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Original Research Article

Optimization of combination of steam distillation and solvent extraction of *Artemisia campestris* essential oil using RSM

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Extraction of essential oil (EO) from *Artemisia campestris* L. leaves using a combination of solvent extraction and steam distillation was investigated. A response surface methodology (RSM), in particular a Box-Behnken design was employed for the optimization of three parameters such as extraction time, liquid-solid ratio and extraction number for the achievement of high yield of extraction by solvent. The optimum extraction conditions were: extraction time of 2h, extraction number equal to 4 and ratio of liquid/solid of 6.5/1 (mL/g). Using these optimal parameters values, the mean obtained value of yield was 26.74 ± 0.11%. EO obtained by the combination of solvent extraction (SE) and steam distillation (SD) yielded 1.3% where the essential oil obtained by only steam distillation (SD) yielded 0.88%. Antibacterial activity showed that essential oil possessed a high activity and significant inhibitory power against *Escherichia coli.* EO isolated by SE-SD showed reducing ability and exhibited antioxidant activity; it had the best reducing power compared to the essential oil isolated by SD.

Key words: *Artemisia campestris* essential oil, extraction, steam distillation, optimization, RSM.

INTRODUCTION

Artemisia is an important genus of family *Asteraceae* and tribe *Anthemideae*. It comprises about 500 species widely distributed over the world (Vallés et al., 2001; Salido et al., 2004; Singh et al., 2008). According to Rustaiyan et al. (2011), the genus *Artemisia* has always been of great botanical and pharmaceutical interest. It has been used in folk medicine to treat malaria, hepatitis, rheumatoid arthritis, asthma, cancer, inflammation, bacterial, viral and fungal infections (Wang et al., 2009; Adams et al., 2009). *Artemisia campestris* (*A.campestris*) is one of the 11 species of *Artemisia* found in the Algerian flora distributed in the arid and semi-arid regions (Quezel and Santa, 1963; Baba Aissa, 1991). It is a perennial under shrub, spontaneous, aromatic and medicinal plant widespread all over in Europe, in northern Africa and in northern part of the United States (Baba Aissa, 1991; Valant-Vetschera et al., 2003). The aerial part of the plant was used by the popular medicine as antihelminthic, desinfectant, cholagogue, tonic, hypotensif and antivenom (Akrout et al, 2010). In Algeria, it was used for the treatment of stomach and for the menstrual pain (Dob et al., 2005; Djeridane et al., 2006).

Despite the use of *A. campestris* in traditional medicine, there has been an interest for the antimicrobial and antioxidant effects of the leaves. It was demonstrated that the leaf extracts of *A. campestris* contain bioactive compounds (Sefi et al., 2010). Akrout et al. (2011)

evaluated the antioxidant and antitumor properties of *A. campestris* collected in southern of Tunisia. Their results showed that there is a positive correlation between the antitumor and antioxidant activities. Sefi et al. (2012) found that *A. campestris* acts as a beneficial agent against renal dysfunctions developed in alloxan-induced diabetes.

Essential oils (EOs) are usually extracted from plants material by different methods such as steam distillation, hydrodistillation, expression, simultaneous distillation-extraction and organic solvent extraction (Bousbia et al., 2009). The composition of oil may vary to a large extent depending on the used extraction methods (Cassel et al., 2009). In addition, these techniques can induce thermal degradation, losses of some volatile compounds, hydrolysis and water solubilization of some fragrance constituents (Faborde et al., 1996). These disadvantages can be avoided by using a combination of organic solvent extraction and steam distillation (SE-SD). This technology was used in the extraction of *Cuminum cyminum* essential oil for low cost, higher yield quality and shorter time (Li et al., 2009). SE-SD was also employed in the extraction of essential oil from discarded tobacco; it offered the advantages of higher extraction yield, higher tobacco aroma component concentration (Zhang et al., 2012). But, to our knowledge, there were no studies reported on the extraction of essential oil from Algerian *A. campestris* using SE-SD.

To achieve higher quality and higher yield of oil extraction from different plant materials, it is important to select adequate techniques and optimize appropriate extraction parameters. This objective can be achieved by the use of statistical methods of optimization such as response surface methodology (RSM). This method was used successfully by many researchers in the modelling and optimization of extraction conditions (Zaibunnisa et al., 2009; Galladima et al., 2012; Peng et al., 2012; Ranitha et al., 2014). Therefore, RSM allows evaluation of the effects of several process variables and their interactions on selected responses; it is a collection of mathematical and statistical techniques that are useful for the modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response. RSM also quantifies the relationship between the controllable input parameters and the obtained response surfaces (Prakash et al., 2001; Khuri et al., 2010).

