

A Kinetic Study on Microwave- assisted Extraction of Bioactive Compounds from Rosmarinus Officinalis L

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Abstract

Due to the importance of the extraction process in many engineering and medical industries, in addition to great interest in medicinal plants, in this research, microwave-assisted extraction has been applied to extract some active compounds from Rosmarinus officinalis leaves. The optimal extraction conditions were then determined by calculating the ratio and extraction efficiency. The process has also been described through kinetic study by applying five kinetic models, the Hyperbolic diffusion model, Power law model, the First order reaction model, Elovich's model, and Fick's second law diffusion model and determining their compatibility with the studies operation, and determining the kinetic constants for each model. The results of extracts showed that the best conditions are: the solvent is ethyl alcohol 80%, the capacity is 720W, and time is 6.5 min. For the kinetic study, all the studied models were appropriate to the applied extraction tip with high correlation coefficients, therefore, the kinetic constants of all these models were determined.

Keywords: Extraction, Kinetic, Microwave, models, Rosmarinus officinalis L.

Introduction

Recent years have witnessed a great deal of interest in medicinal plants, this is for its biological effectiveness, few side effects, and they contain many effective compounds¹. Plant extracts are of great importance in the treatment of many diseases, this because they contain many effective compounds. Polyphenols are one of most important active compounds extracted from medical plants². Rosemary (Rosmerinus officinalis L) is one of the widely used medicinal plants due to its content of high concentrations of carsonic acid, Carsonol acid, Rosmarinic acid, Polyphenols, Alkinds, Tanins, Flavonoids, and others³. Its volatile oil contains

Camphor, α -pinene, Cineol, acetatBornyle, Borineol, and Camphene. Its extracts are used as antibacterial and antifungal⁴. The main compounds responsible for the antimicrobial and anti-oxidant activity are Rosmarinic acid, Carnosol acid, Camphor, and 1,8 Cineole⁵.

The scientific name is Rosmerinus officinalis L, the common name is Rosemary, it is useful to activate memory, hair care, useful in treating respiratory diseases, Hepatitis, relieve, muscle pain. It is also useful in the treatment of diseases which caused by cell depreciation such as Cancer⁶. It is anti-bacterial⁷, anti-oxidant⁸, used to treat headaches,

liver, spleen diseases, colds, and cough⁵. Rosemary is perennial plant, that reaches 1-2 m in height, grown in countries of the Mediterranean sea and south Europe such as Spain, Turkey, Syria, Tunisia, Italy, Russia, and also in the United States⁹.

Khamal et al.2018, studied the effect of Rosemary oil on several kinds of bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *klebsiella pneumonia*), the study showed that the Rosemary oil has anti- bacterial activity against bacteria between concentration 5000-10000ppm, with inhibition zone 1.2-2 cm.⁷ Haidar et al., confirmed in a study conducted 2018 the effectiveness of Rosemary extract against several types of bacteria; *Escherichia coli*, *Staphylococcus aureus*, *klebsiella pneumonia*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. The results showed the most affected bacteria was *Pseudomonas aeruginosa*⁴.

On the other hand, Microwave-assisted extraction is one of most important modern extraction methods, it's effectiveness has been proven in many studies, this is due to the mechanical and thermal effect of Microwave,¹⁰ However, the characterization of the

Materials and Methods

Preparation of Plant Leaves:

Rosemary leaves were obtained from Homs city at September 2021, and dried in the shade for seven days, then ground separately to a fine powder. The ground particles were stored in freezer until use.

The Extraction

For each sample, an amount of 5 g of dry plant powder (which was previously prepared) was placed in each airtight glass containers, and an amount of 50 ml of solvent (Ethyl alcohol or water) was added to it, the containers were put in the Microwave (MC-9287BR(LG), at (180,360,540,and 720)W. The samples were placed in the Microwave for 30 seconds, next the samples were removed from the Microwave, cooled, then returned to the Microwave for 30 seconds, and so on. The extraction time is the time the samples spend in the Microwave. To determine the best extraction condition, we have to study the effect of solvent type and concentration, Microwave capacity, extraction time.

