Global Journal of Medicinal Plant Research, 3(6) November 2015, Pages: 7-12

AENSI Journals

Global Journal of Medicinal Plant Research

ISSN:2074-0883

Journal home page: http://www.aensiweb.com/GJMPR/

Optimisation of extraction of phenolic counpoud and antioxydants in Algerian *Lavendula Stoicha* (Algeria, NW)

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ARTICLE INFO

Article history:
Received 28 September 2015
Accepted 15 December 2015
Available online 24 December 2015

Keywords:

ABSTRACT

Lavendula stoicha is medicinal Algerian plant known in the folk medicine. The present work is optimisation of the extraction of phenolic compound and antioxidant in *Lavendula stoicha* sepcies. The results are treated using response surface methodology test and we have found a significant models

To Cite This Article: Mustapha Mahmoud Dif, Fouzia Benali-Toumi, Mohamed Benyahia, Sofiane Bouazza, Abbes Dellal, Slimane Baha, Mounir chihab., Optimisation of extraction of phenolic counpoud and antioxydants in Algerian *Lavendula Stoicha* (Algeria, NW). **Glob. J. Med. Plant Res,** *3(6): 7-12, 2015*

INTRODUCTION

L. stoicha has analgesic, antiseptic, antispasmodic and antimicrobial properties. It contains oleanolic acid, ursolic acid, vergatic acid, b-sitosterol, a-myrin, lupeol, rythrodiol, vitexin and two longipinane derivatives (Ulubelen *et al*., 1988; Ulubelen and Olcay, 1989, Dif *et al*, 2015)

In the last few decades, the use of polyphenols of plant origin has grown intensively in food sectors over the synthetic antioxidants due to undesirable effects of the later on human and animal health. For food applications, crude phenolics are preferably extracted with ethanol as they are biocompatible and more economical

than any other solvents. The extracted substances can also be subjected for further downstream purification of any of the targeted compound of interest Conventional techniques to obtain polyphenols, such as heating, boiling, or refluxing, usually require several hours or even days for the extraction process and a large volume of solvent, and may result in a loss of flavonoids due to hydrolysis, ionisation and oxidation during extraction (Mun *et al* ,2014; Dif et *al* ,2015)

This can be very time-consuming and when interactions exist between the variables, it is unlikely to find the true optimum condition. Response surface methodology (RSM) is a very useful tool for this purpose, which was first introduced by Box and Wilson.Response surface methodology is also capable of effectively evaluating multiple factors and their interactions and is widely used in food analysis, chromatography condition optimization and prescription screening (Elksibi,2014).

The aim of this study is look in the effect of difference Temperature and time in the extraction of phenolic compound and antioxidant of stoicha, L.

MATERIAL AND METHODS

Extraction:

Leaves of *Lavendula Stoeicha* were extracted by maceration (23°C) and decoction (50°C) in different times intervals (15 min, 30min, 45 min) using water solvent. Each extract was analysed using sepctrophotmter assay to search the concentration of Total phenols, Total flavonoids and DPPH scavening method referring to Benhamou *et al.*, 2009.

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Experimental design:

To explore the effect of independent variables on the response within the range of investigation a central composite rotate design with two independent variables (X_1. Extraction time at tree levels 15, 30 and 45min, X_2. Temperature at two levels 23° and 50°) was performed. The phenol compound (TPC), flavonoids compound (TFC) and Antioxidant activity (AOA) was considered as the dependent variables or responses. For a central composite rotate design with two independent variables, 12 experimental runs are required. The experimental results were fitted to a second-order polynomial model, and the regression coefficients were determined. The quadratic model for predicting the optimal point was expressed according to (Equation 1):

$$Y_k = \beta_{k0} + \sum_{i=1}^{2} \beta_{ki} X_i + \sum_{i=1}^{2} \beta_{kii} X_i^2 + \sum_{i=1}^{2} . \sum_{j=i+1}^{2} \beta_{kij} X_i X_j \quad (1)$$

where β_{k0}, β_{ki}, β_{kii} and β_{kij} are constant regression coefficients of the model, while X_i, X_j are the independent variables.

