J. Mater. Environ. Sci. 6 (4) (2015) 942-948 *Haloui et al.*
ISSN : 2028-2508
CODEN: JMESCN

Application of Plackett-Burman design in the essential oil extraction by hydro-distillation process of *Pistacia lentiscus L.* leaves

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Received 8 May 2014; Revised 24 September 2014; Accepted 24 September 2014
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Abstract
In this research, a screening approach permitted the evaluation of the effects of 5 parameters on *Pistacia lentiscus L.* essential oil extraction via hydrodistillation process. Plakett and Burman design is used and because of it we were able to identify the most influential factors and evaluate them according to their importance and influence on the essential oil yield. The effect factors are 53 %, 23.12 %, 17.47 %, 5.25 % and 1.16 % for processing time, individuality, moisture, ratio of plant material / water and division of plant material respectively. The last one revealed that it has a negligible effect on the studied response.

Keywords: Extraction, Hydro-distillation, Essential oil, *Pistacia lentiscus*, Screening, Plackett-Burman design

1. Introduction
Pistacia Lentiscus L. belongs to the family Anacardiaceae, also called pistachio mastic or mastic tree. In Arabic, it is called: Derou, Tadis or Meska and in Berber: Tru, Imeket or tiket [1]. It is an evergreen shrub, dioecious, highly branched, up to 3m or tree up to 6m, grayish bark, younger reddish branches. Leaves alternate, odd pinnate, broadly winged rachis. Unisexual flowers, Small drupe, red to black [2].

Originally from the Mediterranean basin, the mastic tree grows wild in forests, scrubs the plains and low mountains [1] and all kinds of soil [2]. The therapeutic qualities of this species have been known since antiquity, when the Egyptians used the putty pistachio mastic for embalming [3].

P. lentiscus L. is the main species producing oleoresin which has an immense economic and pharmaceutical importance [4]. This resin has anti-inflammatory and antimicrobial effects and may be beneficial in the treatment of gastric and duodenal ulcer [5]. The essential oil of mastic tree has been shown to have anti-bacterial effects [6;7], Antifungal [8], Antioxidants [9] and insecticides [10]. It is also used in cosmetics, perfumes and as a flavoring in food preparations [11]. In Morocco, or mastic grows wild [12], it is used mainly in the form of dried plants and essential oils. To ensure the profitability of the essential oil mastic production, research strategies should be referred to sustainably optimize resources. Thus, improving the essential oil yield is a prime necessity, it becomes more accessible through the application of statistical techniques such as technical design of experiments, which the goal is to provide some experience by variations of all factors simultaneously to obtain maximum information [13;14].

In this study, we used the Plackett and Burman design based on Hadamard matrices [15] to a screening of the acting factors on *P. lentiscus L.* essential oil extraction by hydro-distillation process.

The experiment will highlight the effects of some factors on the studied response to detect what are the factors that seem to be the most influential.

2. Materials and methods
2.1. Plant material
Two sheets of individuals of *P. lentiscus L.* leaves were randomly collected from natural populations at the flowering stage during May 2013 in the Ifrane's forest at the region of Taounate in Morocco.

2.2. Extraction and analysis of essential oil

P. lentiscus L. leaves were submitted to hydro-distillation using a Clevenger- type apparatus [16]. 100g of plant material was extracted in each test. The moisture content of the plant material was determined by drying at 105 ° C for 4 hours [17]. The essential oil yields are based on dry plant material weight:

$$Y = \frac{M_{HE}}{M_S} \times 100$$

where :
Y : essentiel oil yield (%) ; M_{HE} : essential oil mass (g) ; M_S : dry vegetal matter mass (g).

The essential oil is dried over anhydrous sodium sulfate and stored in a glass vial and sealed opaque at 4 ° C in the shade. The essential oil was analyzed using Gas chromatography (GC) coupled to mass spectrometry GC / MS (Polaris Q ion trap MS), analyses were performed on a Hewlett-Packard (HP 6890) gas chromatograph (FID), equipped with a 5% phenyl methyl silicone HP-5 capillary column (30m x 0.25 mm x film thickness 0.25 µm). The temperature was programmed from 50°C after 5 min initial hold to 200°C at 4°C/min. Gas chromatography were as follows: N2 as carrier gas (1.8 ml/min), split mode was used (Flow: 72.1 ml/min, ratio: 1/50), temperature of injector and detector was 250°C,Final hold time was 48 min. The machine was led by a computer system type “HP ChemStation”, managing the functioning of the machine and allowing to follow the evolution of chromatographic analyses. Diluted samples (1/20 in methanol) of 1µl were injected manually.