extraction process in this way is still not clear because of the kinetic study of this method is still limited and insufficient, while the traditional extraction methods received many scientific studies, the mechanism of extraction by these methods still need to be clarified. Zhitong and others applied the Friedman and Kissinger-Akahira- Sunose method on pyrolysis of plastic waste and determine activation energy and R^2 for the process,¹¹ Biljana et al., 2019, also studied the kinetics of extraction Caffien from guarana seedunder ,the effect of ultrasonic field, they applied three kinetic models; Spiro model, Fick's second law model, and unsteady state diffusion model. The results showed that all models are not valid¹². In view of the importance of the kinetic study of one of the most important modern extraction (Microwave-assisted extraction), in this research, a number of kinetic models were applied to determine the most suitable model for the studied extraction process, which would clarify the extraction mechanism and then determine some important constants for the Microwave-assisted extraction of extracting the active compounds from *Rosmerinus officinalis* L.

Concentration and Type Solvent Effect:

In order to determine the best solvent, two types of solvents were tested, Ethyl alcohol (50,60,70, 80,90)%, and water. High polar solvents were chosen because the active compounds are highly polar. On the other hand, microwave do not affect weakly polar materials¹⁰.

Capacity Extraction Effect:

After determining the best solvents, four levels of capacity were studied: 189, 360, 540, and 720 W. Where, the increase in capacity is reflected in the increase in the thermal and mechanical effect of microwaves on extraction process.

Time Extraction Effect:

Five times were studied: (2.5, 3.5, 4.5, 5.5, and 6.5) minutes to choose the best time extraction. This is because increasing the contact time between the solvent and the solid, which would affect the extraction rate.

Yield Calculations

After extraction, the samples were filtered, the solvent was evaporated using rotary vaporizer at 40 °C and for 20 minutes. Then, the samples were derided at 40 °C until the weight is stable.

The yield was calculated from Eq.1:

$$q = \frac{m_f}{m_i} \dots\dots\dots 1$$

mi : sample initial weight

mf : sample weight after drying

Kinetic Models:

To describe the process, five kinetic models were applied to the extraction process the compounds from plant, the hyperbolic diffusion is expressed by the Eq. 2s¹³:

$$y = \frac{C_1 t}{1 + C_2 t} \dots\dots\dots 2$$

The hyperbolic is compatible with the two- stage mechanism; first fast step (washing step), and second slow step (diffusive step). In this sense, the hyperbolic diffusion parameter C₁ Extraction rate (min⁻¹), C₂ constant related to the maximum extraction rate (min⁻¹)¹¹. The power- law form is expressed in Eq.3 as:

$$q = B.t^n \dots\dots\dots 3$$

This model is suitable for extraction mechanism in non-swellable materials, where B is constant, n is the diffusion exponent, it is often <1.¹⁴The first and second order models are among the most important kinetic models applied to solid- liquid extraction process.^{15,16} First order model as described by Balyan et al¹⁷ .where the extraction speed (re) is dependent on the driving force (C_s-C_t). The first order model is expressed by the Eq. 4:

$$r_e = \frac{dC_t}{dt} = k(C_s - C_t) \dots\dots\dots 4$$

where C_t is the concentration at time t, C_s is the concentration at balance point, and k is the extraction constant (min⁻¹). The liner equation of the First order model is obtained from the integration of equation .3 and expressed by the Eq. 5:

$$\ln \frac{C_s}{C_s - C_t} = k.t \dots\dots\dots 5$$

This equation was deduced to equalize the relationship between extraction rate and temperature, so the effect of temperature on the extraction, Balyan and Sakar¹⁷. The kinetic constants can determined by the relation from Arrhinius equation Eq.6 (Shewale and Rathod ¹³). The values of (k_o , E) are determined by drawing ln k with 1/T Eq.6.

$$K = K_0 e^{\frac{-E}{R.T}} \Rightarrow \ln K = \ln K_0 - \frac{E}{R.T} \dots\dots\dots 6$$

When, E activation energy (kJ/kmol), k constant rate (min⁻¹), k_o frequency coefficient (min⁻¹), R general gas constant 8.314 kJ/ kmol.K.¹⁸The Elovich's model is expressed by Eq.7.

$$q = E_0 + E_1 \ln t \dots\dots\dots 7$$

Sense E₀ , E₁ are constants of the Elovich's model¹⁹. The Fick's second law diffusion is modeled using Eq .8

$$\ln \frac{M_\infty}{M_\infty - M_t} = \ln \frac{\pi^2}{6} + \frac{D \pi^2 t}{R^2} \dots\dots\dots 8$$

Sense: M_∞ is the maximum quantity of extracted compounds (mg/g); M_t is the quantity of extracted compounds (mg/g) at time t, the D is diffusion coefficient (m²/s), and the R is the particle's radius of (m)²⁰.