Statistical analysis:

All data collected from extraction experiments were centred by using three parallel measurements of mean ± SD. The data of RSM were analysed using Statistical Analysis System (STATISTICA version 10, StatSoft. Inc. 2011. Tulsa, USA) and used to design central composite rotatable design and analyse the experimental data. The analysis of regression model (ANOVA) is also realized. P-value <0.05 was regarded as significant and P-value <0.001 was regarded as very significant.

Antioxydante activity was compared using two-way ANOVA and a post hoc Tukey test

RESULTS AND DISCUSSION

Results:

Polyphenol compounds:

The effect of extraction time and temperature on the total phenol compounds (TPC) and total flavonoids (TFC) are shown in Figure 1 and Figure 2 respectively. The relationship between each of TPC (Y_1) and TFC (Y_2), and extraction parameters (X_1 and X_2) are indicated in (Equation 2 and 3). After analysis, the regression model can be described by the following quadratic polynomial in terms of coded values:

$$Y_1 = 0.371 + 0.242\, X_1 + 0.004\, X_2 + 0.083\, X_2^2 - 0.005\, X_1 X_2 \quad (2)$$

$$Y_2 = 0.166 + 0.013\, X_1 + 0.002\, X_2 - 0.001\, X_2^2 + 0.001\, X_1 X_2 \quad (3)$$

The coefficients of the above (Equation 2,3) were calculated, and the linearity and quadratic effect of the treatment variables, their interactions and coefficients on the response variables were obtained by analysis of variance (ANOVA) (Table 1,2). For TPC, results suggested a good fit with the (Equation 2) because the model was acceptable at p = 0.0001 and adequate with satisfactory coefficient of determination (R^2) of 99.4%. The predicted model seemed to reasonably represent the observed values. Thus, the response was sufficiently explained by the model. The factor F-test value (2894.004) and p-value (p < 0.001) correspond to temperature (X_1), and F-test value and p-value corresponding to time extraction (X_2) were smaller 0.541 and (p = 0.48). These results suggested that the temperature was directly related to TPC. In addition, the value of R^2 (0.994) implied that the sample variations of 99.4% for the TPC was attributable to the independent variables, and the adjusted R^2 (R^2_{Adj}) of the equation was 0.962 (Table 1), suggesting an excellent correlation between the independent variables. The same results are shown for TFC and were detailed in table 2.

These results also suggested that the extraction concentration was primarily determined by the linear and quadratic terms. Also, both extraction conditions, showed that the linear effect was positive (Temperature: p<0.001 and Time: p=0.48) (Equation 2). The quadratic effect of extraction time was positive. The coefficients of interaction were insignificant. Furthermore, the predicted results, according to the model for TPC, were close to the observed experimental responses, indicating that the generated models adequately explained the data variation and significantly represented the actual relationships between the reaction parameters.

Table 1: Regression coefficient, standard error, and ANOVA for the regression model of TPC

Factors	SS	df	MS	F	P
Temperature (°C)(X_1)	0.702610	1	0.702610	2894.004	0.000000**
Time (min)(X_2)	0.000131	1	0.000131	0.541	0.489753
Time (min)(X_2^2)	0.018616	1	0.018616	76.677	0.000123**
X_1 by X_2	0.000230	1	0.000230	0.948	0.367788
Lack of Fit	0.002863	1	0.002863	11.792	0.013905*
Pure Error	0.001457	6	0.000243		
Total SS	0.725907	11			
R^2 = 0.994					
R^2_{Adj} = 0.990					

SS: sum of squares; df: degrees of freedom; MS: mean of squares; F: F-test values; P: P-value; R^2_{Adj}: adjusted R^2; (*) significant p < 0.05; (**) very significant p < 0.001.

Table 2: Regression coefficient, standard error, and ANOVA for the regression model of TFC

Factors	SS	df	MS	F	P
Temperature (°C)(X_1)	0.000050	1	0.000050	51.754	0.000365**
Time (min)(X_2)	0.000004	1	0.000004	3.933	0.094591
Time (min)(X_2^2)	0.002085	1	0.002085	2138.800	0.000000**
X_1 by X_2	0.000016	1	0.000016	16.145	0.006974**
Lack of Fit	0.000006	1	0.000006	6.474	0.043823**
Pure Error	0.000006	6	0.000001		
Total SS	0.002168	11			
R^2 = 0.994					
R^2_{Adj} = 0.991					

