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The application of Plackett and Burman design in screening the parameters acting on the hydrodistillation process of Moroccan rosemary (*Rosmarinus officinalis* L.)

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ABSTRACT: Rosemary, *Rosmarinus officinalis* L., is an herb widely used throughout the world. It is, without doubt, one of the most popular plants in Morocco. For the purpose of examining the factors affecting the extraction of the essential oil of this plant by hydrodistillation, a screening study by Hadamard matrix type Plackett and Burman was conducted. After an appropriate choice of seven variables, sixteen experiments lead to a mathematical model of first degree connecting the response function (yield) to factors. Later than the realization of the experiments and data analysis, we concluded that six factors have a significant effect on the hydrodistillation process, namely: the extracting time, the individuality effect, the harvest period, the mass plant/water ratio, the drying of plant material and the temperature of heating. As for the cutting of plants, it presents a statistically negligible effect. Our study proved the great efficiency to applying the experiments design methodology for characterizing the operational parameters that affect the hydrodistillation. It is about a relevant and economical way which allows to obtain the maximum of information in a short time and, especially, with a minimum of experiments.

KEYWORDS: *Rosmarinus officinalis* L**.**, hydrodistillation, screening, Plackett and Burman design, yield.

1 INTRODUCTION

The aromatic and medicinal plants represent a considerable value to the Moroccan economy [1]. Among these plants, *Rosmarinus officinalis* L. which represents all alone an annual production in essential oil estimated by seventy tonnes [2] It is one of the most widespread plants of the *Labiatae* family which contains a great quantity of essential oil (up to 1%) [3]. The oil has tonic stimulant properties; it is used as a pulmonary antiseptic, a choleretic and a colagoguic, it has also stomachic, antidiarrhoic, antirheumatic properties [4] and it is a strong antioxidant [5]. Thus, it is essential to understand the factors' effects which act on the process of hydrodistillation, and see their close link with the improvement of essential oil yield. To achieve this objective, we preceded by the application of the statistical techniques such as the experimental designs to make this improvement increasingly accessible. These methods, which allow the experimentation in a minimum number of experiments [6], give the opportunity to screen the factors from the most to the least influential, and also optimize the operating conditions to achieve the best possible result.

The use of experiments designs in the analysis and the optimization of the extraction of essential oil process was evoked by several authors. Some have used other types of designs such as complete factorial design [7], [8], [9], and others have passed directly to the optimization by using response surface methodology [10], [11].

In this article, we have made a screening of the factors acting on the operation of hydrodistillation of *Rosmarinus officinalis* L.. We have used the screening designs which are best known for factors with 2 levels: the Hadamard matrices or Plackett and Burman design [12]. The experimentation highlights the effects of some factors on the studied response [8]. The choice of screening design for our study instead of the complete factorial design is based on the number of the studied factors which is seven. This number, which is higher compared to the number of factors of the studies that have used a complete factorial design, will cause an increase in the number of experiments (2^7 = 128 experiments for seven factors). As for the response surface designs, they are generally used for the optimization. In our case, a screening design of Plackett and Burman type is more advocated. The objective of this study of screening is to determine the most important factors acting on hydrodistillation process of studied plant with a view to a more detailed study of parameters optimization. The optimization study will come into perspective and it will be concerned only with factors which are considered influential on the hydrodistillation process.

2 MATÉRIALS AND MÉTHODS

2.1 VEGETABLE MATERIALS

Rosmarinus officinalis L. plants were collected from the National Institute of Medicinal and Aromatic Plants garden in Taounat.

2.2 EXTRACTION MATERIALS

The apparatus used for the hydrodistillation is of the type Clevenger [13] according to the method recommended by the French Pharmacopoeia [14]. The process operates at the atmospheric pressure and is equipped with a recycling system, permitting the mass plant/water ratio to be maintained at its initial level. During every experiment, plant material and water were placed, in determined proportions, in a one liter capacity glass ball. The mixture was heated to boiling temperature and the liberated steams crossed up the column and passed out of the condenser in a liquid state. At the end of the distillation, two phases were observed, an aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water. The obtained essential oil was dried over anhydrous sodium sulphate and was stored in the refrigerator at 4°C in dark glass bottles until use.