Table 1 shows the general and linear of the applied model equations.

Table 1. general and linear equations

<i>Kinetic Model</i>	<i>General equation</i>	<i>linear equation</i>
hyperbolic model	$y = \frac{C_1 t}{1 + C_2 t}$	$\frac{1}{y} = \frac{1}{C_1} + \frac{C_2}{C_1 t}$
Power-law	$q = B t^n$	$\ln q = \ln B + n \ln t$
first-order reaction model	$\ln \frac{C_s}{C_s - C_t} = k.t$	$\ln \frac{C_s}{C_s - C_t} = k.t$
Elovich	$q = E_0 + E_1 \ln t$	$q = E_0 + E_1 \ln t$
Fick's second law diffusion	$\ln \frac{M_\infty}{M_\infty - M_t} = \ln \frac{\pi^2}{6} + \frac{D\pi^2 t}{R^2}$	$\ln \frac{M_\infty}{M_\infty - M_t} = \ln \frac{\pi^2}{6} + \frac{D\pi^2 t}{R^2}$

To determine the most suitable model, each correlation coefficient R^2 , root-mean square RMS and absolute relative deviation ARD, are calculated using the following equations^{21,22}.

$$RMS = \left[\frac{1}{N} \sum_{i=1}^N \left(\frac{q_{exp} - q_{cal}}{q_{exp}} \right)^2 \right]^{0.5} \dots\dots\dots 9$$

$$ARD = \frac{1}{N} \sum_{i=1}^N \left(\frac{q_{exp} - q_{cal}}{q_{exp}} \right) \times 100 \dots\dots\dots 10$$

Sense: N is the number of experiments. q_{cal} is the calculated values, q_{exp} is the experimental values in eqs 1.2. 4. 6 and 7. The model that gives the highest value for R^2 and the lowest value for RMS and ARD, is the most suitable model.

Results and Discussion

Concentration and Type Solvent Effect:

The results in Table 2, show that the extraction yield increases with increasing polarity of solvent. This means that compounds contained in the plant

are high polarity. However, Ethyl alcohol was better than water, this agrees with Kashaninejad et al,²¹ and Tomšik et al.²²

Table 2. Concentration and type solvent effect on yield extraction

<i>q%</i>	<i>Time(min)</i>	<i>Capacity(W)</i>	<i>Concentration%</i>	<i>Solvent</i>
6.91	5.5	540		Water
4.42	5.5	540	50	Ethyl Alcohol
5.00	5.5	540	60	
6.97	5.5	540	70	
8.45	5.5	540	80	
7.87	5.5	540	90	

Capacity Effect:

The results in Fig. 1 show that the extraction percentage increases with increasing capacity, this is due to the fact that the temperature increase, the diffusion coefficient increases since the diffusion coefficient is calculated from Arrhenius relation Eq.11:

$$D = D_0 e^{-\frac{E}{RT}} \dots\dots\dots 11$$

D diffusion coefficient (m²/sec), D₀ diffusion coefficient at maximum temperature (m²/sec), E activation energy (J/mol), T temperature (K)¹⁶.

