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**Green-extraction of essential oil of the species *Ruta chalepensis* L.: gas chromatography-mass spectroscopy-infra red analysis and response surface methodology optimization**

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**Abstract:** *The objective of the present study was the optimization of the parameters affecting the hydrodistillation of *Ruta chalepensis* L. essential oil using response surface design type Box-Behnken. After an appropriate choice of three parameters, 15 experiments were performed leading to a mathematical second-degree model relating the response function (yield of essential oil) to parameters and allowing a good control of the extraction process. The realization of the experiments and data analysis was carried out by response surface methodology (RSM). A deduced second-order polynomial expression was used to determine the optimal conditions necessary to obtain a better essential oil yield. These optimized operating conditions were: a granulometry of 2 mm, a condensation-water flow rate of 3.4 mL/min and an extraction time of 204 min. Analysis of variance (ANOVA) indicates that the generated second-order polynomial model was highly significant with*

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$R^2=0.9589$  and  $P<0.006$ . The gas chromatography-mass spectrometry analysis of essential oil extracted from the *Ruta chalepensis* L. aerial parts revealed the presence of 2-undecanone, 2-nonanone and 2-decanone as major components.

**Key words:** Response surface; optimization; Hydrodistillation; *Ruta chalepensis* L.; Essential oils.

## Introduction

Essential oils are natural products of very complex composition extracted from aromatic plants by different techniques. They are fragrant, thermolabile and present only in relatively small amounts [1-3]. These complex mixtures have an important therapeutic potential through their broad-spectrum activities, including antimicrobial, antioxidant, anti-inflammatory and antiviral [4,5].

Various methods can be used for the extraction of essential oils, namely: distillation (hydro and steam), solvent extraction, supercritical fluid extraction, etc. The quality and quantity of the oil yield depend on the extraction conditions and the used technique [6,7]. Today, under the term "green-extraction" there is no place for simple extraction, it is necessary to choose the right extraction technique and optimize its parameters in order to obtain a high yield and good quality oil. Among the previously mentioned techniques, distillation is the most used because of its practical simplicity with an interesting oil quality, process cost, and eco-friendship [8,9].

In the framework of improving essential oil yield, studies have been conducted on the optimization of extraction operating parameters through the use of the response surface methodology (RSM) [7-12]. The objective of the study of the response surfaces is to model the studied phenomenon. It is a natural sequence of the detection steps which constitutes an effective way of optimizing the experimental device or the studied process [13]. This method (RSM) is used in various fields such as industries, agriculture, medicine, analysis, electronics, automation with the main objective is to optimize, develop and improve the system's response [14,15]. In this study, our choice is based on the methodology of the response surface, which has several properties, namely the optimization of the process in terms of time and quantity in addition to information on the interactions of these parameters [16].

The medicinal plant *Ruta chalepensis* is an aromatic plant, belonging to the family Rutaceae, commonly called by the local population "Fidjel". It is spontaneous, widely-spread in North Africa, particularly in Algeria. It is frequently found in rock gardens, lawns and dry slopes [17]. It is used as

laxative, anti-inflammatory, analgesic, antispasmodic, abortive, antiepileptic, emmenagogue and for the treatment of cutaneous pathologies [18].

In addition, its essential oil is intended for the perfumery and the agro-alimentary industry [18]. Therefore, improving its yield of essential oil becomes of a primary necessity. The present work provides the parametric hydrodistillation optimization of the essential oil of the plant *Ruta chalepensis* of Algerian origin using the response surface method (RSM), in order to study the effects of the extraction parameters, namely: the granulometry; the condensation-water flow rate; and the extraction time on the yield of oil. The composition of the oil obtained was analyzed by infrared spectroscopy (IR) and gas chromatography coupled with mass spectrometry (GC-MS).

## **Material and methods**

### **Plant material**

The aerial parts of *R. chalepensis* were harvested in December 2016 in the semi-arid region around Oum el Bouaghi in northeastern Algeria in the following geographical coordinates: latitude 36°03'34 " North, longitude 6°58'30 " East and an altitude of 841m. The plant species has been air-dried at room temperature under shade before being crushed and sieved through a vibrating sieve system that separates the particles according to their granulometry.

### **Methods**

#### **Essential oil extraction**

The extraction of essential oil was carried out by hydrodistillation using a Clevenger type apparatus. 100g of plant material was introduced into a glass flask (2L) to which 1500ml of water was added. The extraction time was measured after the appearance of the first drop of water at the outlet of the condenser. The recovered oil was dried with calcium chloride and stored in tightly sealed opaque glass flask at low temperature (4°C). The yield of essential oil is expressed by the following relationship (01):

$$\text{oil yield(\%)} = \frac{\text{mass of extracted oil}}{\text{mass of plant material}} \times 100 \quad \dots (01)$$

#### **IR analysis**

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Infrared spectroscopy is a method of qualitative analysis used to characterize the main functions of an organic molecule. IR-600 type apparatus was used in this analysis.

