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ECO-EXTRACTION PROCESS OF ESSENTIAL OIL OF THE VEGETAL SPECIES *EUCALYPTUS CAMALDULENSIS* DEHNH.: PARAMETRIC MODELING AND OPTIMIZATION BY BOX-BEHNKEN PLAN

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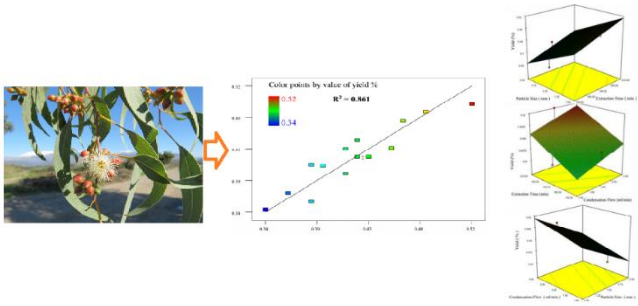
This study was aimed to implement response surface methodology to optimize the hydrodistillation parameters of the essential oil of the plant species *Eucalyptus camaldulensis* (RSM). The distillation time, condensation flow rate, and particle size were the desired parameters. To generate factor combinations, a three-factor Box-Behnken design was used. The response surface analysis yielded a linear model, which was used to calculate the top yield of extracted oil based on the optimized conditions. ANOVA shows that the generated polynomial model was highly noteworthy, with R^2=0.861. The peak yield was 0.513663% at the optimal conditions, which were 1 mm as particle size, 3.4 mL/min as condensation flow rate, and 210 min as extraction time. The GC/MS analysis revealed the presence of p-cymene (26.22%), spathulenol (16.71%), and 1.8-cineole (14.44%) as major components.



INTRODUCTION

The Response Surface Methodology (RSM) is a group of mathematical and statistical techniques for building and analyzing empirical models where the optimal interest response is subjected to many variables.[1] The RSM is a popular method in the development and optimization of various systems and is considered as an important topic in statistical design of experiments. The methodology, which is based on the principles of experimental design (DOE), entails the use of various types of experimental models, the generation of polynomial mathematical relationships, and the mapping of the experimental domain response to select the optimal formulation.[2]

In recent years, RSM has been widely used to optimize, advance, and enhance system response for critical applications in the design and origination of novel products in industries, medicine, electronics,

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automation, chemistry, agriculture, analysis, and other fields. Not only does this method save time and reagents, but it also provides data on parameter interactions.[3]

Because of their vast spectrum of activities, comprising antifungal, antibacterial, anti-inflammatory, antioxidant and antiviral properties, essential oils are considered a potential therapeutic source.[4] As a result, optimizing the extraction processes is required to achieve maximum oil yield.

Many studies on the optimization of extraction processes using response surface methodology have been conducted in order to improve the mass yield of essential oil.[5] Specifically; Galadima *et al*.[6] and Muazu *et al*.[7] investigated the optimization of the hydrodistillation operating parameters of *Eucalyptus tereticornis* and *Eucalyptus Citriodora* essential oils.

Recently *Eucalyptus genius* has gained special virtue because of its availability and its large therapeutic spectrum mainly against respiratory ailments. In fact during COVID-19 pandemic period, research worldwide precipitated the investigations on drugs suspicious to combat the virus amongst which *Eucalyptus* species and this incited us to deepen the research through the optimization of essential oil extraction methods.[8-11] Therefore, the current paper attempts to optimize the parametric hydrodistillation of essential oils from Algerian *Eucalyptus camaldulensis* leaves using the RSM, specifically the effects of extraction parameters (particle size, condensation flow rate, and extraction time) and their interactions on the extracted oil yield. The oil's composition was determined using gas chromatography in conjunction with mass spectrometry.

RESULTS AND DISCUSSION

Experimental yield and kinetics of hydrodistillation

The estimate of the essential oil yield obtained by hydrodistillation divulges a value between 0.34% and 0.52% (Table 1), where the higher yield (0.52%) was recorded for a particle diameter of 1mm, a condensation flow rate of 2.4 mL/min and an extraction time of 210 min. A yield of 0.48% was found at the following conditions: 1 mm, 3.4 ml/min and 180 min respectively.