The aim of the present study was to investigate the applicability of a combination of organic solvent extraction and steam distillation (SE-SD) for the extraction of essential oil from Algerian specie A*. campestris*. For this purpose, a response surface methodology (RSM) and in particular a Box-Behnken design (BBD) was employed in order to investigate the effect of operating parameters and to optimize the extraction process. This design is suitable for the exploration of quadratic response surfaces and generates a second degree polynomial model, which in turn is used in optimizing a process using a small number of experimental runs (Khadjeh, 2011; Tekindal et al., 2012; Zhu et al., 2013). In addition, the antioxidant and the antibacterial activities of the essential oils against some of the common pathogen bacteria were further examined.

MATERIALS AND METHODS

Plant material

Artemisia campestris (*A. campestris*) plant samples were collected in the Sub-sahara highland, located at 250 km south of Algiers, in the central part of North Algeria. Systematically, plant samples were identified and authenticated by the botanic laboratory of Agriculture department (University of Blida1, Algeria) and by the botanic laboratory of El Hamma (Algiers, Algeria) according to the Algerian flora and voucher specimen.

Chemical Reagents

The solvents used for the experimental extraction were hexane, petroleum ether, ethyl acetate and ethanol. They were of analytical grade and were for their majority purchased from Sigma-Aldrich (Steinheim, Germany) with exception for ethyl acetate purchased from Panreac Quimica (Barcelona, Spain).

Bacterial strains

The used micro-organisms were selected for their contamination. They were raised on the foodstuffs and their pathogenicity (negative Gram bacteria: *Escherichia coli* (ATCC 10536), positive Gram bacteria: *Staphyloccocus aureus* (ATCC6538) and *Klesbiella pneumoniae* (ATCC13883). The micro-organisms were supplied from Saidal (Pharmaceutical Company, Algeria).

Extraction by solvent

The dried leaves of *A.campestris* were ground in a blender to produce a powder (60 meshes). For each extraction, 30g of the sample was put into 500ml round bottle flask where a volume of 150 ml of solvent (hexane, ethyl acetate and ethanol) was added. The extraction was carried out in a water bath with a reflux pipe and a magnetic stirrer during 2h. The operation was carried out in triplicates. After extraction, the mixtures were vacuum filtered and the filtrates were vacuum evaporated to obtain the extracts. The extracts were weighed and the yield of *A. campestris* extract (R) was calculated by the formula:

$$R\ (\%) = (W_f/W_0)*100 \quad (1)$$

Where W_f is the quantity of *A. campestris* oleoresin and, W_0 is the quantity of *A. campestris* powder.

Box-Behnken experimental design

After determining the preliminary range of extraction variables through single-factor test, a Box-Behnken factorial design (BBD) was employed for optimization study. The effect of three independent variables: extraction

Table 1. Coded and uncoded levels of factors for the three factors Box-Behnken design

Level	Extraction time (H) X_1	Extraction number X_2	Liquid-solid ratio (mL/g) X_3
-1	2.0	2	5.00 :1
0	2.5	3	5.75 :1
+1	3.0	4	6.50 :1

time (X_1), number of extraction (X_2) and liquid-solid ratio (X_3) were evaluated using this design. The dependent variable or response (Y) was the yield of extract (%). The level of each variable was coded as: -1 (low), 0 (central point or middle) and +1 (high). The factor combinations are summarized in Table 1.

The relationship of the independent variables and the response was modelled using a second order polynomial equation of the response surface (Eq.2):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (2)$$

Where Y represent the yield of extract, X_1, X_2 and X_3 are the independent variables, β_0 is a constant; β_1, β_2 and β_3 are the linear coefficients; β_{12}, β_{13} and β_{23} are the interaction coefficients between the variables. β_{11}, β_{22} and β_{33} are the quadratic coefficients.

The construction of the experimental design and statistical analysis of the validation results were performed using statistical software Moode 6 (Umetrics).

Isolation of essential oil from Artemisia campestris extract

Artemisia campestris leaves extract was placed into a bottle flask related with a condenser. Steam was introduced into the flask. Essential oil was rapidly carried out from the extract using the steam (30 min). The oil collected was weighed and the yield (YEO) was calculated as follows:

$$YEO(\%) = (W_{EO}/W_{Art})*100 \quad (3)$$

Where : W_{EO} is the essential oil weight and W_{Art} is the *Artemisia* leaves powder weight.

Extraction of essential oil by steam distillation

Samples of *A. campestris* leaves were subjected to steam distillation for 3h. The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C for further analysis.