### **GC-MS analysis**

The identification of the chemical constituents of *R. chalepensis* essential oil was made by a gas chromatographic (HP 5890-SERIE II) equipped with a HP5 MS capillary column (30m long, 0.25 mm inner diameter and 0.25 $\mu$ m film thickness) coupled to a mass spectrometer (HP-MSD 5972). The analytical conditions were: injector temperature 250°C; oven temperature programmed at 60°C for 8min, then gradually increased to 280°C at 2°C/min; detector temperature 290°C. The vector gas is helium (0.5 mL/min). The spectra were recorded at 70eV. The spectral analysis of the compounds was carried out by comparing authentic samples using the Wiley 275 mass spectra data bases.

### **Selection of the studied parameters**

The optimization study was conducted on the operating parameters considered influential in hydrodistillation process namely the granulometry, the condensation water flow rate, and the extraction time. The choice of parameters to optimize and their experimental domains is based on literature data [19,20]. The three parameters thought to affect the hydro distillation process are all continuous and quantitative factors; i.e. can be controlled, and adopt real numerical values in the selected domain.

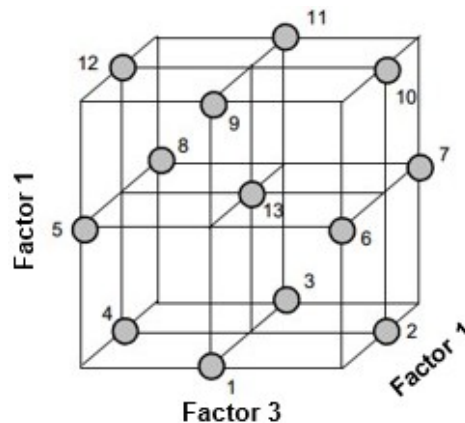
### **Process of optimization by the response surface methodology (RSM)**

The Response Surface Methodology (RSM) is a useful mathematical and statistical technique for modeling and analyzing the effect of multiple quantitative variables on the response of interest. This methodology saves time and effort by reducing the number of experimental trials and providing optimized and statistically significant results [13]. Optimizing extraction parameters is important for efficient extraction. Consequently, the RSM method was used to evaluate the effects of the hydrodistillation parameters on the yield of essential oil, namely: the granulometry, the condensation-water flow rate and the extraction time, in order to find the optimal conditions with a better yield. In

this work the Design-expert® software version 7.0.0 (Stat-Ease, Inc., Minneapolis) was used to design the experiments and evaluate the results.

**Box-Benhken design**

Experimental plans are techniques designed to highlight the effects of various factors on a response and to optimize them in specific experimental domains. A series of tests is organized to manipulate the factors in order to describe the suitable method so as to obtain the optimal response [21]. The Box-Behnken plan is a surface response method used to examine the relationship between several response variables or a set of experimental parameters [16]. The Box-Behnken plan requires fewer implementations and each factor requires only three levels instead of five necessary for the other plans, in cases of three or four variables [22]. The three-factor Box-Behnken plan requires only 6 factorial points and 6 axial points in addition to replicas at the center point, Figure 1 represents a Box-Behnken three-factor design.



**Fig. 1.** Box-Behnken three-factor design.

The number of experiments is given according to the equation (02):

$$N = 2k(k-1) + cp \quad (02)$$

where k is the number of factors, and cp is the number of replications at the central point. Accordingly, Box-Behnken design is considered as an effective option in response surface methodology and a best alternative to central composite designs [16].

### Domains and experimental matrix

The experimental plan is based on a Box-Benhken plan for three parameters and three levels (Table 1). Given 3 central points, a total of 15 tests were generated in Table 2, consisting of 6 factorial points and 6 axial points.

**Table 1. Experimental domains of the studied parameters.**

Parameter	Symbol	Unity	Minimum	average	Maximum
granulometry	$x_1$	mm	1	1.5	2
Condensation-water flow rate	$x_2$	ml/min	1.4	2.4	3.4
Extraction time	$x_3$	min	150	180	210

### Mathematical model

The postulated mathematical model is a polynomial of order 2 such as the equation (03):

$$Y(\%) = \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_0 \quad (03)$$

With:

Y is the theoretical yield of essential oil (Response).

$\beta_0$  represents the model constant.

$\beta_1, \beta_2$  and  $\beta_3$  are the coefficients of the linear terms.

$\beta_{11}, \beta_{22}$  and  $\beta_{33}$  are the coefficients of the quadratic terms.

$\beta_{12}, \beta_{13}$  and  $\beta_{23}$  are the coefficients of the interaction terms.