2.3 PLACKETT AND BURMAN DESIGN

Based on a process or phenomenon, the first problems which the experiments design can provide information about, are those of screening parameters. A screening study may be defined as a step for identifying rapidly, in a large number, those factors that are actually influential on a process in a fixed experimental field.

The most known matrices screening experiments are the matrices of Hadamard [15] or matrices of Plackett and Burman, for which the number of experiments is close to the numbers of the studied factors [16]. These designs are matrices with orthogonal columns composed only of values +1 or -1 [17]. These deigns are generally saturated and the mathematical model is a model without interactions [18].

The Plackett and Burman design is a fractional factorial design and the main effect (the contrast coefficient) of such a design may be simply calculated as the difference between the average of measurements made at the high level (+1) of the factor and the average of measurements made at the low level (-1). Contrast coefficients allow the determination of the effect of each factor. A large contrast coefficient either positive or negative indicates that a factor has a large impact on response; while a coefficient close to zero means that a factor has little or no effect [19].

2.4 EXPERIMENTAL DOMAIN OF FACTORS AND RESPONSES

The levels of factors were selected by taking into account the operating experimental limits, the literature data on hydrodistillation conditions [20], and the previous studies [7], [9], [10], [11], [21].

The factors that could affect the essential oil yield can be divided into two categories:

Continuous or quantitative factors:

- The extracting time varies between 150 and 210 minutes.
- The ratio between the vegetable material and water in the distillation flack: this factor varies between 1/12 and 1/4 (x 100g/100ml).
- The heating temperature of the flask which is directly related to the steam flow leaving the heated flask and hence in the flow of condensation. To test this parameter, two heating temperatures are used: 250 °C and 350 °C.

Qualitative factors:

- The harvest period of plant material which takes two modalities: the beginning of January and the end of June.
- The drying of the studied plants with both modalities: fresh plant and dried plant. The drying of the plant is done in the shade during eight days in a room in which the temperature is fixed at 25 °C.
- The effect of the change of the treated individual: we have studied two separate plants, so we have the modalities "Individual 1" and "Individual 2".
- The cutting of plants in small pieces before the hydrodistillation, which has the both modalities: entire plant and cut plant.

The table 1 shows the seven factors which were simultaneously studied to quantify the effect of each one on the hydrodistillation operation.

The studied response is an essential oil yield of *Rosmarinus officinalis* L. which results in the expression:

$$Y = Y_{HE}(\%) = \frac{M_{HE}}{M_s} \times 100$$

Where M_{HE} = essential oil mass (g), M_s = dry vegetal matter mass (g) and Y_{HE} = essential oil yield (%).

2.5 EXPERIMENTAL MATRIX

Since we have seven factors, the experimental design will be a matrix of eight experiments. For more precision, we have duplicated the chosen design, which thus leads to a matrix of sixteen essays. Three repetitions were carried out for each of the experiments.

2.6 MATHEMATICAL MODEL AND STATISTICAL ANALYSIS

The resulting mathematical model is a polynomial of order one as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + \varepsilon$$

With:

$Y = Y_{HE}$ (%) : yield of essential oil (response).

b_0 represents the average theoretical value of the response.

b_1, b_2, b_3, b_4, b_5, b_6 and b_7: Principal effects of coded factors X_1, X_2, X3, X_4, X_5, X_6 and X_7 respectively.

ε is an error term.

The statistical significance was checked by an F-test (analysis of variance) at the 95% significance level. The mean squares (MS) are obtained as follows:

$$MS = {}^{SS}/_{DF}$$

Where SS is the sum of squares of each variation source and DF is the respective degree of freedom.

The ratio between the mean square regression (MS_R) and the mean square residual (MS_r), $F_{ratio(R/r)}$, was used in order to establish whether the model was statistically significant [7]. The greater F value from unity adequately explains the variation of the data around its mean, in addition the estimated factor effects are real [22], [23].