2.3. Plackett and Burman design

The plan used for screening is a Plackett and Burman design. It is saturated for the first order model as the design matrix X configuration contains only the levels (-1 and +1).

This type of experimental design is a great practical interest because it combines minimum number of experiments (optimal cost) and minimum number of levels (facilitated changes in levels of various factors) [13].

These plans are often saturated. The mathematical model is then a model without interaction [18].

2.4. Study methods

2.4.1. Study parameters

Factor levels were chosen taking into account the operating limits of experimental apparatus and considering literature data about hydro-distillation conditions [19].

The experimental factors are:
- Processing time of hydro-distillation: that will vary between 60 min and 180 min.
- Ratio of plant material and water in the distillation flask: varied between 1/2 and 1/4 (g / ml).
- Moisture content was studied by both modalities: fresh plant and dried plant, drying plant is in the shade for 7 days.
- Individuality: we studied two different individuals designated 1 and 2.
- Division of the plant material: full leaves and cut leaves.

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

Factor	Variable Code	Number of levels	Levels	Code level
Individuality	X_1	2	Individual 1	-1
			Individual 2	1
Moisture	X_2	2	Fresh	-1
			Dry	1
Division of the plant material	X_3	2	Full	-1
			Cut	1
Ratio Plant material /water	X_4	2	½	-1
			¼	1
Processing time	X_5	2	60	-1
			180	1

2.4.2. Mathematical model

The Plackett-Burman design has permitted the response to be modeled by the first-order polynomial, which can be expressed as the equation:

$$Y = b0 + b1\,X1 + b2\,X2 + b3\,X3 + b4\,X4 + b5\,X5$$

Where Y is the response function (essential old yield %). X_1, X_2, X_3, X_4 and X_5 are the independent variables. b_0 is the average theoretical value of the response. b_1, b_2, b_3, b_4 and b_5 are the factor effects of X_1, X_2, X_3, X_4 and X_5 respectively.

2.4.3. Statistical analysis

The first-order polynomial regressed equations were established on the basis of the experimental data. Statistical significance was evaluated using the analysis of variance (ANOVA) and $p<0.05$ was considered as [20]. The analysis of results was performed with statistical and graphical analysis software Nemrodw [21].

2.4.4. Experimental matrix

The Plackett and Burman design with 8 experiments for 5 factors were carried out as a screening approach to find the significant factors affecting the response. It gave us an experimental matrix in eight tests, that is the translation of the coded variables shown in table 2 with the parameter levels and 8 tests described by the experimental matrix are conducted.

Table 2: Experimental matrix

Number Test	X_1	X_2	X_3	X_4	X_5
1	1	1	1	-1	1
2	-1	1	1	1	-1
3	-1	-1	1	1	1
4	1	-1	-1	1	1
5	-1	1	-1	-1	1
6	1	-1	1	-1	-1
7	1	1	-1	1	-1
8	-1	-1	-1	-1	-1

2.4.5. Experimental design

The results of experimental design of *P. lentiscus L.* essential oil extraction by hydro-distillation process are given in the following table:

Table 3: Experimental design

number test	Individuality	Moisture	Division of plant material	Ratio Plant material / water	Processing time (min)	Response (%)
1	Individual 2	Dry	Cut	1/2	180	0.33
2	Individual 1	Dry	Cut	1/4	60	0.23
3	Individual 1	Fresh	Cut	1/4	180	0.25
4	Individual 2	Fresh	Full	1/4	180	0.30
5	Individual 1	Dry	Full	1/2	180	0.26
6	Individual 2	Fresh	Cut	1/2	60	0.20
7	Individual 2	Dry	Full	1/4	60	0.26
8	Individual 1	Fresh	Full	1/2	60	0.14

3. Results and Discussion

3.1. Chemical Composition of the Leaves Essential Oils:

The results of chromatographic analysis of *Pistacia lentiscus leaves* essential oils are presented in Table 4. In order to simplify the analysis of the results, only compounds with more than 0.5% abundance were selected. 40 constituents, which represented 87.79% of the total essential oils, were identified. The major constituents of the *Pistacia lentiscus* essential oils were caryophyllene (8.94 %) while sabinene (7.69 %), terpinen-4-ol (6.58 %), caryophyllene oxide (5.87 %), tricyclene (5.54 %), 3-carene (4.37 %), α-terpineol (4.08 %), p-cymene (3.67%), viridiflorene (3.09 %) and bornyl acetate (2.97%).

