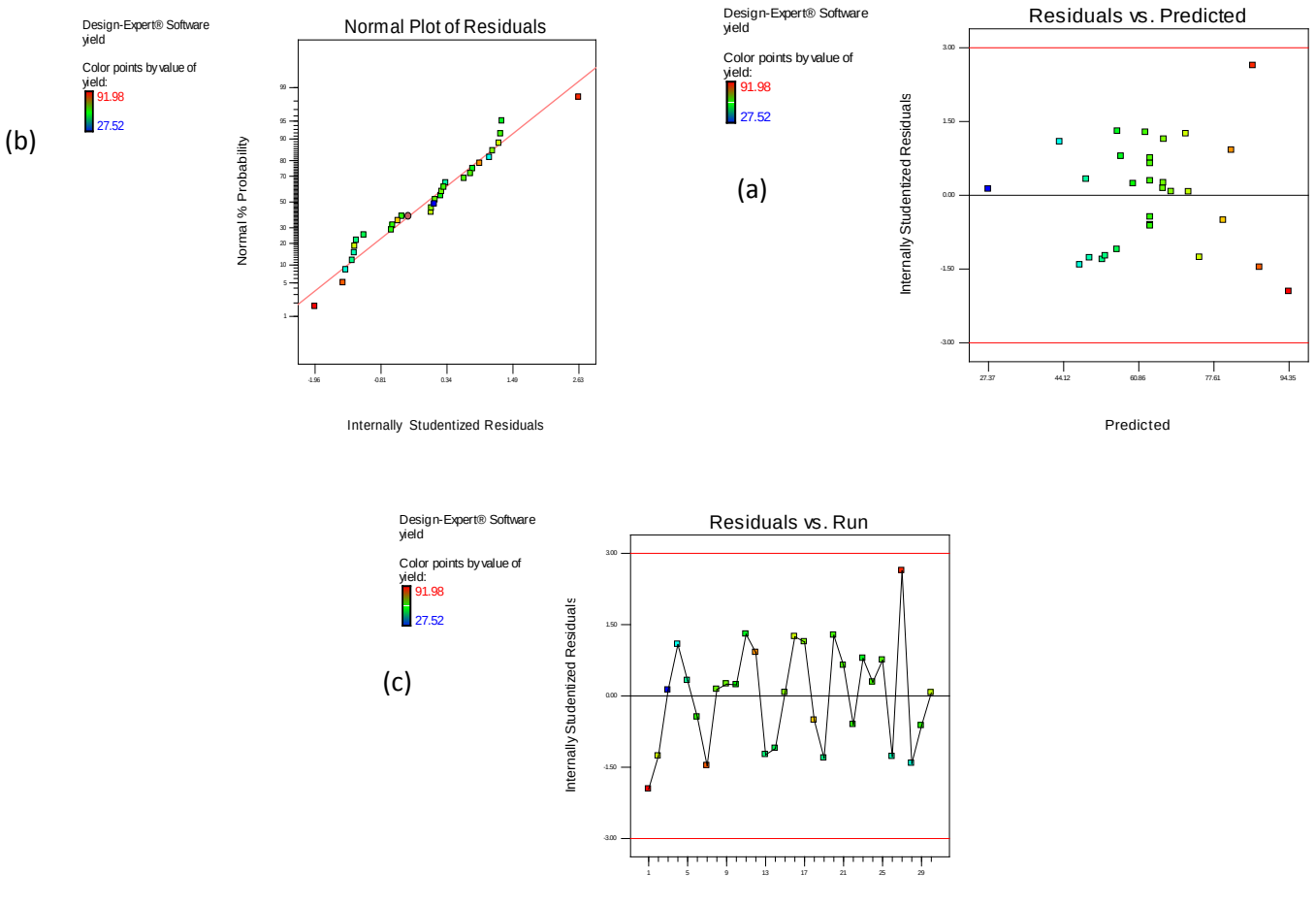


Figure 13. Model adequacy checking: (a) Normal probability plot of the residuals, (b) Plot of residual Vs the order of the data, and (C) Plot of residuals versus fit values for tannin acid extract yields.

Interaction plots in Figure 15 show the interaction between parameters. Parallel lines illustrate the lack of interaction and effect between those parameters. This extraction method for tannin can be developed for industrial extraction process of various oaks to produce and prepare a good antioxidant, food additive and preservative, and a drug substance with an inhibition effect for growth of microorganisms and fungi and antibacterial effect.