The quality of fitting the first-order polynomial was also expressed by the coefficient of regression, R^2. The R^2 measures the proportion of total variation about the mean response explained by the regression, in fact it is the correlation between observed and predicted response and it is often expressed as a percentage by multiplying by 100 [25].

The model coefficients were considered significant for values of $p < 0.05$. The statistical significance of the model coefficients was determined by using the t-test (only significant coefficients with p-value < 0.05 are included). During this study, we have used the conception and the treatment software of experiments design Nemrodw [25].

3 RESULTS

The observed response values with different combinations of seven studied variables are listed in Table 2.

3.1 STATISTICAL VALIDATION OF THE USED MODEL

According to the table of the analysis of variance (Table 3), we can conclude that the regression explains the studied phenomenon well since the significance of the risk ($p_{value} < 0.0001$) is lower than 0.05. Obviously, the calculation of $F_{Ratio(R/r)}$ which is equal to 89.96, has shown that it is 25 times higher than the value of $F_{(0.05;7,8)}$ at the 95% confidence level which is equal to 3,5. As a practical rule, a model has a statistical significance if the calculated F value is at least three to five times greater than the theoretical value [26].

The correlation coefficient R^2=98.7% is very sufficient. The value gives a good agreement between the experimental and predicted values of the adapted model.

These results are clearer on the graph (Figure 1) which shows that the curve of the observed values in term of the predicted values has perfectly the shape of a line.

3.2 STUDY OF THE FACTORS EFFECTS

The main effects of the seven studied variables are shown in table 4. Each coefficient is associated with the values of t and p value. The values of t are employed to determine the significance of the regression coefficients of each of the parameters and the values of p are defined as the lowest level of importance leading to the rejection of the null hypothesis [7]. In general, the more the magnitude of t is larger, the more the value of p is smaller, and the more the corresponding coefficient term is significant [27]. The value of the constant b_0 is equal to 1.75. This value does not depend on any factor.

The results show also that only the factor b_7, which is related to the cutting of plants, doesn't have any influence on the hydrodistillation operation, since the other six factors have a risk's signification inferior than 0.05. These results are clearer on the two graphs of the factors' effects (Figure 2).

3.3 FITTED MODEL

The statistical mathematical model that represents the response in terms of the most influential variables is:

$$Y = 1.75 + 0.028\,X_1 + 0.015\,X_2 + 0.06\,X_3 - 0.032\,X_4 - 0.038\,X_5 - 0.012\,X_6 + \varepsilon$$

The experimental error (ε) represents the residual standard deviation of the response. It can be calculated from the replications of the experiments and it has been found that it has the value of 0.014.

4 DISCUSSIONS

4.1 STATISTICALLY NEGLIGIBLE PARAMETERS

4.1.1 CUTTING OF PLANTS

For the cutting, we estimated that this factor have a large influence on the yield because it permits important surface contact to occur between the plant material and water, therefore facilitating the essential oil extraction process. Figure 3 shows that there is a small increase in yield in passing from the entire to cut leaves, However, the results obtained (table 4) revealed a weak effect of cutting and shows that this factor doesn't have any influence on the hydrodistillation operation, since the risk signification (0.3) is more than 0.05.

4.2 Statistically significant parameters

4.2.1 Extracting time

Both graphs show that the time (factor b_3) is the most influential factor on the hydrodistillation operation with a coefficient of 0.06. It contributed all alone by 48.78% in the variability of the studied response. Obviously, the time influences directly the hydrodistillation operation and its impact on such operation has been demonstrated by several authors [7], [9], [10], [11], [21].

4.2.2 Individuality effect

The individuality effect is the second factor that affects the hydrodistillation operation with a negative coefficient of -0,038 and 20.35% of contribution in the variability of yield. The negative sign shows that the passage from the individual 1 to individual 2 in the realization of the experiments generates a decrease in the essential oil yield. This change from one individual to another can be explained by the stage of development of the plant organ (leaf, flower and fruit ontogeny) [28] or due to factors such as the plant age [29], [30], or even to genetic factors [30], [31].