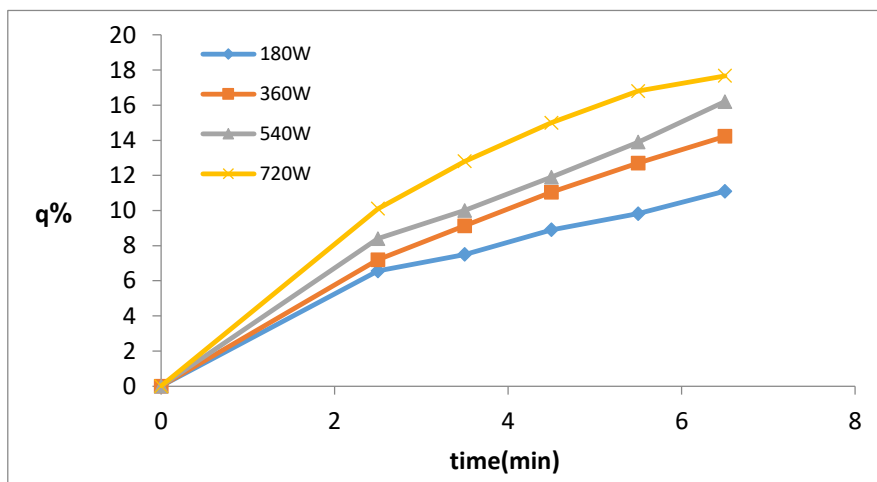


Figure 1. Capacity and time effect on the yield extraction of Ethyl alcohol

Kinetic Modeling of the Microwave-assisted Extraction:

The relationship between yield extraction, time extraction, and Microwave capacity using are shown in Fig.1. It is clear from the extraction curves that the extraction process took place in two steps: fast increases in the bioactive compounds concentrations at first (washing step), and gradual increases in the advanced time (solute diffusion step). Bioactive compounds concentrations increased quickly during the first 5 minutes, then tending to almost constant value. This is the behavior of most solid–liquid extractions²³.

Kinetic models were applied to the Ethyl alcohol extract for Microwave- assisted extraction. The hyperbolic Eq.1, power law Eq.2, first order Eq.3, Elovich’s Eq.6, and Fick’s second law diffusion is modeled using Eq .7. The experimental results were applied to the equations of kinetic models, and the constants for each model were determined, the results are shown in Table 2. Figs. 2–7 show the results of applying the experimental values to the kinetic models. With the linearized hyperbolic model, power-law, first order, second order, hyperbolic, Elovich’s, and Fick’s law equations.

These models have been applied to study the extraction kinetics of compounds from several plants such as Asteraceae plant, pomegranate peels, and chicory roots by-products²⁰. To determine the most appropriate model, T correlation coefficient (R²), the ARD and RMS are calculated.

Table 3. shows the result of kinetic study for Ethyl alcohol extracts , RMS, and ARD results ,it is clear from the table that the values of R²> 0.94, the RMS and ARD < 10% for all models and at all conditions. So, it can be considered that all applied models describe the mechanism of Microwave-assisted extraction of compounds Rosemary leaves²⁴. However, as reported by Lafka et al.²⁵, it must be underlined that because they are empirical models, it is difficult to assign physical meaning to their parameters. Fig. 2 shows the form of linearized hyperbolic equation for Microwave- assisted extraction for active compounds from Rosemary leaves using the Ethyl alcohol. The hyperbolic equation was the one with the good suitable model. It behaves an extraction kinetic behavior of the first order, with the yield growing linearly with time in the first step and zero order in the very late stage.

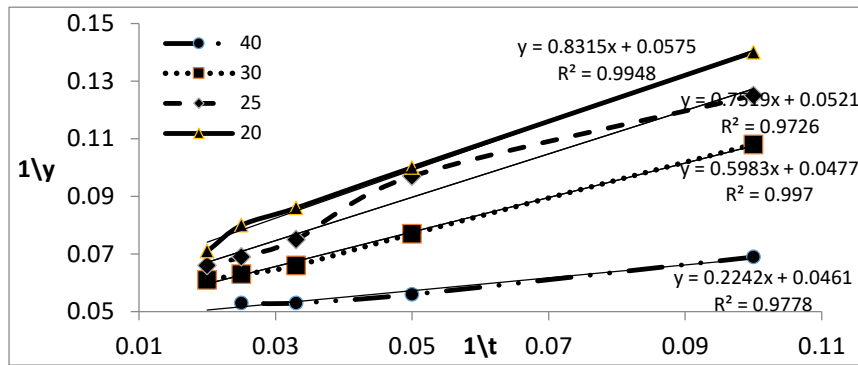


Figure 2. Plot of hyperbolic model

Fig. 3. shows the plot linearized power-law equation for Microwave- assisted extraction for active compounds from Rosemary leaves using the Ethyl alcohol. Where, R^2 (0.98–0.99), RMS (0.01–

0.05), and ARD (0.6–5). So, the model is well fit the process. The power-law describes the extraction process for a non-swelling device with a diffusion exponent $n < 1$ for plants²³.