$x_1, x_2$  and  $x_3$  are the values of the parameters.

### Statistical analysis

The model has been validated by the ANOVA. The model, the regression coefficients and the non-fit test will be considered significant when the probabilities of the significance of the p-value risk are less than 0.05 [19,23]. In addition to the analysis of the variance, we will use other tools namely the multiple determination coefficient  $R^2$  and the coefficient of variance CV, a variance coefficient value less than 10% indicates a very high degree of precision between experimental and predicted values [7].

### Optimization study

After the determination of the model and the proof of its validity, and to explore the optimal operating conditions which lead to the desired response by a better optimization, we use the response surfaces that can be generated within the experimental domain from the model equation. These surfaces explain the variations of our response according to two parameters while maintaining the third constant, i.e. the graphical representation of the results (estimated model) to be able to derive optima. Then, to find the exact optimal operating conditions with a certain percentage of compromise, we use the function "Desirability". This function, which makes it possible to give an exact optimal adjustment, varies between 0 and 1. Indeed, the value 0 is assigned when the factors lead to an unacceptable (undesirable) response and that of 1 when the response represents the maximum desired performance for the considered factors [20].

### Results and discussion

#### Experimental yield

From the experimental results of Table 2, it is found that the highest yield value 0.81% was recorded for two experiments (8 and 12) having the same extraction time (210min) with a granulometry and a condensation-water flow rate of: 2mm and 2.4mL/min, 1.5mm and 3.4mL/min respectively. The smallest value of the yield (0.49%) is recorded in experiment 5 under the following operating conditions: 1mm, 2.4 mL/min and 150min extraction time.

**Table 2. Experimental matrix and experimental yield values.**

Exp	$x_1$ : granulometry (mm)	$x_2$ : Condensation-water flow rate (mL/min)	$x_3$ : Extraction time (min)	Experimental yield (%)
1	1	1.4	180	0.62
2	2	1.4	180	0.69
3	1	3.4	180	0.68

4	2	3.4	180	0.78
5	1	2.4	150	<b>0.49</b>
6	2	2.4	150	0.69
7	1	2.4	210	0.61
8	2	2.4	210	<b>0.81</b>
9	1.5	1.4	150	0.57
10	1.5	3.4	150	0.72
11	1.5	1.4	210	0.7
12	1.5	3.4	210	<b>0.81</b>
13	1.5	2.4	180	0.71
14	1.5	2.4	180	0.72
15	1.5	2.4	180	0.72

On the other hand, the reading in the results of the extraction yield of *R. chalepensis* essential oil of different origins obtained by hydrodistillation generally shows that the yields found in our sample are in agreement with those mentioned in literature (Table 3). Indeed, Bendriss, Merghache et al., and Attou [24,18,25] conducted studies on the same specie harvested from different localities and at different periods and reported yield values of the order of 0.28%-1.28%, 0.12%-0.8% and 0.41%-1.9% in the region of Tlemcen, Chlef and Ain Temouchent respectively.

**Table 3.** Essential oil yields of *R. chalepensis* extracted by hydrodistillation.

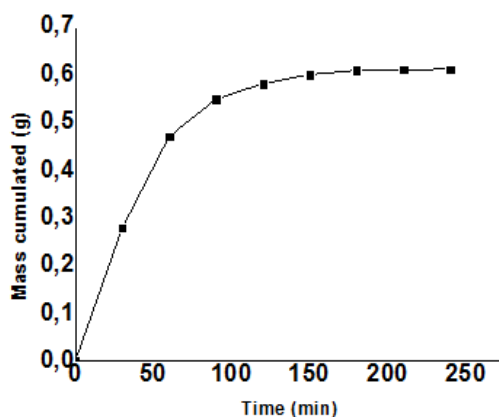
Pays	Region	Yields	Reference
Algeria	NortheastAlgeria (Oum El Bouaghi)	0.61%-0,81%	Our study
	Central Algeria (Chlef)	0.12%-0,8%	[24]
	Central Algeria (Ain Témouchent)	0.41%-1,9%	[25]
	North West Algeria(Tlemcen)	0.28%-1,28%	[18]
Tunisia	Southern Tunisia (Mednine)	0.34%	[26]

**Kinetics of hydro distillation**

The kinetics of extraction consists in following the evolution of the cumulative extracted oil mass as a function of time. For this purpose and in order to assess this variation, sufficient time is left until



the extraction of almost all the essential oil. The amount of oil extracted after 180min and up to 240min is about 0.37% of the total amount of oil extracted (Figure 2).



**Fig. 2.** Variation of the cumulated mass of the essential oil as a function of the extraction time.

Based on the concept of minimizing extraction time and reducing energy consumption, the time required for the hydrodistillation is set as 180 min.