4.2.3 Vegetable material / Water Ratio

The third factor that showed a significant influence on the yield is evidently the ratio between vegetable material and water in the distillation flask. This factor has a coefficient of -0.032 and a contribution of 14.31%. The minus sign indicates that the passage of the min level (the ratio 1/ 12) to the max level (the ratio 1/4) causes a fall in the essential oil yield. Several studies have shown that the increase in the ratio of vegetable material and water generates a decrease in yield [7], [8], [10]. This decrease can be explained by the fact that a high amount of plant material in the water prevents the water vapor formed in the bottom of the flask to be mounted in the tube of condensation which causes a reduction in yield [32].

4.2.4 Harvest period

The harvest period comes in the fourth position with a coefficient of 0.028 and a contribution of 11.2%. This result indicates that the yield becomes more important at the end of June compared to the beginning of January. These results are in accordance with Moretti's ones [33] that indicated that summer is the best period for better exploiting the rosemary essences, and also those reported by other authors [34], [35] which show that the best essential oil yields of *Rosmarinus officinalis* L. are found in June.

4.2.5 Drying

The fifth factor that had a significant effect on the studied response is the drying of plant with a coefficient of 0.015, a contribution of only 3.05%. Indeed, the study showed that the drying of the plant for a week causes an increase in the yield, which coordinates with the results found on rosemary [36], [37] which say that the drying in the shade induces a significant improvement in the obtained yield. This increase in the essential oil yield can be explained by an important physiological activity. The biosynthesis of essential oils continues and accelerates after the harvest of vegetable material in response to water stress [37], [38], [39].

4.2.6 Heating temperature

The last factor having a significant effect on the yield is the heating temperature, with a coefficient and a contribution of -0.012 and 2.12% respectively. Its growth leads to an increase in the flow of condensation. This increase has a negative effect on yield. Indeed, a big increase in the flow of condensation leads to a decrease of the residence time of the condensate in the decanter, and it does not leave time for essential oils to be separated from the liquid [40]. These results accord with those found by other authors [7], [8], [32].

5 TABLES AND FIGURES

Table 1. Parameter levels and coded values used in the experimental design

Factors	levels	Units	Coded variables	Coded levels
Harvest period	Biginning of January		X1	-1
	End of June			1
Drying	Fresh plant		X7	-1
	Dried plant			1
Extracting time	150	Min	X5	-1
	210			1
Mass plant/water ratio	1/12	x 100g/100ml	X4	-1
	1/4			1
Individuality effect	Individual 1		X2	-1
	Individual 2			1
Heating temperature	250°	°C	X6	-1
	350°			1
cutting of plants	Cut plants		X3	-1
	Entire plants			1

Table2. Experimental design of the hydrodistillation process of Rosmarinus Officinalis L. with the answers recorded for each experiment

N° of Experiment	Harvest period	Drying	Extracting time	Mass plant/water ratio	Individuality effect	Heating temperature	Cutting of plan	Y_{HE} (%)
1	1	1	1	-1	1	-1	-1	1,85
2	1	1	1	-1	1	-1	-1	1,87
3	-1	1	1	1	-1	1	-1	1,78
4	-1	1	1	1	-1	1	-1	1,8
5	-1	-1	1	1	1	-1	1	1,73
6	-1	-1	1	1	1	-1	1	1,7
7	1	-1	-1	1	1	1	-1	1,61
8	1	-1	-1	1	1	1	-1	1,63
9	-1	1	-1	-1	1	1	1	1,66
10	-1	1	-1	-1	1	1	1	1,67
11	1	-1	1	-1	-1	1	1	1,89
12	1	-1	1	-1	-1	1	1	1,89
13	1	1	-1	1	-1	-1	1	1,75
14	1	1	-1	1	-1	-1	1	1,77
15	-1	-1	-1	-1	-1	-1	-1	1,74
16	-1	-1	-1	-1	-1	-1	-1	1,72

Table 3. Analysis of variance for the fitted model

Source of variance	DF	Sum of squares	Mean square	F Ratio	p-value
R	7	0,118075	0,016868	89,96	<0,0001*
r	8	0,0015	0,000188		
Total	15	0,119575			
R^2	98,70%				