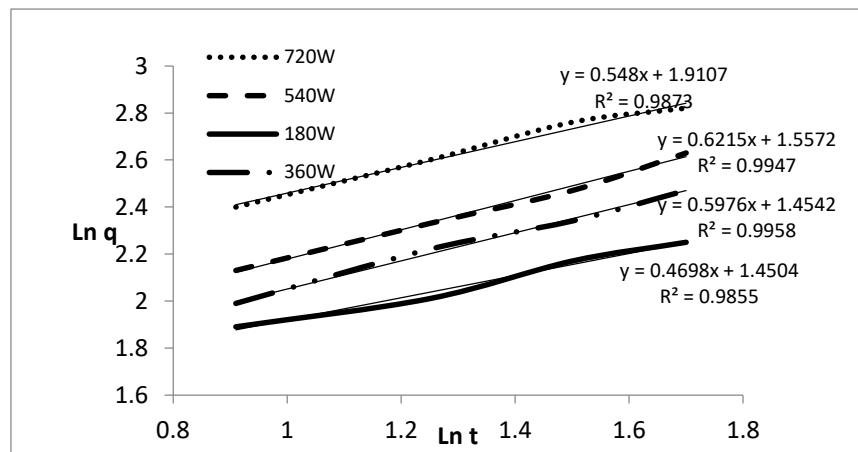


Figure 3. Plot of power-law model

Fig. 4, shows the plot of linearized First order equation for Microwave- assisted extraction for active compounds from Rosemary leaves using the Ethyl alcohol. R^2 (0.97–0.99) and low RMS (0.01–

0.1) and ARD (4–9), rate constant increase with capacity, and activation energy is 27725 J/mol. With this values we can consider that this model is suitable for this process.

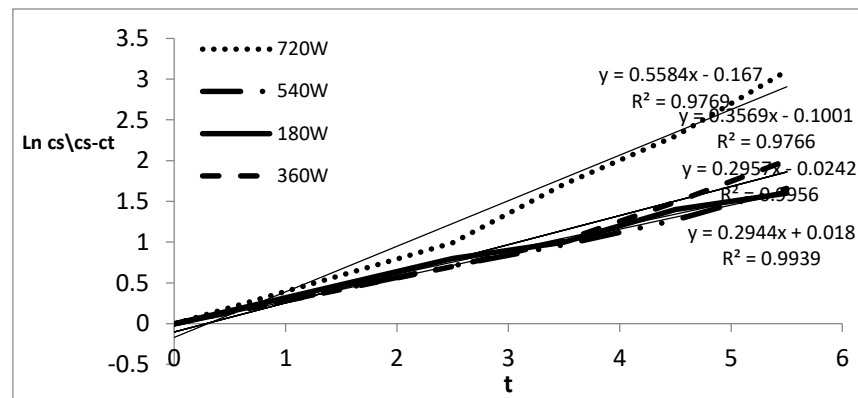


Figure 4. Plot of first order model

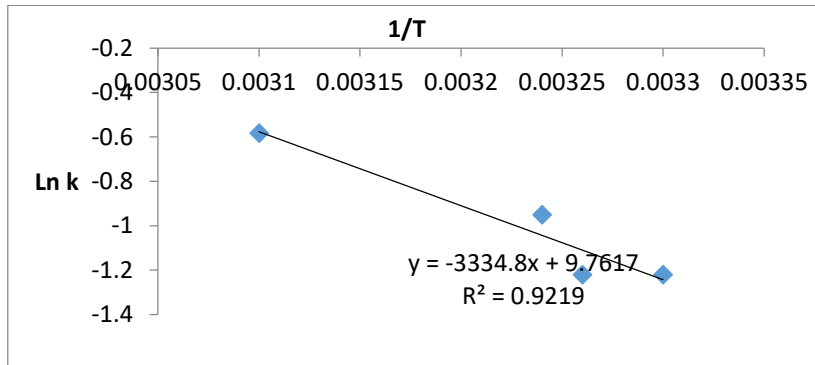


Figure 5. Arrhenius relationship

Fig. 6. displays the plot of linearized Eloviche's equation for Microwave- assisted extraction for

active compounds from Rosemary leaves using the Ethyl alcohol. R^2 (0.97–0.98) and low RMS (0.01–0.1) and ARD (0.4–4).