### Physicochemical properties of extracted essential oil

The physicochemical properties of oil extracted by hydrodistillation were evaluated. Indeed, the essential oil of *R. chalepensis* L. is a clear green liquid, an odor similar to the vegetable matter (herbaceous characteristic) and an acid index of 1.4025. The refractive index was measured at 20°C using a branded refractometer (ABBE) having a temperature indicator. The relative density and refractive index were 0.830 and 1.431 respectively. All these physicochemical properties are close to all essential oil marketing standards (New Directions Aromatics Inc., 2016) as shown in Table 4.

**Table 4.** Physicochemical properties of essential oil extracted.

Properties	Present study	New Directions Aromatics Inc., 2016
Aspect	Green clear Liquid	Pale yellow
Odor	Herbaceous characteristic	Characteristic odor
Relative density at 20°C	0.830	0.826-0.834

Refraction Index at 20°C	1.431	1.429-1.436
Acid Index	1.4025	ND

ND: undetermined.

On the other hand, our results are generally in line with the literature data as shown in Table 5.

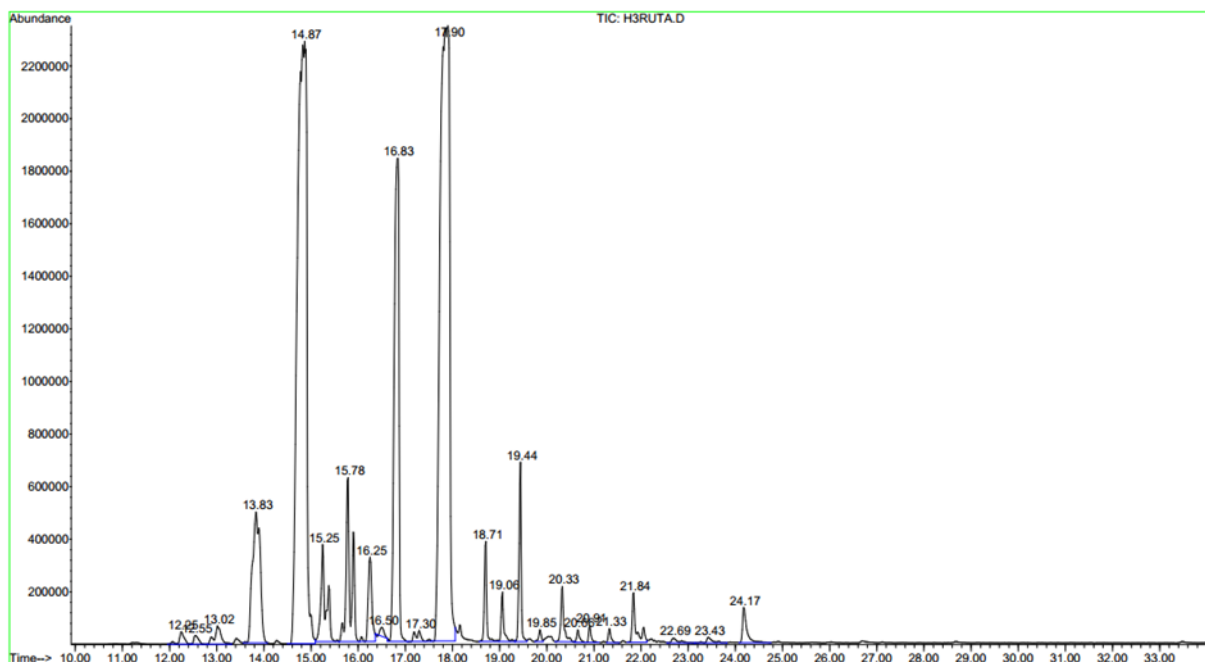
**Table 5.** Physicochemical properties of *R. chalepensis* essential oil.

Reference	Relative density at 20°C	Refraction Index at 20°C	Acid Index
[18]	0.8193-0.8378	1.4307-1.4328	2.93-5.61
[24]	0.932	1.5326	2.32
[25]	0.8631-0.9295	1.344-1.5	2.09-9.53
[27]	0.8363	1.4325	//

### Chemical composition of the essential oil extracted

The percentage of individual components of the essential oil extracted from the aerial parts of the *R. chalepensis* from the semi-arid region of Oum el Bouaghi in eastern Algeria is shown in Table 6. CG-MS qualitative and quantitative analysis (chromatogram is shown in Figure 3) revealed 37 compounds representing 99.99% of the oil.

The reading of the results shown in Table 6 shows that the essential oil is prevailed by 2-undecanone (33.37%) followed by 2-nonanone (32.87%) and 2-Decanone (11.11%).