R: regression; r: residual; DF: degrees of freedom

Table 4. Estimated regression coefficients for the Plackett and Burman design

Name of parameter	Coefficient	Effect	Standard error	Ratio t	Prob
Constant	b0	1,75375	0,003423	512,3	<0,0001*
Harvest period	b1	0,02875	0,003423	8,4	<0,0001*
Drying	b2	0,015	0,003423	4,38	0,0023*
Extracting time	b3	0,06	0,003423	17,53	<0,0001*
Mass plant/water ratio	b4	-0,0325	0,003423	-9,49	<0,0001*
Individuality effect	b5	-0,03875	0,003423	-11,32	<0,0001*
Heating temperature	b6	-0,0125	0,003423	-3,65	0,0065*
Cutting of plan	b7	0,00375	0,003423	1,1	0,3

** Statistically significant for a level of confidence of 95%.*

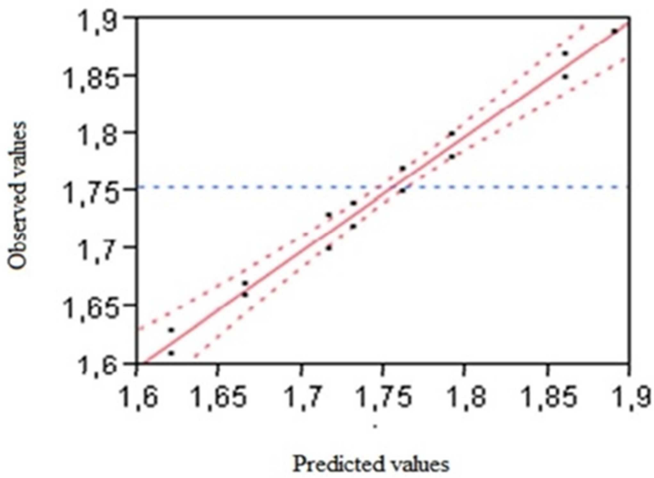

Fig. 1. Curve of the observed values in terms of the predicted values

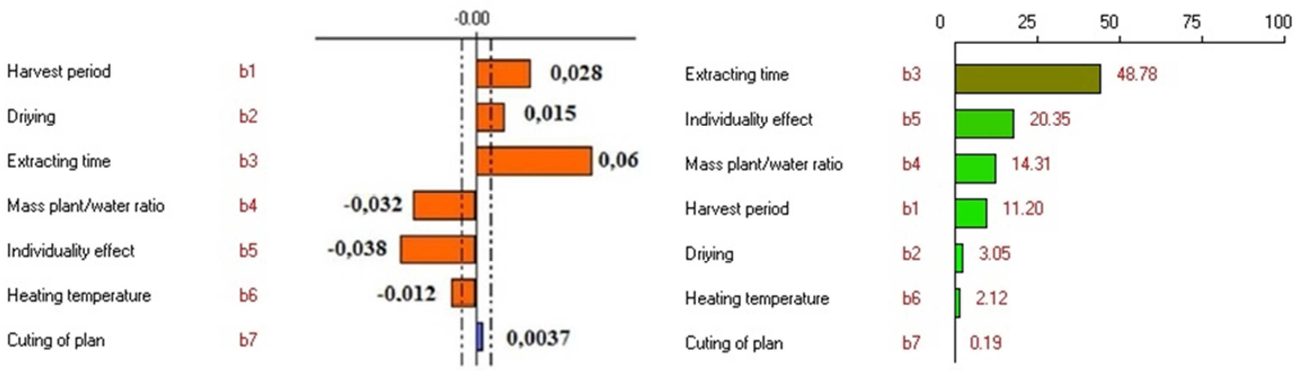

Fig. 2. On the Left graph of the factors effects, on the right graph which shows the percentage of contribution of each factor in the variation of the studied response