As a whole our findings are in conformity with the literature. Similar results were stated by Nait[12] and Foudil[13] in peripheries of Tizi-Ouzou and Algiers, with yields of 0.42% and 0.33% respectively. However, Mehani reported a yield of 0.89% in a study carried out in Ouargla (arid region).[14] In Morocco, yield values between 0.84% and 1.4% were reported.[15]

Table 1

Experiment matrix and experimental yield values

Experiment	x_1:Particle size (mm)	x_2: Condensation flow rate (mL/min)	x_3: Extraction time (min)	(Y) Experimental yield (%)
1	1	1.4	180	0.42
2	2	1.4	180	0.38
3	1	3.4	180	0.48
4	2	3.4	180	0.41
5	1	2.4	150	0.45
6	2	2.4	150	**0.34**
7	1	2.4	210	**0.52**
8	2	2.4	210	0.39
9	1.5	1.4	150	0.36
10	1.5	3.4	150	0.38
11	1.5	1.4	210	0.41
12	1.5	3.4	210	0.46
13	1.5	2.4	180	0.42
14	1.5	2.4	180	0.43
15	1.5	2.4	180	0.43

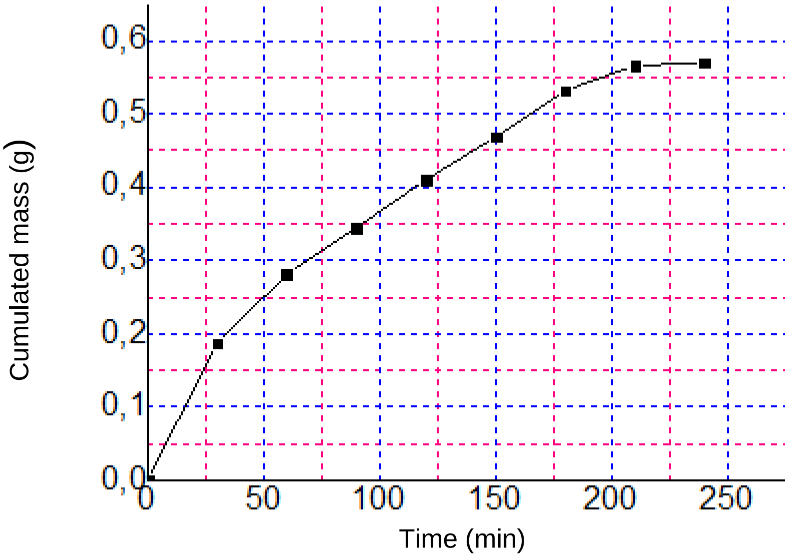

Fig. 1 – Variation of the cumulated mass of *E. camaldulensis* essential oil *vs.* extraction time.

The extraction kinetics involves tracking the evolution of the cumulative mass of oil extracted over time. The amount of oil extracted between 180 and 240 minutes represents approximately 4% of the total amount of oil extracted (Fig. 5). We can deduce that the appropriate time for hydrodistillation is around 180 minutes based on the concept of reducing extraction time and energy consumption.

Physicochemical properties and chemical composition of the extracted oils

E. camaldulensis essential oil is a yellow gilded liquid with a eucalyptol odor. The refraction index was measured at 20°C using an ABBE refractometer with a temperature indicator. The extracted oil's relative density and refraction index were 0.9087 and 1.4731, respectively. As shown in Table 2, all physicochemical properties are in accordance with marketing standards (New Directions Aromatics Inc, 2016) and the literature.

The GC/SM chromatogram of the essential oil is illustrated in Table 3 and revealed the presence of 35 compounds representing 99.99% of the total oil.

It comes out from this analysis that the oil of *E. camaldulensis* of Oum el Bouaghi is mainly composed of: *p*-Cymene (26.22%); spathulenol (16.71%); 1.8-Cineole (14.44%); Limone (7.34%), α-Terpineol (7.33%) and Terpinen-4-ol (3.14%).

The chemotype recorded for this essential oil is definitely different from that of the same species of the area of Ouargla. Indeed, Mehani[14] published that 1-methyl-4-(1-methylethyl)- benzene is the major constituent (19.96%), followed (+) spathulenol (17.05%), Sabinene, Bicyclo [3,1,0] hexane (4.36%), 2-cyclohexen-1-one, 4-(1methyleth (4.13%) and of 3-cyclohexen-1-ol (3.02%). Another study reported by Singh *et al.* showed that 1.8-cineole is the main component in various species of *Eucalyptus.*[18]