Antibacterial activity

The test of antibacterial activity was carried by the disc diffusion method using Mueller-Hinton Agar with determination of inhibition zones (IZ). The Muller Hinton (MH) agar was used as culture medium for the bacteria. The test was performed in sterile Petri dishes (90mm diameter) containing MH agar medium**.** A volume of 100 µl of standardized inoculum suspension containing 10^7CFU/mL (colony-forming units/mL) of tested microorganisms was spread on the MH agar. Sterile paper discs (6mm in diameter/Whatman) were impregnated with 20 µl of essential oil. These discs were deposited on the surface of the media previously inoculated on the surface using a bacterial suspension. After 24h of incubation at 37°C, diameter of inhibition zones was measured and expressed in millimeters (mm). Each assay in this experiment was replicated three times. Inhibition zone can be symbolized by signs according to susceptibility testing of strains via the essential oil: **1.** Not sensitive (-) or resistant: diameter<8mm. **2.** Sensitive (+): diameter comprised between 8 and 14 mm. **3.** Highly sensitive (++): diameter between 14 and 19 mm. **4.** Extremely sensitive (+++): the diameter>20 mm (Durraffourd et al., 1986; Elaissi et al., 2011).

Reducing power

The reducing power test is based on reduction of ferric to ferrous by the antioxidant potency. The reductive potential of *A. campestris* essential oil isolated by SD and by SE-SD was determined according to the method of Oyaizu (1986). Different concentrations of essential oil were mixed with 2.5mL of phosphate buffer solution (0.2M, pH 6.6) and 2.5 ml of potassium ferricyanide solution ($K_3Fe(CN)_6$, 1%). The mixture was incubated at 50°C for 20 min, and then 2.5 ml of trichloroacetic acid (10%) was added to the mixture which was then centrifuged at 300 rpm for 10 min. 2.5 ml of upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride solution (0.1%) and the absorbance was measured at 700 nm using an UV-Vis spectrophotometer**.** Ascorbic acid was used as positive control.

RESULTS AND DISCUSSION

Solvent extraction

Figure 1 shows the effect of solvent nature on the yield of extract. The results indicate that ethanol gave the best extract yield; so it was selected as the best solvent extraction with a yield of around 15.40%.

Model development and statistical analysis

Based on BBD, a total of 15 runs were performed (Table 2).

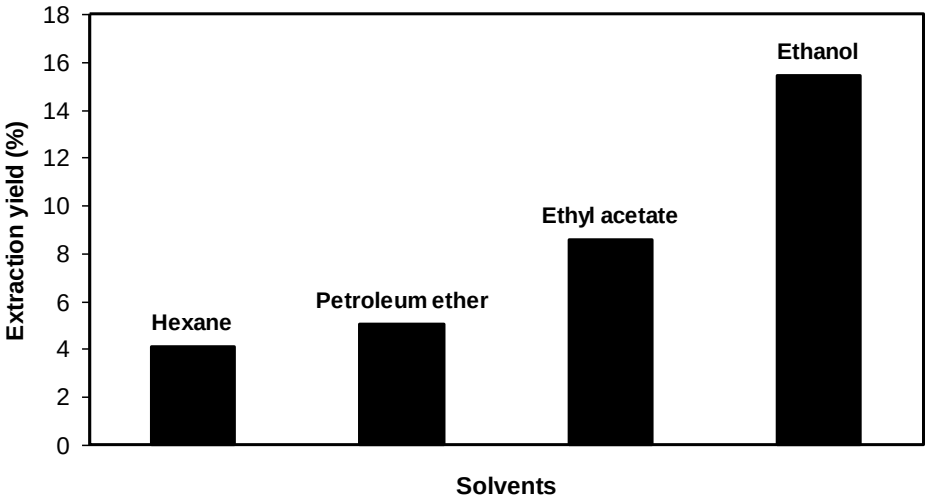

Figure 1: Effect of solvent nature on the yield of the extract

Table 2. Box-Behnken experimental design and results of extract yield

Exp No	X_1	X_2	X_3	Y
1	2.0	2	5.75	15.10
2	3.0	2	5.75	14.16
3	2.0	4	5.75	22.38
4	3.0	4	5.75	20.35
5	2.0	3	5.00	19.59
6	3.0	3	5.00	20.81
7	2.0	3	6.50	25.25
8	3.0	3	6.50	15.21
9	2.5	2	5.00	15.30
10	2.5	4	5.00	20.22
11	2.5	2	6.50	12.33
12	2.5	4	6.50	20.35
13	2.5	3	5.75	13.75
14	2.5	3	5.75	14.30
15	2.5	3	5.75	14.45

Generally, a second-order (quadratic) polynomial response surface model (Eq.2) can be applied to fit the experimental results obtained by the Box-Behnken design. The function representing the relationship between the operating parameters and the chosen response is illustrated by the following model (Eq.4):

$$Y = 14.025 - 1.473\ X_1 + 3.301\ X_2 - 0.347\ X_3 - 0.272\ X_1X_2 - 2.815\ X_1X_3 + 0.775\ X_2X_3 + 3.568X_1^2 + 0.403\ X_2^2 + 2.621\ X_3^2 \quad (4)$$