**Fig. 3.** GC-MS Chromatogram of *Ruta chalepensis* essential oil.

According to the Merghache et al, GC-MS analysis of the essential oils of the different parts of *R. chalepensis* of the Tlemcen region identifies 20 constituents, among which 2-undecanone (20.40-82.74%) is the major constituent in addition 1-decanol and 2-undecanol [18]. These chemotypes are in agreement with our findings. The same fallouts have been observed for *R. chalepensis* essential oil from the Chlef region where 2-undecanone was the major compound with 62.84% [24].

Furthermore, it was stated that 2-undecanone is the major constituent of the essential oil of *R. chalepensis* of Argentina, Turkey, Iran and India with the following proportion (66.5%, 38.1%, 41.3%-67.8% and 52.5% respectively) [27-30].

**Table 6.** Chemical composition of *R. chalepensis* hydro-distillated essential oils.

N°	Compound	RT (min)	KI	(%)	N°	Compound	RT (min)	KI	(%)
1	2-Heptanone	6.323	890	0.31	20	Geyrene	10.513	1142	0.24
2	$\alpha$ -thujene	6.623	935	0.25	21	Camphor	10.624	1144	1.39
3	$\alpha$ -Pinene	7.093	940	0.12	22	2-octyl acetate	11.282	1146	0.28

4	camphene	7.167	954	0.25	23	Geijerene	11.459	1150	2.22
5	2-heptanal	7.417	966	0.13	24	2-Decanone	12.31	1192	11.11
6	sabinene	7.556	976	0.12	25	Methyl chavicol	12.758	1205	0.57
7	$\beta$ -pinene	7.707	997	0.2	26	Octyl acetate	13.175	1215	0.12
8	$\alpha$ -Phellandrene	7.948	1005	0.09	27	Cuminic	13.291	1251	0.15
9	p-Cymene	8.108	1025	1.2	28	aldehyde	13.549	1295	33.37
10	Limonene	8.186	1032	2.02	29	2-undecanone	14.737	1315	1.26
11	1,8-Cineole	8.254	1033	1.75	30	Nonyl acetate	15.297	1374	0.8
12	$\gamma$ -Terpinene	8.426	1063	0.24	31	1-Undecanol	15.868	1455	1.84
13	$\alpha$ -terpinolene	8.815	1089	0.11	32	2-Tridecanone	17.063	1461	0.69
14	2-nonanone	9.573	1094	32.87	33	$\alpha$ -Humulene	18.079	1478	0.21
15	2-Nonanol	9.649	1098	0.67	34	1-Dodecanol	18.584	1549	0.65
16	Nonanal	9.722	1105	0.71	35	Elemol	18.689	1576	0.14
17	$\alpha$ -Thujone	9.851	1117	2.19	36	spathulenol	18.833	1590	0.24
18	$\beta$ -Thujone	10.037	1120	0.51	37	Veridiflorol	22.177	2118	0.5
19	dihydro- Linalool	10.357	1134	0.47		Phytol			
							Total		99.99

KI: Kovats index on a HP5 MS column.

On the other hand, the essential oil from Italy contains two major constituents: 2-nonanone (49.9%) and 2-undecanone (30.0%) [31]. the essential oil of the plant collected in Saudi Arabia contains 2-undecanone only as 4.5% [32]. The chemical composition of essential oils varies according to several abiotic factors namely climate, altitude, soil type, pH, harvest period and drying and extraction technique [33-38].

### Statistical validation of the model

Variance Analysis (ANOVA) is a statistical tool that is widely used in model validation and comparison as well as in data analysis. This method uses variance measures to assess the significance of factors and models. The interest of this analysis is to be able to test in an absolute way the influence of the factors on the variations of a given answer. The results of modeling were statistically tested by analysis of variance (ANOVA) (Table 7). the analysis showed that the experimental data were very adapted to the second order polynomial model.