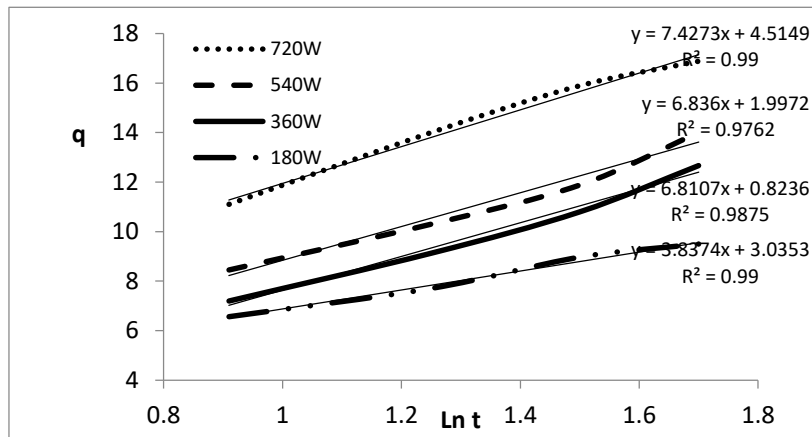


Figure 6. curves of Elovich's model

Fig.7 displays the plot of linearized Fick's second law diffusion equation for Microwave- assisted extraction for active compounds from Rosemary

leaves using the Ethyl alcohol. R^2 (0.96–0.99) and low RMS (0.06–0.14) and ARD (1–8). D diffusion coefficient increase with capacity, this agrees with Arrhenius equation.

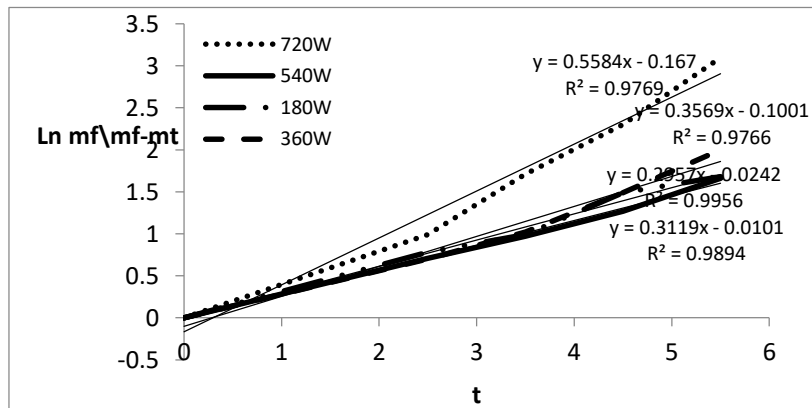


Figure 7. Plot of Fick's second law model

Table 3. Kinetic models, constants, R², RMS, ARD, for hyperbolic, power law, first order, and Elovich's models for Microwave-assisted extraction of bioactive compound from Rosemary.

<i>model</i>	<i>Capacity W</i>			
	180	360	540	720
hyperbolic				
C₁	3.90	4.18	5.15	7.33
C₂	0.21	0.14	0.17	0.25
R²	0.99	0.99	0.98	0.98
RMS	0.028	0.04	0.07	0.06
ARD%	4.0	0.08	0.01	4.00
First order				
K(min⁻¹)	0.294	0.295	0.353	0.532
E(j/mol)	27725			
R²	0.99	0.99	0.97	0.98
RMS	0.01	0.06	0.01	0.14
ARD%	9.0	4.0	4.0	9.0
POWER Low				
B	4.28	4.74	6.75	3.19
N	0.46	0.59	0.62	0.54
R²	0.99	0.99	0.98	0.99
RMS	0.01	0.04	0.03	0.05
ARD%	2.0	3.0	0.6	5.0
Elovich's				
E₀	3.03	0.82	1.99	4.51
E₁	3.83	6.81	6.83	7.42
R²	0.97	0.98	0.98	0.97
RMS	0.01	0.02	0.01	0.1
ARD%	0.4	0.8	4.00	0.9
Fick's law				
D(m²/sec)	0.50x10 ⁻⁹	0.53x10 ⁻⁹	0.60x10 ⁻⁹	0.90x10 ⁻⁹
R²	0.99	0.99	0.96	0.98
RMS	0.07	0.06	0.060.14	
ARD%	5.0	1.0	1.0	8.0