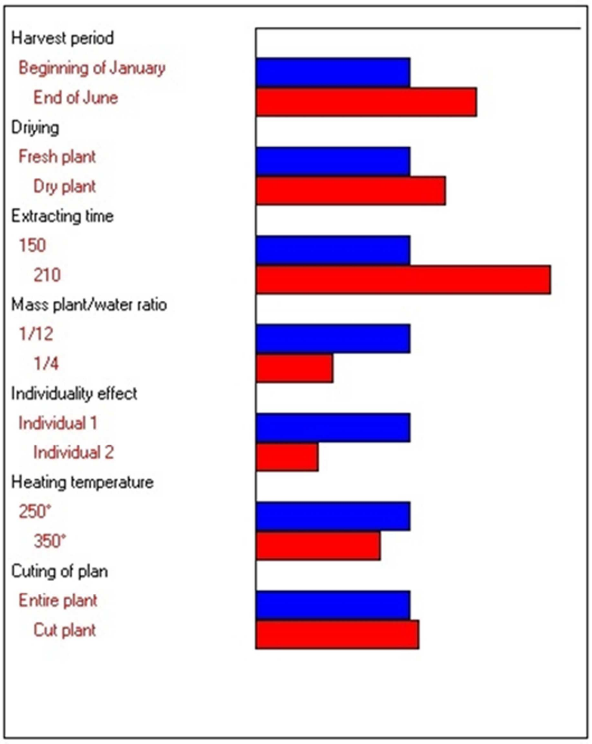

Fig. 3. Variation of the response (Y_{HE} (%)) in terms of the variation of each parameter

6 CONCLUSION

In this study, we were able to evaluate the effect of operating conditions on the essential oil yield of *Rosmarinus officinalis* L., by using as a strategy the experiments design methodology to get the maximum results with a minimum of experiments. The results show clearly that the experimental conception is an appropriate method for screening the factors affecting the hydrodistillation operation of the studied plant. The design of Plackett and Burman which was applied led to a first-order model whose statistically significant coefficients are related to the most influential factors on the response.

After the statistical validation of the obtained model, we passed to the analysis of effects. Thus, this model allowed, on one hand, to show that the Extracting time, the individuality effect, the ratio of vegetable material and water, the harvest period, the drying, and the heating temperature are all the factors which influence the hydrodistillation, having all a significant effect on yield (0.06, -0.038, 0.032, 0.028, 0.015, -0.012 respectively). On the other hand, it showed that although the cutting of the plants increases the exchange surface between water and the matter, this factor presents a negligible effect (approximately 0.003) and is not statistically significant on the essential oil yield.

To complete this study, an optimization study must be brought into play. It will seek the optimal operating conditions to have a better yield through using another type of experiments design developed for this type of study; namely, the response surface methodology by acting only on the continuous operating factors such as time processing, mass plant/water ratio, heating temperature, and the drying time.

REFERENCES

[1] A. Farah, A. Afifi, M. Fechtal, A. Chhen, B. Satrani, M. Talbi and A. Chaouch,, "Fractional distillation effect on the chemical composition of Moroccan myrtle (*Myrtus communis* L.) essential oils", *flavour and fragrance journal*, vol 21, pp. 351–354, 2006.

[2] M. Lahlou and R. Berrada, "Composition and niticidal activity of essential oils of three chemotypes of *Rosmarinus offcinalis* L. acclimatized in Morocco", *flavour and fragrance journal*, vol18, pp.124–127, 2003.

[3] G. Pintore, M. Usai, P. Bradesi, C. Juliano, G. Boatto, F. Tomi, M. Chessa, R. Cerri and J. Casanova, "Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica", *flavour and fragrance journal*, vol 17, pp.15–19, 2002.

[4] P. Oury , *Encyclopédie des Plantes et Fleurs Médicinales*, Editions Alliance, Paris, 1984.

[5] N. Erkan, G. Ayranci and E. Ayranci, “Antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, blackseed (Nigella sativa L.) essential oil, carnosic acid, rosmarinic acid and sesamol”, *Food Chemistry*, vol 110, pp.76–82, 2008.

[6] S. Zeboudj, N. Belhanèche-Bensemra and R. Belabbès, “Use of surface response methodology for the optimization of the concentration of the sweet orange essential oil of Algeria by wiped film evaporator“, *Journal of Food Engineering*, vol. 67 , pp. 507–512, 2005.