SS: sum of squares; df: degrees of freedom; MS: mean of squares; F: F-test values; P: P-value; R^2_{Adj}: adjusted R^2; (*) significant p < 0.05; (**) very significant p < 0.001

Through the Three-dimensional (3D) plots, it is very easy to understand the interactions between two variables and also to determine their optimum levels. The relationship between independent and dependent variables is illustrated in 3D representation of the response surfaces generated by the model for TPC and TFC. As shown in (Figure 1) Temperature exerted a quadratic effect on total phenol production, but extraction time only had a slight effect to the TPC, and the interactions between these two factors were not significant (p value of X_1X_2 = 0.367) (Table 1). The extraction yield was not significantly time-dependant and increased with extended temperature, especially from 40 to 55°C. The results indicated that the efficient extraction period for achieving maximum yield of flavonoids was about 55°C. The results have shown that increasing extraction temperature in the extraction process was better than prolonging extraction time for intervals less then 30min. However, for highest intervals (>30min), TPC increase when temperature was kept at levels upper then 40°C.

The effect of extraction time on the TFC is shown in Figure 2. Again, these results indicated that time have slight effects on the TFC, which is the same as the results in Table 2. The interaction effect was significant (p value of X_1X_2 = 0.0069). extraction temperature had little effect on the yield of flavonoids when the ratio of liquid to material was kept at low levels. As time increased to a high level, the TFC increased with an increase in extraction temperature.

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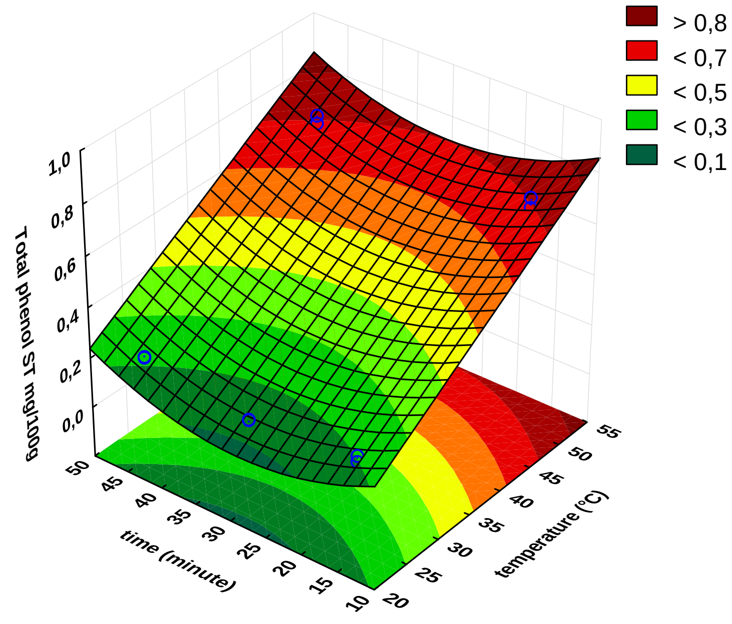

Fig. 1: Response surfaces plots for TPC extracted from *Lavendula Stoicha* extraction temperature (X_1) and extraction time (X_2)

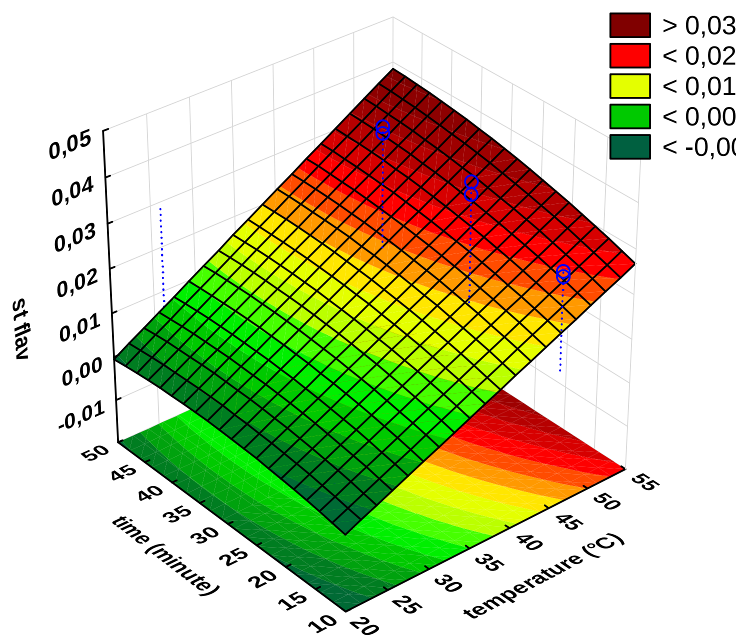

Fig. 2: Response surfaces plots for TFC extracted from *Lavendula Stoicha*. extraction temperature (X_1) and extraction time (X_2)