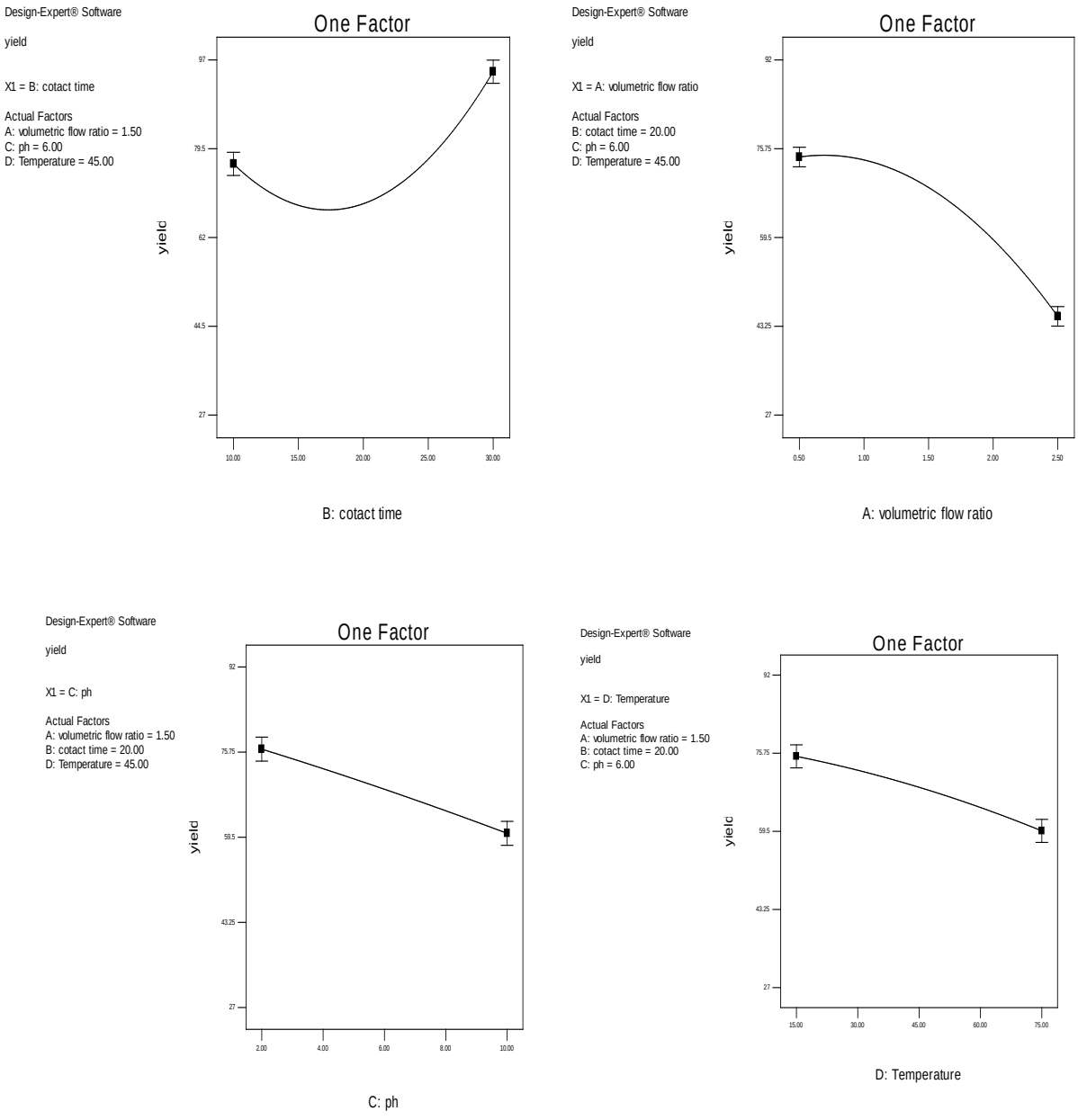


Figure 14. Main effects plot of parameters for extraction yield.