The observed and predicted values of yield were compared (Figure 2). It was noticed that the predicted and experimental values of yield (response) are correlated by a linear equation, confirming the accuracy and the quality of the obtained model. The predicted values match the experimental values reasonably well with a correlation coefficient R^2 of 97.2% and R^2(adj) of 91.0% for the response extract yield corresponding to a good model. In addition, the analysis of variance (ANOVA) was employed to estimate the statistical significance of the factors using the Fisher's statistical test (*F*) and probability (*p*). The *F*-ratio is the ratio of the mean-squared error to the pure error obtained from the replicates at the design centre. The significance of the *F*-value depends on the number of degree of freedom (DF) in the model. The *p*-value provides the exact level of significance of a hypothesis test. A *p*-value lower than 0.05 indicates that the model is significant. The results of ANOVA are summarized in Table 3. The analysis of these results shows that the model is statistically significant where the values of F_{model} and probability are respectively, 15.645 and 0.009.

Effects of factors on the extract yield and optimization

The effects of the process factors and their interactions on

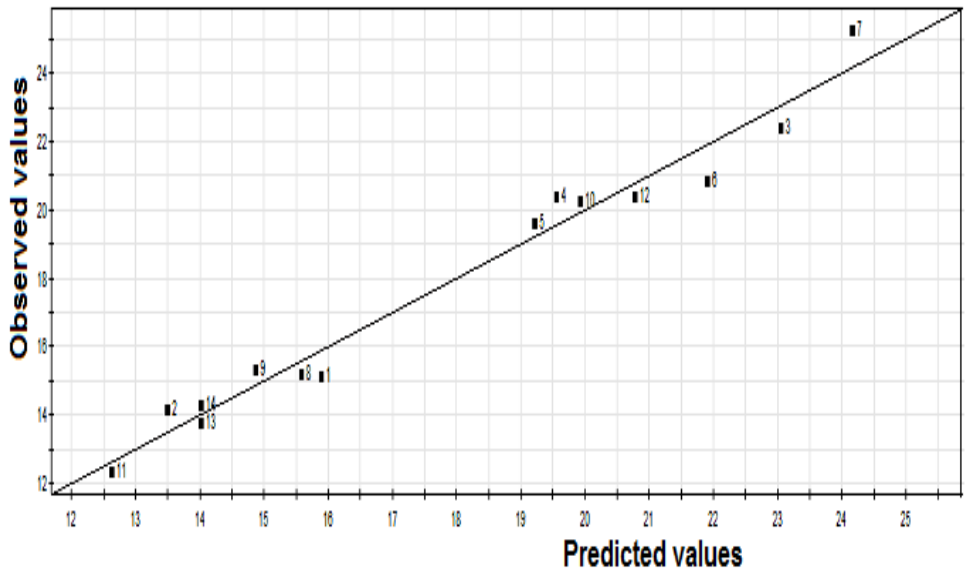

Figure 2: Relation between observed values and predicted values of extract yield

Table 3. Summary of the Anova model statistics

Turbidity removal	DF	SS	MS	F	P	SD
Total	14	4631.88	330.848			
Constant	1	4432.2	4432.2			
Total Corrected	13	199.674	15.3596			3.91913
Regression	9	194.159	21.5732	15.6457	0.009	4.6447
Residual	4	5.51543	1.37886			1.17425
Lack of Fit	3	5.36418	1.78806	11.8219	0.210	1.33718
Pure Error	1	0.15125	0.15125			0.388909

$R^2 = 0.972$; $R^2_{adj} = 0.910$; $Q2 = 0.56$

the yield can be deduced by simulation (Figure 3). These effects represent the coefficients in the surface response model (Eq.4). A positive value represents an effect that favors the extraction, while a negative value has an opposite effect.

The quadratic effect of extraction time (X_1^2) influences significantly and positively the extract yield, followed by the positive effect of the liquid/solid ratio (X_3^2), while the quadratic effect of extraction number (X_2^2) is lower. Simple effect of the extraction number (X_2) is the quantitative variable having the largest influence on the extract yield, followed by the simple effect of the extraction time (X_1) but they are in opposite directions. The effect of the factor X_2 is positive, this means that the response value increases when the factor changes from low to high level. The single-acting factor X_1 is negative; it means that the response drops when the factor goes from low to high level. The interaction between (extraction time-liquid/solid ratio) X_1-X_3 is larger; it negatively affects the response. Hence, the extract yield decreases when the two factors spend their lowest levels to high ones.

To determine the optimal levels of each variable for the solvent extraction, the three dimensional (3D) and contour (2D) plots were constructed according to Eq.4 and are shown in Figure 4, Figure 5 and Figure 6, respectively.