Antioxidant activity:

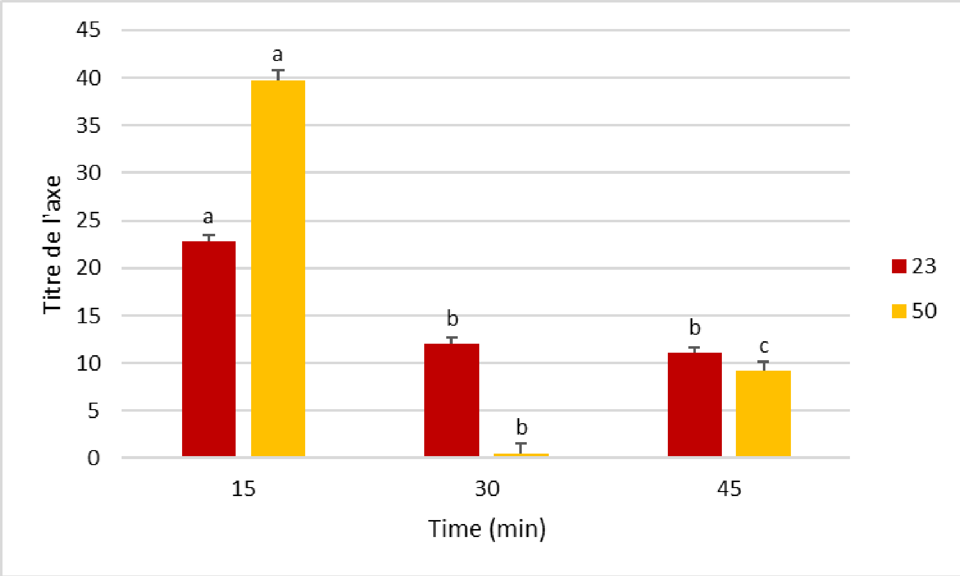

Fig. 3: Antioxidant activity

Different between groups with same letter is not statistically significant for the same level of temperature two way ANOVA analysis showed a significate different of antioxidant activity (reffering to temperature p=0.005) and (referring to time $p<0.001$) also a significate different in interaction between the extraction factors $p<0.001$)

Discussion:

The Effect of time and temprature in the yield is demonstrated in Table1. As can be observed, it is indicated that the colour strength increases with increase of extraction time. The effect of the extraction duration can be correlated with the higher contact time of solvent with powder which grasped more colouring component into solution but further increase resulted in decrease in colour strength which might be due to decomposition of colouring component at higher temperature with more contact time (Kubelka & Munck, 1931; Shaukat, Tanveer, & Rakhshanda, 2009; Spyroudis, 2000).

In fact, Heating also might soften the plant tissue and weaken the phenol–protein and phenol–polysaccharide interactions, thus more phenolic would distribute to the solvent.Despite the positive effects of higher temperatures on the phenolic

extraction, this cannot be increased indefinitely by cause phenolic stability (Wang *et al*., 2007). Apart from environmental factor, reduction of phenolic content with longer extraction time could also be due to the endogenous enzymes in plant tissues which destroyed the phenolic compounds in the extract (Kuljarachanan, Devahastin, & Chiewchan, 2009).

The extraction of antioxydant from the plant sources depends on number of factors like Temperature, Time, solubility of the phenolic acids present, the interaction of these substances with other plant compounds which consequently could lead to development of newer complexes that may be soluble or insoluble in a given solvent.

Conclusion:

The extraction of polyphenols from *lavandula stoicha* plant sources depends on a number of factors. In the present study, it was observed that variation in temperature and time had adverse effects on apparent phenolic content, superoxyde hydrogene and DPPH activity in the leaves of three *lavandula stoicha*. however, their combined interaction had a positive effect on the antioxidant activities.

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