**Table 7.** Analysis of variance (ANOVA) for the quadratic model.

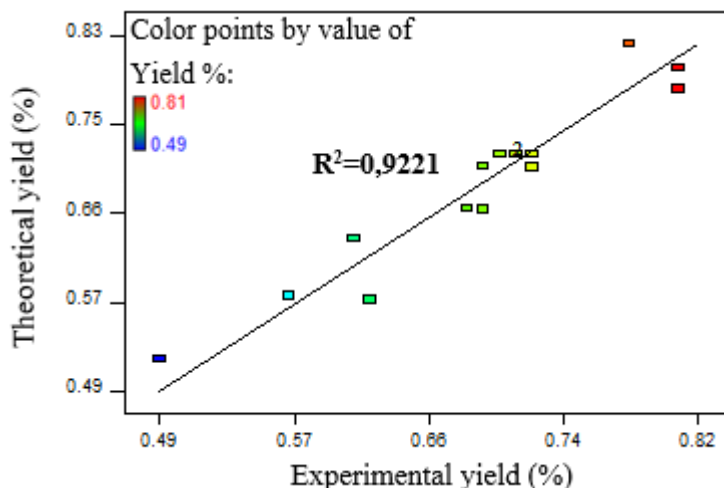
Variance source	Sum of squares	Ddl	Middle square	p-value
Model	0.098	9	0.011	0.0258*
$x_1$ : granulometry	0.041	1	0.041	0.0042*
$x_2$ : condensation-water flow rate	0.021	1	0.021	0.0160*
$x_3$ : Extraction time	0.026	1	0.026	0.0103*
$x_1x_2$	2.250E-004	1	2.250E-004	0.7269
$x_1x_3$	0.000	1	0.000	1.0000
$x_2x_3$	4.000E-004	1	4.000E-004	0.6432
$x_1^2$	5.078E-003	1	5.078E-003	0.1396
$x_2^2$	6.160E-004	1	6.160E-004	0.5677
$x_3^2$	3.231E-003	1	3.231E-003	0.2204
Residues	8.242E-003	5	1.648E-003	
adjustmentFailure	8.175E-003	3	2.725E-003	0.0121*
pure Error	6.667E-005	2	3.333E-005	
Total	0.11	14		
$R^2$			0.9221	
CV (%)			5.9	

\*Significant Terms,  $p$ -value <0.05.

The results displayed in the variance analysis (Table 7) indicate that the model is noteworthy since the probability of significance of the risk-value is less than 0.05, so there is only 0.58% chance that the model becomes invalid because of the noise. In addition, the model does not represent an adjustment lack since the probability of the risk significance of terms "default of adjustment" ( $p$ -value = 0.0444) is also less than 0.05. Thus, we can say that the model is well adjusted. the model, therefore, can be used to navigate the entire space of the experimental domain. The variance analysis also indicates that the terms of the model  $x_1$ ,  $x_2$  and  $x_3$  are significant terms ( $p$ -value <0.05). Nevertheless, the rest of the terms are statistically insignificant:  $x_1x_2$ ,  $x_1x_3$ ,  $x_2x_3$ ,  $x_1^2$ ,  $x_2^2$  and  $x_3^2$ . For a good adjustment of a model, the  $R^2$  value should be a minimum of 0.80 [39]. The coefficient of determination  $R^2=0.9221$  is sufficient because it gives good compatibility between the experimental and predicted values of the adapted model.

Alternatively, a coefficient of variation (CV) of 5.9% indicates a very high degree of precision between the experimental and predicted values [12].

The representative curve of the expected yield values as a function of the experimental yields is shown in Figure 4, where it can be seen from the graph that the cloud of points is not far from the equation line ( $y=x$ ) with a coefficient of determination  $R^2=0,9221$ , which indicates that the model has a good descriptive quality.



**Fig. 4.** Representation of theoretical yield according to the experimental one.

### Mathematical model

The experimental yield is used to determine the values of the regression coefficients of the polynomial (Table 8). The quadratic model made it possible to correlate the essential oil yield extracted by hydrodistillation as function of the three studied parameters.

**Table 8.** Regression coefficients of the polynomial.

Coefficient	Value	Coefficient	Value
$\beta_0$	-1.37943	$\beta_{22}$	0.012917
$\beta_1$	0.55150	$\beta_{33}$	-3.28704.10 <sup>-5</sup>
$\beta_2$	0.026750	$\beta_{12}$	0.015000
$\beta_3$	0.014550	$\beta_{13}$	0.000000
$\beta_{11}$	-0.14833	$\beta_{23}$	-3.33333.10 <sup>-4</sup>

With all the possible combinations of the three parameters, the used mathematical model is given by the following equation (04):

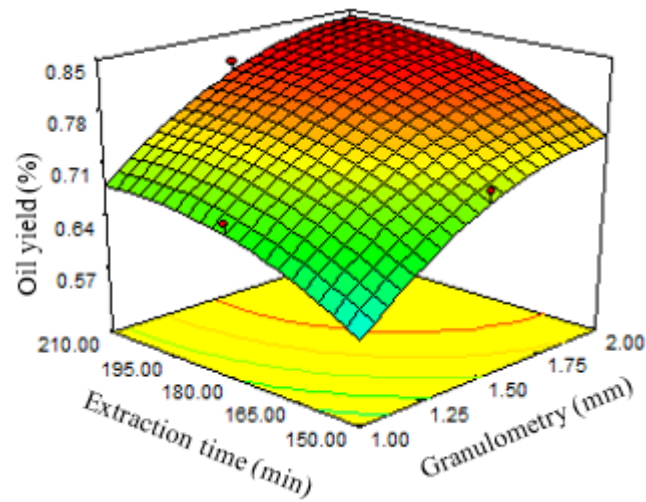
$$Y(\%) = -0.14833x_1^2 + 0.012917x_2^2 - 3.28704 \cdot 10^{-5}x_3^2 + 0.015000x_1x_2 - 3.33333 \cdot 10^{-4}x_2x_3 + 0.55150x_1 + 0.026750x_2 + 0.014550x_3 - 1.37943 \dots (04)$$