Similar findings compounds have been reported at different percentages by other authors [6, 7, 8, 10]. Environmental factors such as geography, temperature and collection period, etc., were considered to play a key role in the chemical composition of Essential oils [6, 7].

3.2. Statistics of the response

The experimental response values with different combination of the five variables used in the experimental design are listed in *table 3.* The regression coefficient is estimated to be acceptable (R^2 = 0.996). If the model allows you to find exactly the value of the measured responses, the R^2 is equal to 1, and the model provides answers calculated equal to the average. R^2 is a measure of model quality [22].

It implies that 99.6 % of the variability in the response could be explained by the model, which proves that it is good and very reliable.

Table 4: Main constituents (%) of leaves of *Pistacia lentiscus* essential oils

RT (min) [a]	Compound [b]	%
8.90	Tricyclene	5,54
9.47	Camphene	1,01
10.35	α- Phellandrene	1,57
10.53	trans-β-Ocimene	2,71
11.05	Sabinene	7,69
12.37	p-Cymene	3,67
12.49	3-carene	4,37
15.18	γ-Terpinene	0,77
15.60	α-Terpinolene	0,23
18.28	Terpinen-4-ol	6,58
18.60	p-Cymenene	0,59
18.85	α-Terpineol	4,08
19.83	Geraniol	0,69
20.67	trans-β-Ocimene	0,62
21.79	Bornyl acetate	2,97
22.10	Undecanone	0,97
23.29	Carveol	0,57
23.88	2-carene	0,71
24.71	α-Cubebene	1,24
25.18	β-Elemen	1,21
26.22	Caryophyllene	8,94
26.99	Benzoic acid, pentyl ester	0,69
27.35	α-Caryophyllene	2,25
27.48	Aromadendrene	0,76
27.96	α-Cadinene	2,08
28.17	α-Muurolene	2,76
28.42	Longicyclene	0,55
28.69	Isoledene	1,71
28.91	α-himachalene	0,51
29.15	γ-Cadinene	0,72
29.28	δ-Cadinene	2,28
29.46	Calamenene	0,96
31.35	aromadendrene	5,87
32.15	Caryophyllene oxide	0,81
RT (min) [a]	**Compound [b]**	**%**
32.33	4,4-Dimethyl-3-(3-methylbut-3 enylidene)-2 methylenebicyclo [4.1.0] heptane	0,57
32.62	α-Copaene	0,9
33.03	β-Guaiene	2,01
33.14	Selina-3,7(11)-diene	1,99
33.48	Viridiflorene	3,09
34.23	α-Longipinene	0,55

a: Retention time.
b: Compounds present in trace amounts (<0.5%) were not registered.

3.3. Effect studies of factors

The study of the effect factors on the response was performed using the analysis design procedure of NemrodW software. The b_i calculated values are listed in table 5.

Each factor who has a high coefficient is delimited by the vertical lines (Figure 1) that corresponds to the 95%¨limit indicating statistical significance.

Table 5: Model coefficients and their significations

b_i	Coefficient	Ecart-Type	t.exp.	Signification %
b_0	0.247	0.002	102.73	0.0134 ***
b_1	0.027	0.002	11.13	0.557 **
b_2	0.023	0.002	9.67	0.772 **
b_3	0.006	0.002	2.50	13.0
b_4	0.013	0.002	5.30	3.05 *
b_5	0.041	0.002	16.84	0.219 **

The individual effects of various variables can be discussed from the Pareto chart illustrated by figure 2.