Figure 4 shows the effect of extraction time and extraction number on the yield of extract at fixed liquid/solid ratio. The extract yield decreases slightly with the increasing of extraction time and it reaches a minimum at the extraction time equal to 2h, after that it increases slightly. However, the extract yield increases rapidly with the increasing of the number of extraction and the maximum was obtained at the maximum value of the number of extractions. The interactions between the extraction time and the liquid-solid ratio are shown in Figure 5 at a fixed extraction number. The extraction time and extraction number showed a quadratic effect and, the maximum of yield was obtained at 2h and a ratio of 6.5/1 (mL/g). Figure 6 shows the effect of the extraction number and liquid-solid ratio on the yield of the extract at a fixed

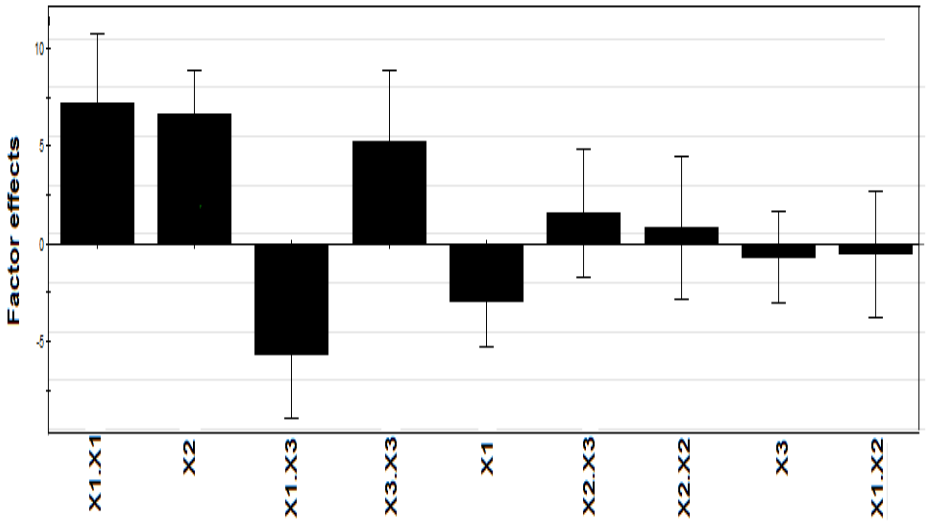

Figure 3: Effect of process factors and their interactions on the extract yield

extraction time of 2.5h. The results indicated that the liquid/solid ratio presents a quadratic effect on the response, however extraction number displayed a linear effect on the response and the maximum value of the yield was reached at a number of 4 extractions and a ratio of 6.5:1 (mL/g).

The optimal parameters obtained using BBD are as follows: extraction time of 2h, extraction number of 4 and liquid-solid ratio of 6.5/1 (mL/g). Experiments were performed using these optimal conditions and, the obtained mean value of the extract yield was 26.74% ± 0.110% (n = 3).

Extraction and isolation of essential oils

The essential oil extracted from dried leaves of *A. campestris* using steam distillation (SD) appears as a fluid, yellowish in colour with a yield of 0.88% (w/w). This value is higher than that obtained from the species of Boussaâda located in the south of Algeria (0.66%) (Belhattab et al., 2011) and that from Turkey (0.7%) (Baykan Erel et al., 2012).

In order to obtain a high yield of *A. campestris* essential oil, the oil was extracted by a combination technology of solvent and steam distillation (SE-SD) under the optimal conditions described above. The oil appeared as a yellow fluid and the yield of essential oil isolated from the *Artemisia* oleoresin was found around 1.3%. This value is higher than that obtained by using steam distillation (SD) alone.

Antibacterial activity

The *in-vitro* antibacterial activity of the essential oils obtained by SD and by SE-SD of *A. campestris* was evaluated by the measure of the diameter of inhibition zone around the discs containing the samples to be tested in the presence of three pathogenic bacteria (*Escherichia coli, Staphylococcus aureus* and *Klesbiella pneumoniae*) after 24 h of incubation at a temperature of 37°C. The qualitative results are presented in Table 4.

After incubation, it was noticed the appearance of zones of inhibition with different diameters around the discs impregnated with the volatile essential oil fraction. The existence of these zones can be explained by the fact that the bacterial strains: *Escherichia coli*, *Staphylococcus aureus* and *Klesbiella pneumoniae* are sensitive. It was shown that *Escherichia coli* is extremely sensitive to the essential oil extracted by SD and SE-SD, with the largest zone of inhibition (23-29 mm) and, *Klesbiella pneumoniae* is very sensitive to the essential oil with a zone of inhibition (15-21 mm). However, *Staphylococcus aureus* was found averagely sensitive which gave an inhibition of 12 mm area for essential oil extracted by SD. It is also very sensitive to the volatile fraction extracted by SE-SD with a zone inhibition of 20 mm. By comparing these results with those obtained in a study carried out on the antibacterial activity realized on the essential oil of the same species coming from Tunisia (Akrout et al., 2010) and Algeria (Ghorab et al., 2013), it was shown that *Escherichia coli* is the most sensitive strain followed by *Klesbiella pneumoniae* and *Staphylococcus aureus* with a light sensitivity.