### Optimization of parameters

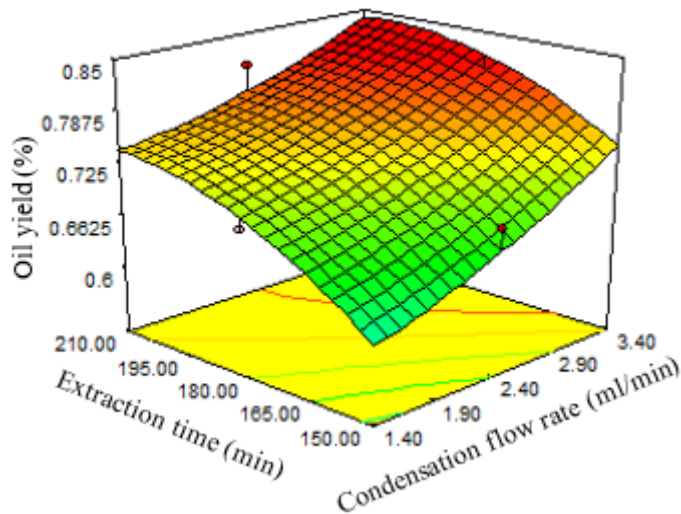
The objective of this study is to improve the yield of essential oil of *R. chalepensis* extracted by hydro distillation, through the optimization of operating parameters affecting the extraction process. By carrying out the experimental plan, we experimentally recorded a maximum yield with a value of 0.81%. The objective is therefore to determine the optimum operating parameters that lead to a better yield. In other words, we look for the optimal operating conditions that lead to the maximum accessible value.

### Response surfaces

The influence of the studied parameters on the oil yield can be graphically illustrated by the representation of the response surfaces in a three-dimensional space (3D). These surfaces make it possible to show the variation of the oil yield according to two factors while keeping the third constant. the three figures (5, 6 and 7) shows the response surface diagrams for the effect of the parameters on the oil yield that can be observed from the nature of the curvature of the response surfaces.

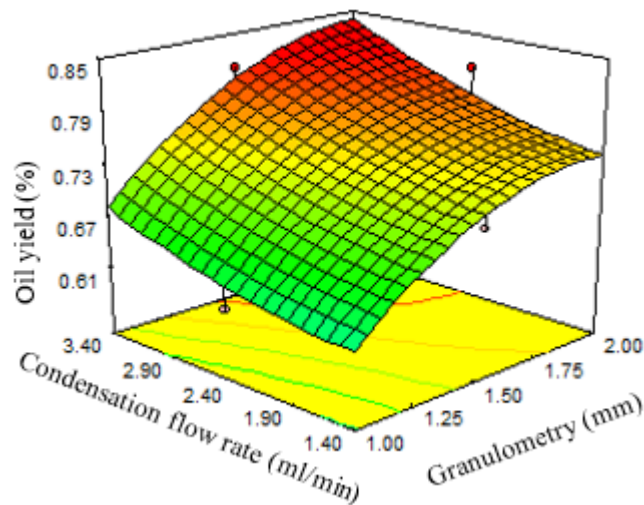


**Fig. 5.** Response surfaces to show the effect of the hydrodistillation parameters on the oil yield (extraction time and granulometry).



**Fig. 6.** Response surfaces to show the effect of the hydrodistillation parameters on the oil yield (extraction time and condensation-water flow rate).





**Fig. 7.** Response surfaces to show the effect of the hydrodistillation parameters on the oil yield (condensation-water flow rate and granulometry).