Table 2

Physicochemical characteristics of *E. camaldulensis* oil

Properties	Present study	New Directions Aromatics Inc., 2016	Reference 10	Reference 16	Reference 17
Aspect	Yellow gilded, Liquid	Colorless to pale yellow clear, Liquid	//	//	//
Odor	Eucalyptol fresh	Characteristic of *eucalyptol* odor	//	//	//
Relative density (g/mL) at 20°C	0.9087	0.8850–0.9280	0.9035	0.9245	0.9642
Refractive index at 20°C	1.4731	1.4550–1.4800	1.4769	1.4911	1.4648
Acid index	1.235	ND	1.1760	4.2075	//

ND: undetermined

Table 3

Chemical composition of *E. camaldulensis* oil

N°	Compound	TR	IK	(%)	N°	Compound	RT	KI	(%)
1	cis-Salvene	6.208	855	0.65	19	Pinocarvone	10.746	1162	0.57
2	α-thujene	6.346	935	1.95	20	Borneol	10.976	1168	0.21
3	α-Pinene	6.645	940	0.24	21	Terpinen-4-ol	11.239	1180	3.14
4	camphene	7.187	954	0.32	22	α-Terpineol	11.464	1190	**7.33**
5	sabinene	7.499	976	0.29	23	Methyl chavicol	11.903	1205	0.62
6	β-pinene	7.71	997	1.78	24	Phellandral	12.248	1237	0.84
7	α-Phellandrene	7.959	1005	0.29	25	Cuminic aldehyde	12.547	1251	2.8
8	p-Cymene	8.132	1025	**26.22**	26	Bornyl acetate	13.219	1288	1.96
9	Limonene	8.225	1032	7.34	27	Patchoulane	13.437	1380	1.69
10	1.8-Cineole	8.28	1033	**14.44**	28	β.-Bourbonene	13.944	1393	1.05
11	γ-Terpinene	8.843	1063	0.49	29	β-Caryophyllene	15.851	1427	0.05
12	α-terpinolene	9.072	1089	0.08	30	α-Humulene	16.457	1461	0.08
13	2-nonanone	9.539	1094	0.58	31	allo-Aromadendrene	16.578	1478	0.55
14	Linalool	9.789	1112	2	32	spathulenol	18.607	1576	**16.71**
15	α-Thujone	10.017	1117	0.67	33	Caryophyllene oxide	18.688	1586	1.57
16	β-Thujone	10.137	1120	0.93	34	T-Muurolol	18.829	1642	0.42
17	dihydro-Linalool	10.419	1134	0.12	35	13-Epimanool	19.115	1961	0.53
18	Camphor	10.603	1144	1.48		Total			99.99

KI: Kovats index on a HP5 MS column.

The above results are quite different from those obtained by Ben Marzoug[19] who inspected the essential oil of *Eucalyptus* in southern of Tunisia to find out 83 components with a prevalence of γ-eudesmol (25%), Spathulenol (16.1%), *p*-cymene (10.6%), 1.8-cineole (8.7%), *p*-cymen-8-ol (4.4%), cis-sabinol (4.2%), p-cymen-7-ol (4.0%) and verbenone (3.7%). Elaissil in his turn reported that the *Eucalyptus* cultivated in the center of Tunisia is dominated by 1.8-cineole (26.1%), α-Pinene (12.3%) and limonene (12.1%).[20]

The major components of *E camaldulensis* essential oil of Morocco around the city Maamora were found to be the 1.8-cineole (42.30%), α-pinene (28.30%), γ-terpinene (7.30%) and of *p*-cymene (6.50%). This composition is relatively comparable with that of the essential oil of *E camaldulensis* in Sidi Amira (Morocco), showing 1.8-cineole (50.69%), *p*-cymene (11.24%), α-pinene (11.23%) and γ-terpinene (1%).[21] Another study done in Cameroun revealed that 1.8-cineole (54.29%), *p*-cymene (14.59%) γ-terpinene (14.80%) and α-pinene (12.13%) are the main components.[22] A study in Taiwan revealed the appearance of two chemotypes: one is characterized by the prevalence of 1.8-cineole (29.6%),[23] whereas the other is marked by the codominance of α-pinene (22.52%), *p*-cymene (21.69%) and of α -phellandrene (20.08%).[24] In Spain, the study of Verdeguer[25] showed a composition dominated by spathulenol (41.46%), p-cymene (21.92%) and the cryptone (7.76%). Another study in Cyprus indicated a predominance of ethanone (25.36%) and 1.8-cineole (13.73%).[26] According to the above mentioned results, it is clear that the essential oil composition of *E. camaldulensis* in Oum El Bouaghi is quite different from that in various parts in the world.