[7] A. H. Ammar, F. Zagrouba and M. Romdhane, “Optimization of operating conditions of Tunisian myrtle (*Myrtus communis* L.) essential oil extraction by a hydrodistillation process using a 2^4 complete factorial design“, *Flavour Fragrance Journal*, vol 25, pp. 503–507, 2010.

[8] T. Silou, M. Malanda and L. Loubaki, “Optimisation de l’extraction de l'huile essentielle de *Cymbopogon citratus* grâce à un plan factoriel complet 2^3“, *Journal of Food Engineering*, vol. 65, pp. 219–223, 2004.

[9] E. L. Wognin, Z. F. Tonzibo, K. A. Toure and Y. T. N’guessan, “Contribution à l’optimisation de la distillation des huiles essentielles extraites des fleurs de *chromolaena odorata* l king & robinson grâce à un plan factoriel complet 2^4“, *Revue Ivoirienne des Sciences et Technologie*, vol. 15, pp. 23–37, 2010.

[10] Q. Tan, X. Kieu, N. Kim and X. Hong, “Application of response surface methodology (RSM) in condition optimization for essential oil production from *Citrus latifolia*“, *Emirates Journal of Food and Agriculture*, vol. 24, pp. 25–30, 2012.

[11] K. Mu’azu, I.A. Mohammed-Dabo and S.M Waziri, “Development of Mathematical Model for the Prediction of Essential Oil Extraction from *Eucalyptus Citriodora* Leave“, *Journal of Basic and Applied Scientific Research*, vol. 2 , pp. 2298–2306, 2012.

[12] R. L. Plackett and J. P. Burman, “The design of optimum multifactorial experiments“. *Biometrika*, vol. 33, pp. 305–325, 1946.

[13] J. Clevenger, “Apparatus for the determination of volatile oil“, .*Journal of the American Pharmacists Association*, vol. 17, pp. 345–349, 1928.

[14] Pharmacopée Française. 10th edn, Maison neuve, Paris (1983).

[15] K.J. Horadam, *Hadamard Matrices and Their Applications*. Princeton University Press, Princeton, New Jersey, 2007.

[16] M. Claeys-Bruno, M. Dobrijevic and M. Sergent, a*nalyse de sensibilite : comparaison entre les plans d'experiences et la methode monte carlo* in: 41èmes Journées de Statistique, Bordeaux, pp. 1-6, 2009.

[17] W. Tinsson, *Plans D’expérience: Constructions et Analyses Statistiques,* Springer, Berlin, 2010.

[18] J. Goupy, *Les plans d’expériences*. Revue MODULAD. Vol. 34, pp. 74-116, 2006.

[19] L. Levin, F. Forchiassin and A. “Viale, Ligninolytic enzyme production and dye decolorization by Trametes trogii: application of the Plackett–Burman experimental design to evaluate nutritional requirements“, *Process Biochemistry*, vol. 40 , pp. 1381–1387, 2005.

[20] L. Ganou, *Contribution à l’étude de mécanismes fondamentaux de l’hydrodistillation des huiles essentielles*. PhD Thesis, Institut Polytechnique de Toulouse , Toulouse, 1993.

[21] M. S. Galadima, A. S. Ahmed, A. S. Olawale and I. M. Bugaje, “Optimization of Steam Distillation of Essential Oil of *Eucalyptus tereticornis* by Response Surface Methodology“, *Nigerian Journal of Basic and Applied Sciences,* vol. 20, pp. 368–372, 2012.

[22] R.H. Myer and D.C. Montgomery, *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, Wiley, New York, 2002.

[23] G.E.P. Box, W.G. Hunter and J.S. Hunter, *Statistics for Experimenters: An Introduction to Design, Data Analysis and Model Building*, Wiley, New York, 1978.

[24] NR. Draper and H. Smith, *Applied Regression Analysis.* 3rd ed., Wiley, New York, 1998.

[25] NemrodW for Windows, experimental design software, 2000.

[26] S. J. Kalil, F. Maugeri and M. I. “Rodrigues, Response surface analysis and simulation as a tool for bioprocess design and optimization“, *Process Biochemistry*, vol. 35, pp. 539–550, 2000.