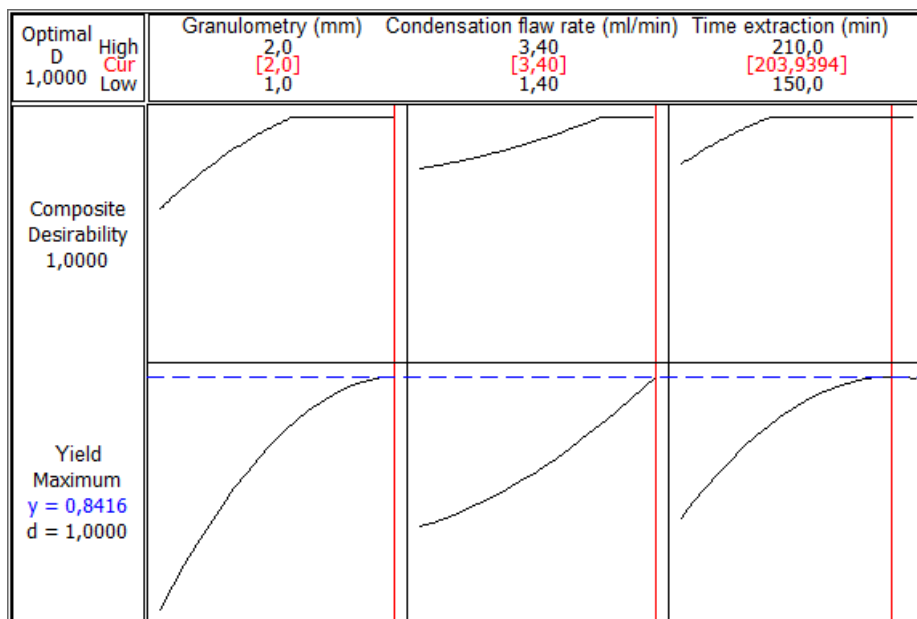
The two surfaces of Figure 5 and 6 highlight the effect of the extraction time on the essential oil yield, where they show that the yield of essential oil increases over the extraction time whatever are the granulometry and the condensation-water flow rate. On the other hand, it appears from Figure 5 and 7 that the increase in granulometry results in a large increase in yield. In addition, the same situation similar to the first two parameters is observed in Figure 6 and 7, where it is noted that the increase in oil yield is perceived with the increase of the condensation-water flow rate.

It appears from the analysis of the 3 surfaces that the oil yield is proportional with the three studied parameters: the extraction time, the particle diameter and the condensation-water flow rate. So, an insight into these surfaces leads to say that granulometry is the most effective magnitude in the parameters, then the condensation-water flow rate and the extraction time.

The reading in the response surfaces allowed knowing the domains of variation of the parameters leading to the desired yield. Thus, obtaining an optimum yield of 0.84% (the maximum accessible value) requires an extraction time of 210 min, a condensation-water flow rate of 3.4 mL/min and a granulometry of 2 mm.

**Desirability study**

The aim of our study is to maximize the yield of essential oil so as to reach the maximum available. The desirability study enables us to define with precision the optimized values of the three studied parameters. Figure 8 indicates that the achievement of the maximum yield value is possible with desirability of the order of 1, while providing, as operating conditions a granulometry of 2mm, a condensation-water flow rate of 3.4 mL/min and finally an extraction time of 204 min.



**Fig. 8.** Prediction profile of optimal hydrodistillation conditions of *R. chalepensis*.

These results indicate that the desired yield requires an increase in the extraction time to the red terminal at the right of the variation range (210 min). Obviously, time has a direct influence on the hydrodistillation operation, and its impact on has been proven by several authors [20].

The increase of the yield is also apparent with the increase of the granulometry. This is perhaps due to the localization of the producing glands of the metabolites in the tissues of this plant. Indeed, several essential oils are stored in specialized histological structures, often located on the surface of the plant such as secretion hairs and therefore do not require a strong grinding to extract them.

The last parameter (condensation-water flow rate) has a proportionally effect on the essential oil yield which reaches its maximum when the condensation-water flow rate is in the higher limit of the variation range (3.4 mL/min). This phenomenon can be explained by the fact that the increase in the flow of condensation-water is due of the increase in the heating temperature and, therefore, a maximum of temperature which allows the burst of the secretion pockets which contain essential oil.

## Conclusion

The results of the study revealed that the essential oil of *Ruta chalepensis* can be easily extracted using the hydrodistillation technique. The physicochemical properties of extracted oil were determined. Analysis of this oil by GC-MS identified 37 constituents representing 99.99% of the crude oil, the majority of which is 2-undécane (33.37%), followed by 2-nonane (32.87%) and 2-Decane (11.11%).

The expected essential oil yield expressed by the second-order polynomial model depends on the linear terms  $\beta_1$ ,  $\beta_2$  and  $\beta_3$ . The quadratic terms  $\beta_{11}$ ,  $\beta_{22}$  and  $\beta_{33}$  are related to the granulometry, the condensation-water flow rate and the extraction time respectively.  $\beta_{12}$ ,  $\beta_{13}$  and  $\beta_{23}$  interaction terms are related to the granulometry/ condensation-water flow rate, granulometry/extraction time and condensation-water flow rate/extraction time interactions respectively. The high regression coefficients ( $R^2=0.9221$ ) of the response with the model value  $p$ -value=0.0258 showed that the developed model is well suited to the experimental data. The optimal value of the expected yield  $Y=0.84\%$  is obtained by the combination of the following operating conditions: granulometry (2mm), condensation-water flow rate (3.4 mL/min) and extraction time (204 min).

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