Statistical validation of the model

The modeling results were tested statistically by ANOVA as displayed in Table 4.

The results of the analysis revealed that the experimental data well suited to the linear polynomial model. The results also showed that the model is noteworthy because the significance probability of the risk p-value is less than 0. 05,[16] implying that there is only a 0.01 % chance that the model will become invalid due to noise. Furthermore, the model does not exhibit a lack of adjustment because the significance probability of the risk of the term "defect of adjustment" (p-value=0.0686) is also close to 0.06, implying that the model is well adjusted.

The model can be used to navigate the entire space of the experimental domain. The examination of variance also specifies that the terms of the model x_1, x_2 and x_3 are significant (p-value <0.05). However, 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.

A coefficient of variation (CV) of 4.69% <10% indicates a high degree of precision between the experimental and predicted values.[27] Contrariwise, for a good model fit, the coefficient of multiple correlation R^2 value should be at least 0.80.[28] R^2 = 0.861 indicates that the experimental and predicted values of the adapted model agree well.

Table 4

Variance Analysis (ANOVA) for the quadratic model

Variance source	Summon squares	Ddl	Average square	*p*-value
Model	0.026	3	8.755E-003	0.0001
x_1 : Particle size	0.015	1	0.015	0.0001
x_2 : Flow of condensation	3.200E-003	1	3.200E-003	0.015
x_3 : Extraction time	7.813E-003	1	7.813E-003	0.0009
Residues	4.248E-003	11	3.862E-004	
Adjustment failure	1.900E-003	9	4.646E-004	0.0686
Pure error	6.667E-005	2	3.333E-005	
Total	0.031	14		
R^2	0.8610			
CV (%)	4.69			

* Significant terms, *p*-value <0.05.

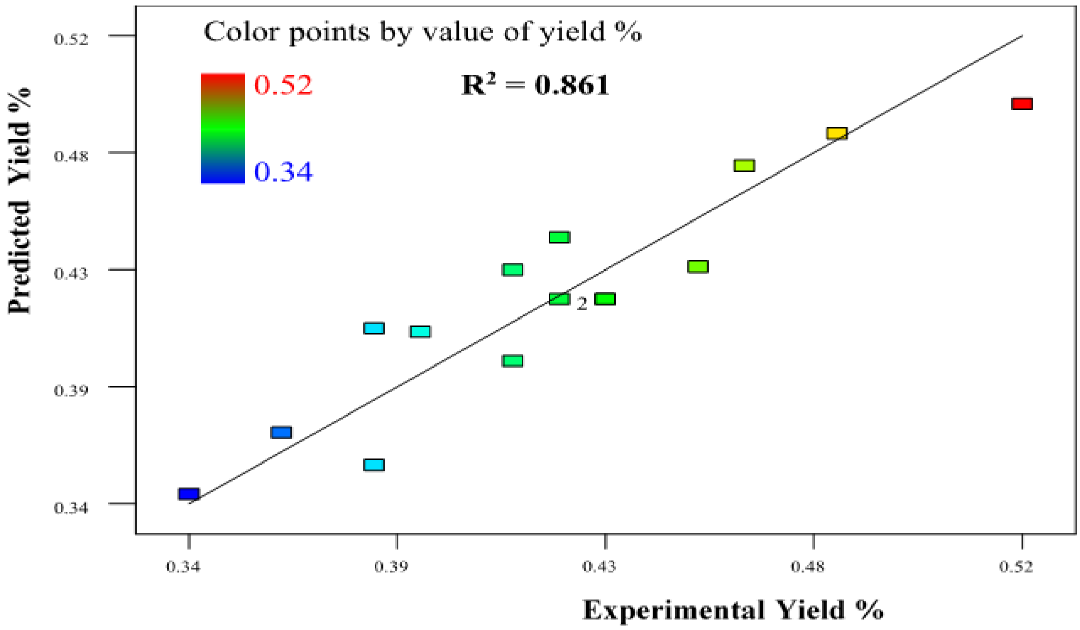

Fig. 2 – Predicted yield according to that experimental.

Table 5

Coefficients of regression of the linear polynomial

Coefficient	Value
β_0	+0.31442
β_1	–0.087500
β_2	+0.02000
β_3	$+1.04167.10^{-3}$

Fig. 2 depicts the curve representing the values of the expected yields as a function of the experimental yields. The graph shows that the point cloud is not far from the line of equation (y = x), indicating that the model has good descriptive quality.