[27] K. Ravikumar, S. Krishnan, S. Ramalingam and K. Balu, “Optimization of process variables by the application of response surface methodology for dye removal using a novel adsorbent“, *Dyes and Pigments*, vol.72, pp. 66–74, 2007.

[28] A. C. Figueiredo, J. G. Barroso, L. Pedro and J.J.C. Scheffer, “Factors affecting secondary metabolite production in plants: volatile components and essential oils“, *Flavour Fragrance Journal,* vol. 23, pp. 213–226, 2008.

[29] M. Bourkhiss, M. Hnach, T. Lakhlifi and A. Boughdad, “Effet de l’Age et du Stade Végétatif sur la Teneur et la Composition Chimique des Huiles Essentielles de Thuya de Berbere“, *Technologies de laboratoire*, vol 6, pp.64–68, 2011.

[30] S. Duriyaprapan and E. J. Britten, “The Effect of Age and Location of Leaf on Quantity and Quality of Japanese Mint Oil Production”, Journal of Experimental Botany, vol 33, pp.810–814, 1982.

[31] F. A. Bekkara, L. Bousmaha, S. A. T. Bendiab, J. B. Boti and J. Casanova, "Composition chimique de l'huile essentielle de *Rosmarinus officinalis* L. poussant à l'état spontané et cultivé de la région de Tlemcen", Biologie & Santé, vol 7, pp.6–11, 2007.
[32] J. Rabesiaka, R. Pierre and B. Razanamparany, "Optimization and Extrapolation to Pilot Scale of Essential Oil Extraction from *Pelargonium Graveolens,* by Steam Distillation", *Journal of Food Processing & Technology*, vol. 04, pp. 2–7, 2012.
[33] M.D.L. Moretti, A.T. Peana, G.S. Passino and V. Solinas, "Effects of Soil Properties on Yield and Composition of *Rosmarinus officinalis* Essential Oil", journal of essential oil research, vol 10, pp. 261–267, 1998.
[34] Y. Pan, H. Bai, H. Li, C. Jiang and L. Shi, "Effect of Location, Harvest Season and Plant Age on Chemical Composition and Antibacterial Activity of Essential Oils from *Rosmarinus officinalis"*, Chinese Bulletin of Botany, vol 46, pp. 625–636, 2012.
[35] M. G. Miguel, C. Guerrero, H. Rodrigues and J. Brito, *Essential oils of Rosmarinus officinalis L., effect of harvesting dates, growing media and fertilizers* in the 3rd IASME/WSEAS International Conference on Energy, Environment, Ecosystems and Sustainable Developmen, pp. 65–70, Greece, 2007.
[36] K. Jalal, M. Rahmat, F. T. Mohammad and N. Himan, "Influence of Drying Methods , Extraction Time , and Organ Type on Essential Oil Content of Rosemary (*Rosmarinus officinalis* L.)", Nature and Science, vol 7, pp. 42–44, 2009.
[37] B. Benjilali, *le matériel végétal et l'extraction* in : Huiles essentielles : de la plante à la commercialisation – Manuel pratique, Corporation Laseve, pp. 61–80 , Université Du Québec, Chicoutimi, 2005.
[38] S. Zrira, B. Benjilali and G. Lamaty, "Effet du séchage à l'air libre des feuilles d'*E. camaldulensis* sur le rendement et la composition de l'huile essentielle ». Actes de l'institut Agronomique et Vétérinaire, vol 15, pp. 27–35, 1995.
[39] M. Bourkhiss, M. Hnach, B. Bourkhiss, M. Ouhssine, A. Chaouch and B. Satrani, "Effet de séchage sur la teneur et la composition chimique des huiles essentielles de *Tetraclinis articulata* (Vahl)", *Agrosolutions*, vol. 20, pp. 44–48, 2009.
[40] N. Herzi, Extraction et purification de substances naturelles: comparaison de l'extraction au CO2-supercritique et des techniques conventionnelles, PhD Thesis, Institut National Polytechnique de Toulouse, Toulouse, 2013.