Reducing power

The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity (Şerbetçi et al., 2012). According to Gůlçin et al. (2010), antioxidants can be

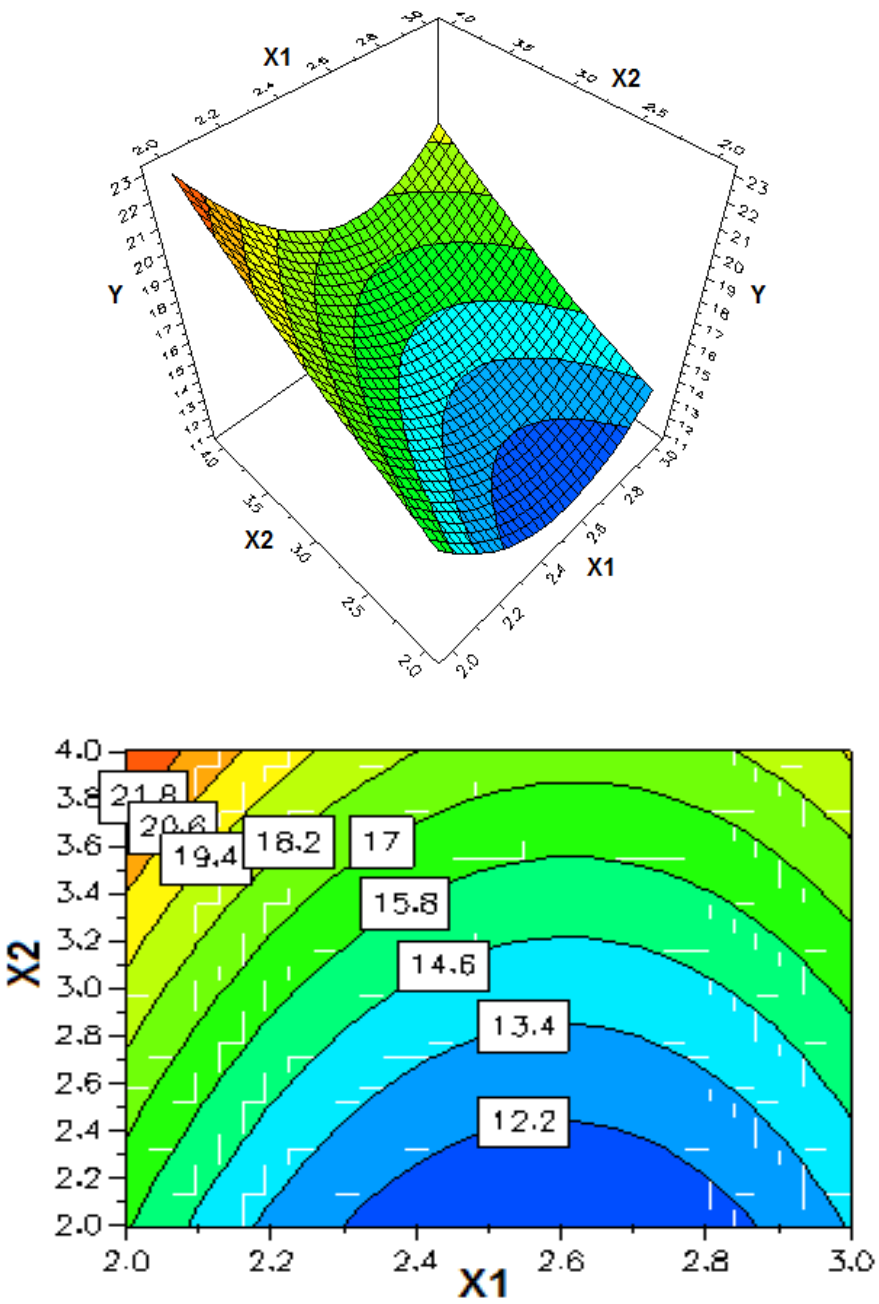

Figure 4: Three-dimensional (3D) and contour (2D) plots of extract yield (Y). X_1: Extraction time, X_2: Extraction number

designated as reductants, and inactivation of oxidants by reductants can be described by redox reactions in which one reaction species is reduced at the expense of the oxidation of the other. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Figure 7 shows the concentration-response curves for the reducing power of ascorbic acid, plant essential oil obtained by SD and by SE-SD. The antioxidant activity of the essential oil which was reflected through the reduction of Fe^{3+} to Fe^{2+}, was increased with increasing concentration. Increased absorbance of the reaction mixture indicates an increase of reduction capability (Gůlçin et al., 2010). The obtained results show that *A. campestris* essential oil has reducing ability and exhibits antioxidant activity, but which is significantly less than that of the reference (ascorbic acid). By comparing samples, the essential oil isolated by SE-SD shows the best reducing power compared to the essential oil isolated by SD with a maximum optical density of 1.18 at a concentration of 5