Table 5 shows the values of the polynomial regression coefficients. The linear model correlated the mass yield of essential oil extracted from three plants by hydrodistillation according to the three considered parameters.

With all the probable arrangements of the three parameters, the selected mathematical model is given by the equation (04) following:

$$Y(\%) = +0.31442 - 0.087500\, x_1 + 0.02000\, x2 + 1.04167.10^{-3}\, x3 \qquad (04)$$

Optimization of the parameters by response surfaces and study of the desirability

The aim of this research is to increase the essential oil yield of *E. camaldulensis* extracted by hydrodistillation by optimizing the influential operational parameters. We obtained a maximum yield of 0.52% by carrying out the experimental design for this plant. The objective will thus be to find the optimums of operational parameters that result in a maximum yield greater than that of the experiment. In other words, we seek the optimal operating conditions that result in the greatest available value.

The representation of response surfaces in space can graphically illustrate how the studied parameters affect oil yield (3d). These surfaces demonstrated how the oil yield changed depending on two variables while keeping the third constant. According to the nature of the surfaces of the answer curve, Figs. 3a, 3b and 3c show the diagrams of the response surfaces of the effect of the parameter interactions on the oil yield.

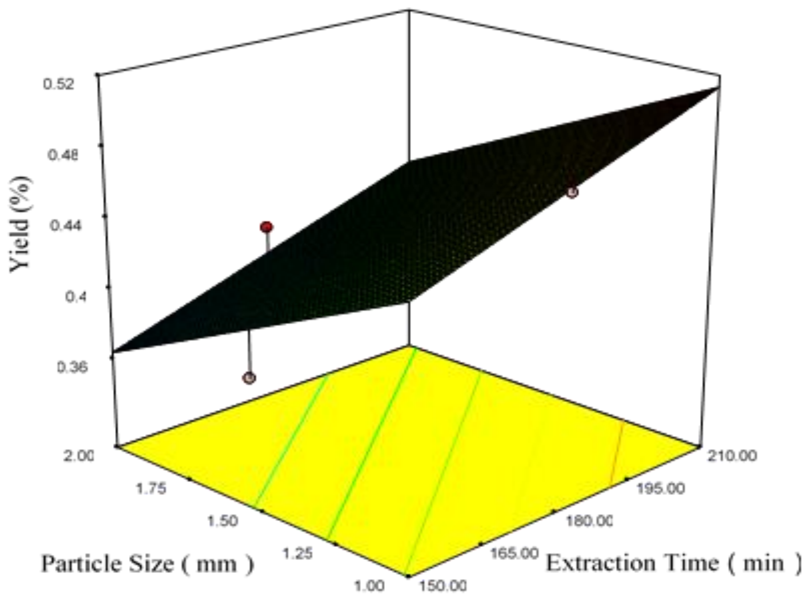

Fig. 3.a – Response surfaces demonstrating the effect of extraction time and particle size parameters on essential oil yield.

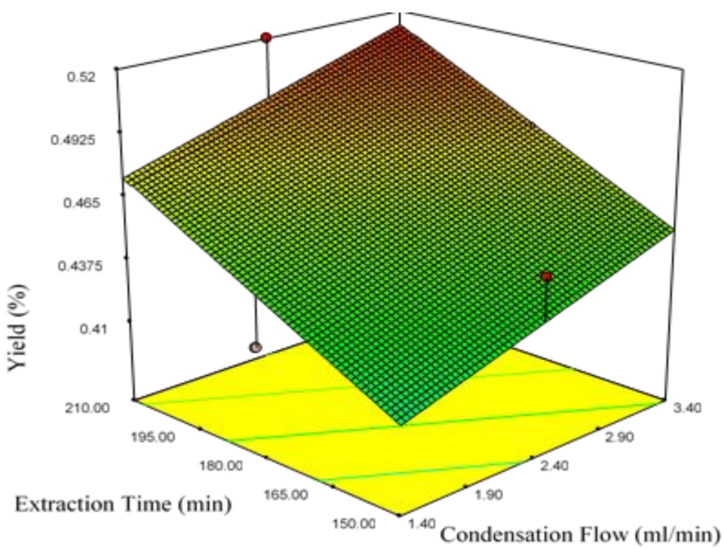

Fig. 3.b – Response surfaces demonstrating the effect of condensation flow rate and extraction time parameters on essential oil yield.