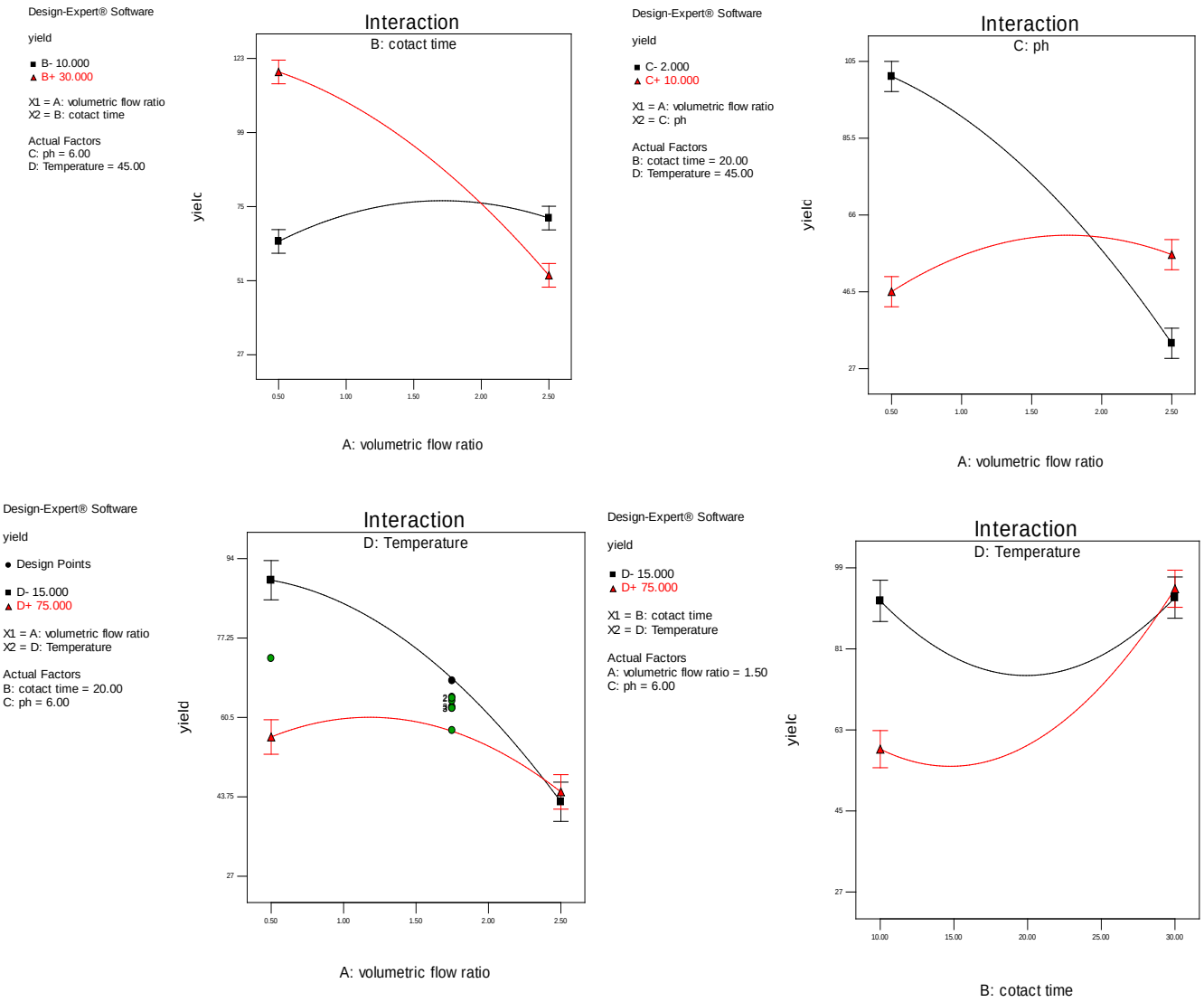


Figure 15. Interaction factors' effects plot for extraction yield.

5. Conclusions

Demand for extraction of nutrient and biologically active substances needs economically affordable extraction methods and environmental considerations. Excessive use of solvent, particularly organic solvents in traditional extraction methods of effective substances of medicinal herbs causes many environmental problems. Using large amount of solvent results in environmental issues in addition to an increase in operating costs. Microfluidic system is a new method to extract useful substances of medicinal herbs as a suitable substitution for traditional methods. Advantages of this method include low use of solvent, short time of extraction, high mass transfer, easy scale up, and high efficiency.

In this study, extraction of tannin from oak leaf is investigated using microfluidic system. To find the optimal conditions, the effects of different parameters, such as microchannel confluence angle, solvent, pH, temperature, volumetric flow ratio, and contact time of the two phases, on extraction yield were investigated. It was found that the microchannel confluence angle of 180° was the best geometry for this work. The best solvents were chosen to be ethylacetate as organic solvent and ethanol 10 % (water 90 % + ethanol 10 %) as aqueous phase. Response surface methodology was successfully applied

for using the microfluidic system in extraction of TA from Quercus leaves. According to the result, experimental design of the RSM model appropriately matched the experimental results. The high regression coefficients of the second-order polynomial showed that the model well fitted the experimental data. ANOVA tables show that volumetric flow ratio, temperature, Ph, and contact time of the two phases have a significant effect on efficiency. The VR, pH, and T have shown a negative effect on extraction yield, while the CT has shown a positive effect on extraction yield. The predicted optimized condition by experiment design includes volumetric flow ratio OF 1.2, pH of 2, CT of the two phases 25.35s, and temperature of 33.1 ℃. Table 5 shows real and predicted efficiencies, and the difference between them is low. In other words, the results match with the RSM model.

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