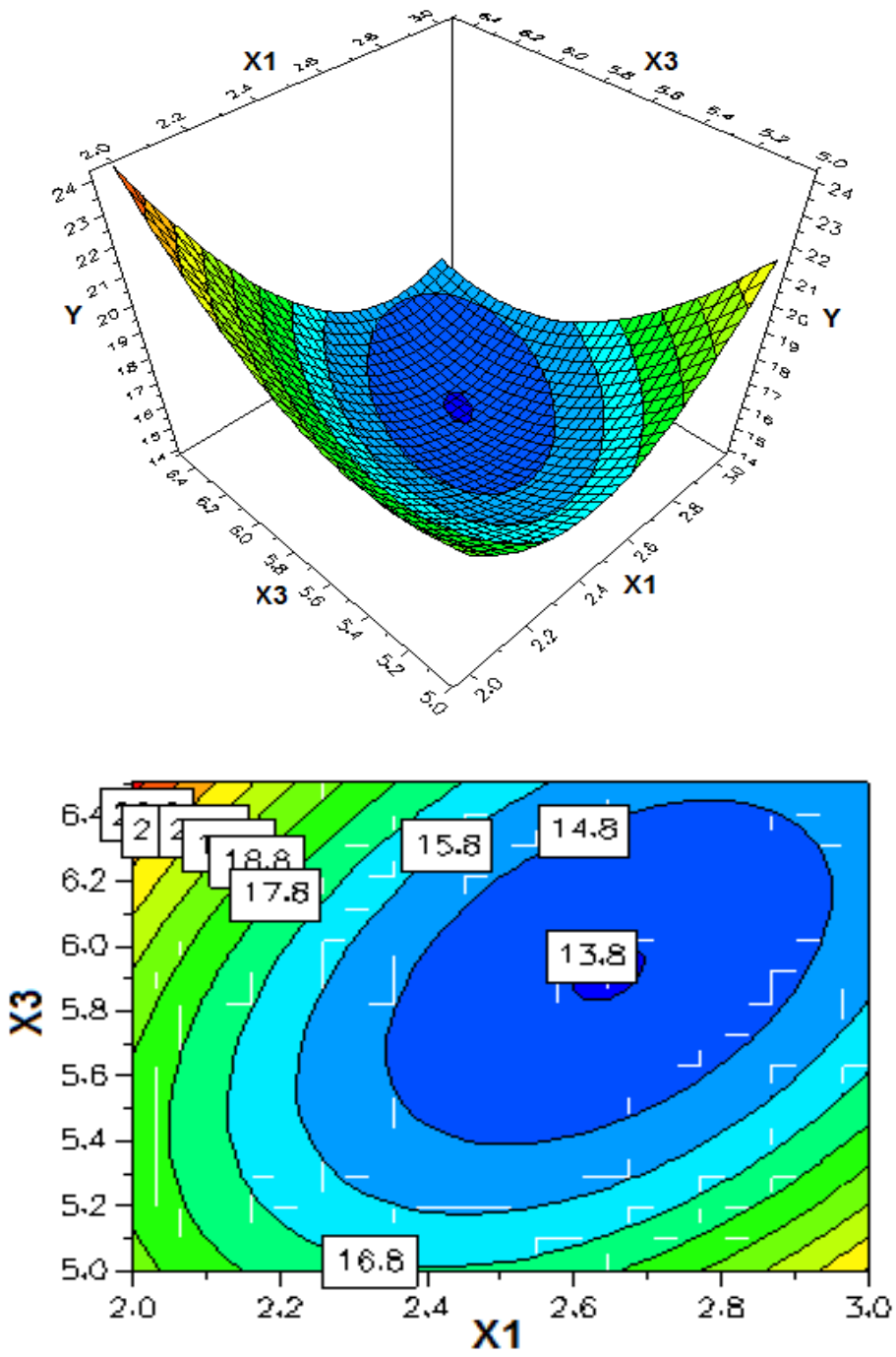

Figure 5: Three-dimensional (3D) and contour (2D) plots of extract yield (Y). X_3: Ratio liquid/solid, X_1: Extraction time.

mg/mL.

Conclusion

This study was carried out in order to investigate the extraction of *A.campestris* essential oil using a combination of organic solvent extraction and steam distillation (SE-SD). The extraction conditions were optimized using a Box-Behnken design in order to produce oil with high quality. Polynomial equation was used to predict the yield of extract and evaluate the effect of extraction parameters and their interactions on the selected response.