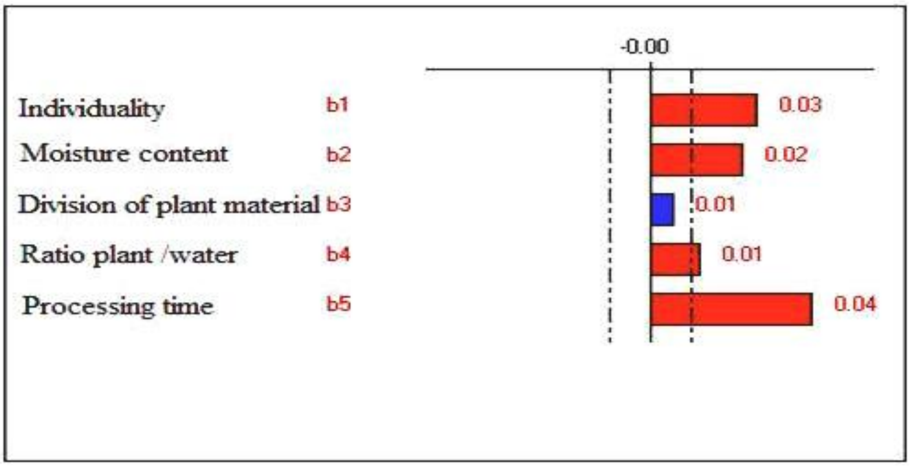

Figure 1: Graphical study of the factors effects

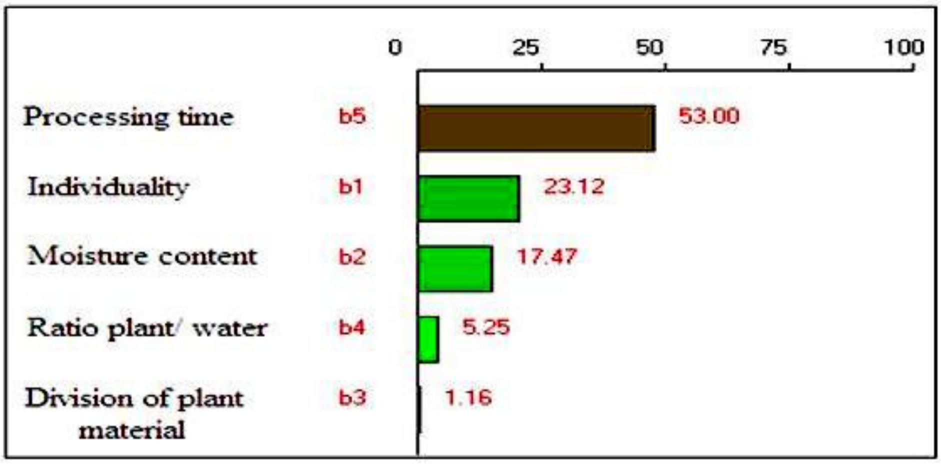

Figure 2: PARETO chart of individual factors effects

3.3.1. Individuality

Individuality has been revealed as a factor affecting the hydro-distillation process with a coefficient of 0.027 and a positive effect of 23.12% on the response. Leaves from individual 2 were the most recommended for a higher yield.

The change from one individual to another can be explained by the growth cycle of the individual [23], to factors such as the age of the plant [25] or even genetic factors [26].

3.3.2. Moisture content

Moisture has a significant and positive influence on the *P. lentiscus* essential oil yield with a coefficient of 0.023. Moisture content contributes to 17.47% on the variation of the response studied. Accordingly, the maximum level of this variable is recommended.

These results coordinate with results found in studies on other plants [26; 17; 27] this increase in the concentration of essential oils can be explained by an important physiological activity (enzymatic reactions). Biosynthesis of essential oils continues and accelerates after the harvest of plant material in response to water stress [28].

3.3.3. Division of the plant material

This factor didn't significantly effect on the response studied since the b3 coefficient was significant risk greater than 5%. Therefore, the minimum level of this variable is recommended.

3.3.4. Ratio plant material and water

This factor showed a significant effect on performance, with a coefficient of 0.013, and a positive effect on the studied response of 5.25% (Fig. 2).

Several studies on other plants showed that there was an effect of the relationship between the plant material and water on the essential oil yield [29; 30].

3.3.5. Processing time

This variable has a significant influence on the performance and has emerged as the most influential factor on the *Pistacia lentiscus* essential oil extraction by hydro-distillation with a coefficient of 0.041 and a positive effect on the response.

The variation of the extraction time contributes to 53% on the essential oil yield. Consequently, the maximum level of this variable is recommended.

The impact of the extraction time on this operation has been shown by several authors [29; 30].

3.4. Fitted model

These design permitted the response to be modeled by fitting first-order polynomial, which can be expressed the essential oil yield as the following equation:

$$Y = 0.247 + 0.027\,\mathrm{X1} + 0.023\,\mathrm{X2} + 0.013\,\mathrm{X4} + 0.041\,\mathrm{X5}$$

Conclusion

The implantation of Plackette-Burman design led to evaluate the effect to the operative conditions of *P. lentiscus L.* essential oil yield. In this study, the effects of differents parameters such as processing time, ratio plant material and water, division of the plant material, moisture content and individuality have been identified as influential in the hydro-distillation process. In the choice of the experimental field, it is recommended to maintain the high level for four influential factors. The factor who doesn't reveal a significant effect can be kept low.

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(2015); http://www.jmaterenvironsci.com