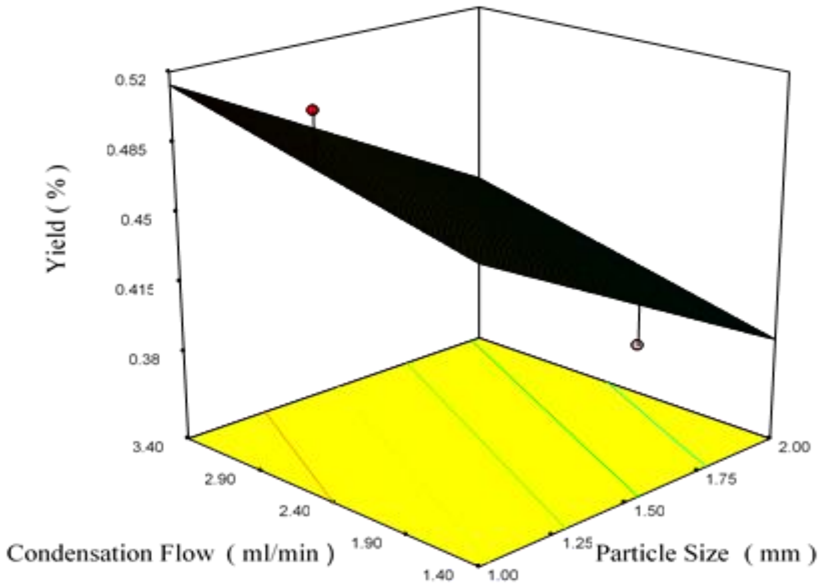

Fig. 3.c – Response surfaces demonstrating the effect of condensation flow rate and particle size parameters on essential oil yield.

Figs. 3a and 3b highlight the relationship between the extraction time and the yield of essential oils, demonstrating that regardless of the particle size and condensation flow rate, longer extraction time result in higher oil yields. Figs. 3b and 3c on the other hand show that an increase in condensation flow rate entrained an increase in yield. Figs. 3a and 3b show that, in contrast to the first two parameters, the reduction in particle size is perceived to increase oil yield Fig. 3c.

We were able to determine the areas of parameter variation necessary to achieve the desired yield by reading in the response surfaces. Therefore, an extraction time of 210 minutes, a condensation flow rate of 3.4 mL/min, and a particle size of 1 mm are needed to achieve an optimal yield of 0.52% (the highest accessible value).

The desirability study will enable to precisely define the heightened values of the three studied parameters.

Fig. 4 shows that, under the conditions of an extraction time of 210 minutes, a condensation flow rate of 3.4 mL/min, and a particle size of 1 mm, the maximum yield value can be achieved with a desirability of the order of 1.

These findings suggest that in order to achieve the desired yield, the extraction time must be increased toward the terminal shown in red to the right of the parameter's range of variation. Time clearly has an impact on the hydrodistillation process, and various authors have demonstrated this impact.[12]

The decrease in particle size is also accompanied by an increase in yield, which can be attributed to the secreting sites' deep mesophylle locations (endogenous sites).[29] In fact, crushing broadens the contact surface and facilitates water absorption into the plant's pores.

The maximum yield is indicated by the condensation flow rate of 3.4 mL/min. In fact, this phenomenon can be elucidated by the fact that an increase in temperature causes the condensation flow rate to increase, causing the pockets secretaries containing essential oil to burst.

EXPERIMENTAL

Plant material

The leaves of *Eucalyptus camaldulensis* were collected in spring 2016 in Oum El Bouaghi (a semi-arid region in the northeast of Algeria) at the coordinates: 36° 03' 34" North, 6° 58' 30" East, and an altitude of 841 m. The leaves were shade-dried, crushed, and passed through sieves with a vibrating system to recover particles with the desired diameters.

Methods

A Clevenger type apparatus was used for the hydrodistillation. 100 g of plant material was placed in a 2 L glass flask, followed by 1 L of water. The extraction time was measured from the appearance of the first drop of distillate at the refrigerant outlet. The recuperated essential oil was dried on calcium chloride ($CaCl_2$) and stored at 4°C in tightly sealed opaque glass vials. The following relationship expresses the yield of essential oil:

$$\text{yield of oil (\% weight)} = \frac{\text{weight of oil (g)}}{\text{weight of plant material (g)}} \times 100 \ldots\ldots\ldots\ldots \quad (01)$$