Conclusion

In this study the following finding were concluded:

- The extraction yield increases with the increasing polarity of solvent, this is due to the fact that the Polyphenols are organic, polar compounds, so that hydrophilic compounds are located inside the cell, while the hydrophobic

in the cell wall, therefor, ethyl and methyl alcohol are the best solvent . The extraction yield increases with the increasing capacity, thus with increasing temperature, this is due to the fact that the diffusion coefficient increase with temperature, the solvent viscosity

decrease, which facilitates the process of transferring the compounds.

- The extraction yield increases with the increasing the time extraction, this due to the increase the contact time between the samples and solvent.
- The best condition for extraction is: the solvent is ethyl alcohol 80%, time extraction is 6.5 minutes, and capacity of Microwave is 720W.
- All kinetic models fitted the process with high value for $R^2 > 0.94$ and low value for RMS and

ARD < 10%, this means the Microwave-assisted extraction agrees with the two step mechanism, fast increases in the bioactive compounds concentrations at first (washing step), and gradual increases in the advanced time (solute diffusion step), this agrees with the most extraction processes applied on plants.

- The most suitable model was hyperbolic diffusion model where $R^2(0.98-0.99)$, RMS(0.028-0.06), ARD(0.01-4).

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Authors' Declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Furthermore, any Figures and images, that are not mine, have been included with the necessary permission for re-publication, which is attached to the manuscript.

- The author has signed an animal welfare statement.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Al-Baath.

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دراسة حركية الاستخلاص المحفز بالأمواج الميكروية للمركبات الفعالة من أوراق إكليل الجبل

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الخلاصة

نظراً لأهمية عملية الاستخلاص في الكثير من المجالات الهندسية والطبية، وتزامناً مع العودة إلى الاهتمام الكبير بالنباتات الطبية، تمّ في هذا البحث دراسة العوامل المؤثرة على عملية الاستخلاص المحفز بالأمواج الميكروية للمركبات الفعالة من أوراق إكليل الجبل وتحديد شروط الاستخلاص الأمثل التي تعطي القيمة الأعلى لنسبة الاستخلاص. ومن أجل توصيف عملية الاستخلاص والتوصل إلى معرفة آلية العملية، تمّ إجراء الدراسة الحركية بتطبيق خمسة نماذج حركية على الاستخلاص المحفز بالأمواج الميكروية وللمستخلص الكحولي الذي أعطى أفضل النتائج. تؤكد النتائج ان الكحول الإيثيلي 80% كان الأفضل حيث اعطى أعلى نسبة استخلاص عند الزمن 6.5 دقيقة واستطاعة قدرها 720 واط. أما بالنسبة للنماذج الحركية فقد تمّ تطبيق خمسة نماذج هي : نموذج الانتشار بشكل قطع زائد، نموذج قانون الطاقة، نموذج القانون الأول، نموذج ايلوفتش، ونموذج قانون فيك الثاني في الانتشار، وقد بينت النتائج ان جميع النماذج المدروسة تلائم عملية الاستخلاص المحفز بالأمواج الميكروية للمركبات الفعالة من أوراق إكليل الجبل بقيم مرتفعة لمعامل الارتباط اكبر من 0.96 وقيم منخفضة لكل من الجذر التربيعي النسبي و الانحراف النسبي المطلق حيث كانت جميع القيم أقل من 10%، وعليه فقد تمّ حساب الثوابت الحركية الخاصة بهذه النماذج.

الكلمات المفتاحية: استخلاص، نموذج، ميكرويف، حركية، إكليل الجبل.