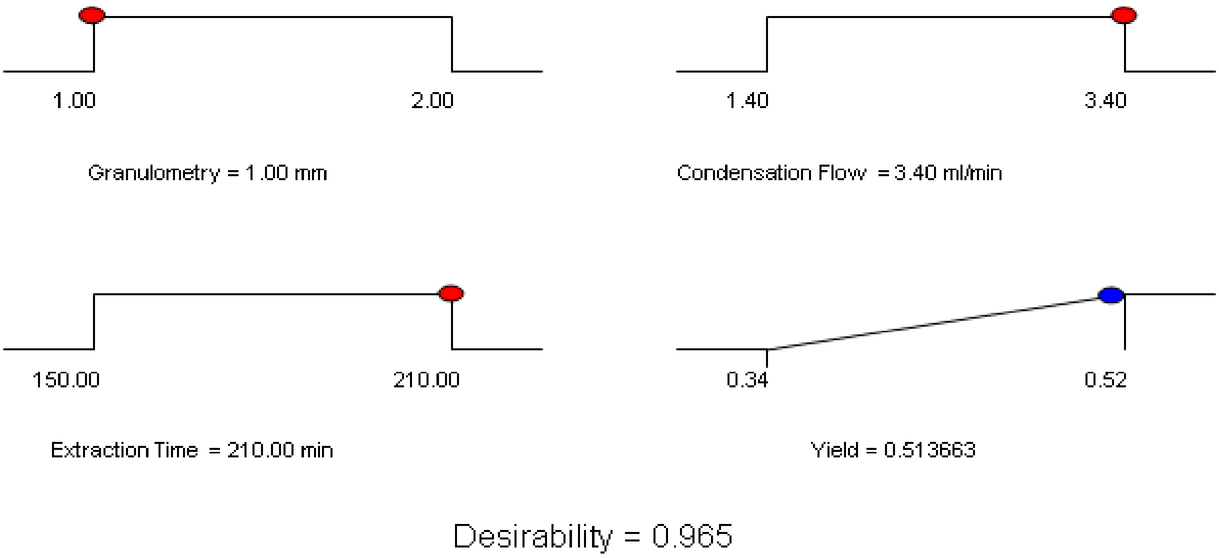

Fig. 4 – Prediction profiles of optimal hydrodistillation conditions.

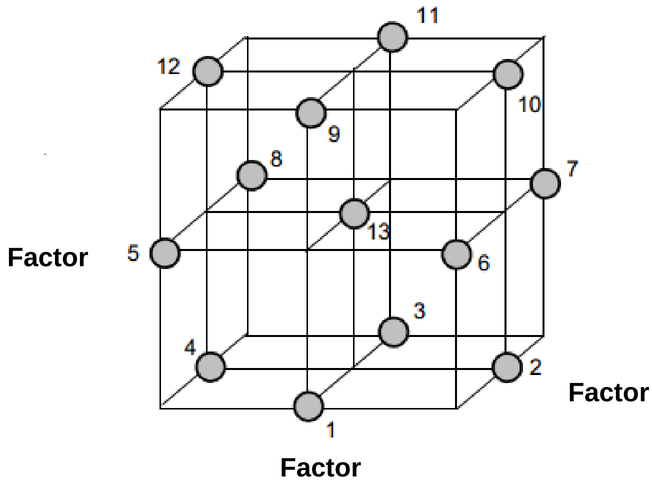

Fig. 5 – Design Box-Behnken for three parameters and three levels.

Table 6

Experimental domains of the considered parameters

Parameter	Symbol	Unit	Minimum	Average	Maximum
Particle diameter	x_1	mm	1	1.5	2
Condensation flow rate	x_2	mL/min	1.4	2.4	3.4
Extraction time	x_3	Min	150	180	210

GC/MS analysis

The chemical constituents of essential oil were identified using a gas chromatographic chain (HP 5890-SERIE II) outfitted with an apolar HP5 MS capillary column (30 m long, 0.25 mm internal diameter, and 0.25 m film thickness) coupled to a mass spectrometer (HP-MSD5972). The vector gas helium was introduced into the column at a rate of 1mL/min. In single mode, 1L of essential oil was injected. The injection and detector temperatures were both the same (280°C). The column temperature was programmed to rise from 40°C to 250°C at a rate of 3°C/min. The spectra were taken at 70eV. The spectral analysis was carried out by comparison to counterparts using WILEY275 spectrophotograms.

Experimental design and optimization by response surface methodology

The RSM, as a useful mathematical and statistical approach for modeling and investigating the effect of a number of quantitative variables on the response of interest, reduces the number of experimental trials while providing optimized and statistically significant results.[5] In this case, the RSM was used to evaluate the effect of particle size, condensation flow rate, and extraction time on essential oil mass yield, resulting in optimal extraction conditions. The experiments were designed and appraised using Design-expert® software version 7.0.0 (Stat-Ease, Inc., Minneapolis).

- **Choice of operating parameters**

The operating parameters considered to have an impact on the hydrodistillation process were particle size, condensation flow rate, and extraction time. The literature[30] was used to guide the selection of parameters and experimental domains. The three parameters chosen are continuous and quantifiable, that is: they can be monitored and can take any numerical value in the domain of interest.

- **Box-Behnken design**

Experimental designs are techniques for quantifying and optimizing the impact of various factors on a response in specific experimental areas. The method for obtaining the optimal response is described by a set of manipulative trials.[31] The Box-Behnken plan is a surface response method used to investigate the correlation between a set of experimental parameters and several response variables.[3] The Box-Behnken plan involves fewer executions in cases of three or four variables, and each factor requires only three levels rather than the five necessary by the other plans[32] unless the alpha is greater than the unity, which may be more factual and simple to achieve.[33]

The three-factor Box-Behnken plan needs only six factorial points and six axial points plus replicas at the midpoint; Fig. 5 denotes a three-factor Box-Behnken design.

The number of experiments is calculated using the equation (02):

$$N = 2k\,(k-1) + cp \;\ldots\ldots\ldots \qquad (02)$$

where k represents the number of factors and cp represents the number of replications at the central point. As a result, the Box-Behnken design is regarded as an effective option in the RSM and a viable alternative to composite design power stations.[34]

- **Fields and experiment matrix**

For three parameters and three levels, the experimental design is based on a Box-Behnken plan (Table 6). Using three central points, a total of 15 tests were generated (Table 1), with six factorial points and six axial points. A comparison between the Box-Behnken design and other response surface designs (central composite, Doehlert matrix and three-level full factorial design) has demonstrated that the Box-Behnken design and Doehlert matrix are slightly more efficient than the central composite design but much more efficient than the three-level full factorial designs.[35]

- **Mathematical model**

The hypothesized mathematical model is a 2nd order polynomial such as equation (03):

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

by taking Y like predicted response (oil theoretical yield), β_0 like constant of model; β_1, β_2 and β_3 coefficients of the linear terms; β_{11}, β_{22} and β_{33} coefficients of the quadratic terms; β_{12}, β_{13} and β_{23} coefficients of the interaction terms; and x_1, x_2 and x_3 as the parameters values.

- **Statistical analysis**

The variance test was used to validate the model (ANOVA). When the probabilities of the significance of the risk p-value are less than 0.05, the coefficients of regression and the test of adjustment defect are considered significant.[34] We will use other tools besides the analysis of variance, such as the coefficient of multiple determination (R^2) and the coefficient of variance CV. A CV value of less than 10% designates that the accuracy between the experimental and expected values is very high.[36]

- **Parametric optimization**

After determining the model and verifying its validity, we use response surfaces engendered in the experimental domain from the model equation to search for optimum working conditions that result to the desired response. These surfaces translate the variations in our response as a function of the two parameters while keeping the third one, that is, the graphical representation of the results (estimated model), in order to develop optimums from them. The “Desirability” function, which provides an exact optimal setting ranging from 0 to 1, is then used to find the optimal operating conditions with a certain level of compromise.

Indeed, the value 0 is assigned when the factors result in an unacceptable (unwanted) response, and the value 1 is assigned when the response signifies the full performance desired for the considered factors.[30]

CONCLUSION

The carried out study revealed that the hydrodistillation process of the essential oil of the plant species *Eucalyptus camaldulensis* can be optimized by using the response surface methodology. The GC/MS analysis revealed 35 constituents, accounting for 99.99 % of the oil. The main components are p-Cymene (26.22%), Spathulenol (16.71%), 1,8-Cineole (14.44%), Limonene (7.34%), Terpineol (7.33%), and Terpinen-4-ol (3.14%).

The expected yield stated by the linear model relies on the terms β_1, β_2 and β_3, and the response regression coefficients $R^2 = 0.861$ with the model value p-value = 0.0001 demonstrated that the developed model well suited to experimental data, including the optimal (maximum) value of the expected yield Y = 0.513663 % obtained by combining the following operating conditions: particle size (1 mm), condensation flow rate (3.4 mL/ min), and extraction time (210 min).

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