According to the mathematical model generated in this study, optimal parameters leading to high yield are as follows: extraction time of 2h, extraction number of 4 and

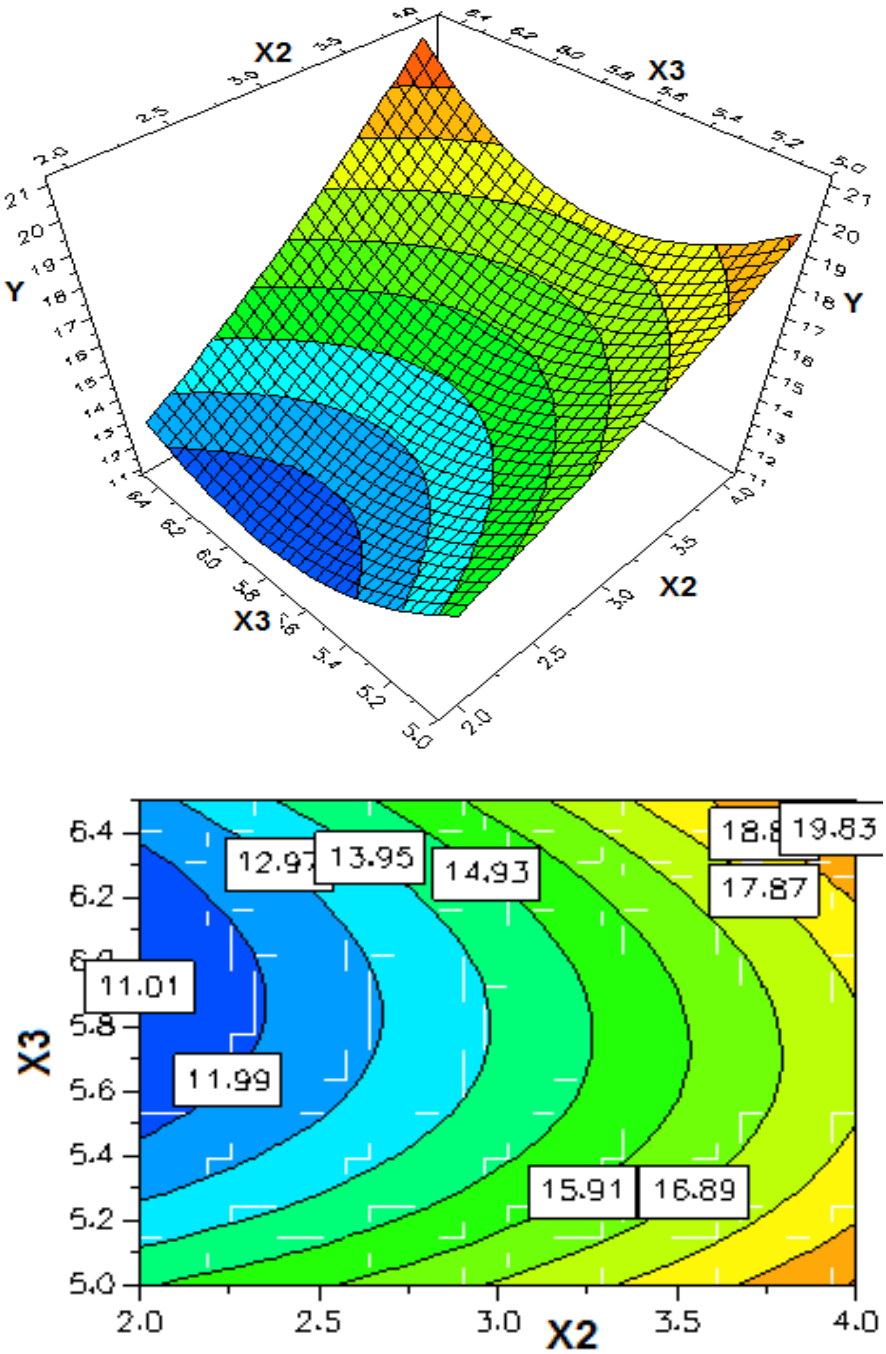

Figure 6: Three-dimensional (3D) and contour (2D) plots of extract yield (Y). X_3: Ratio liquid/solid, X_2: Extraction number.

Table 4. Diameter of inhibition zone

Strains	Gram	Essential oil (SD) (mm)	Essential oil (SE-SD) (mm)
Escherichia coli	-	29	23
Staphylococcus aureus	+	12	20
Klesbiella pneumoniae	-	15	21

liquid-solid ratio of 6.5/1 (mL/g). Using these conditions, the mean value of the extract yield was about 26.74% ± 0.110%. The essential oil obtained by SD yielded 0.88% against 1.3% for SE-SD. In addition, the antibacterial

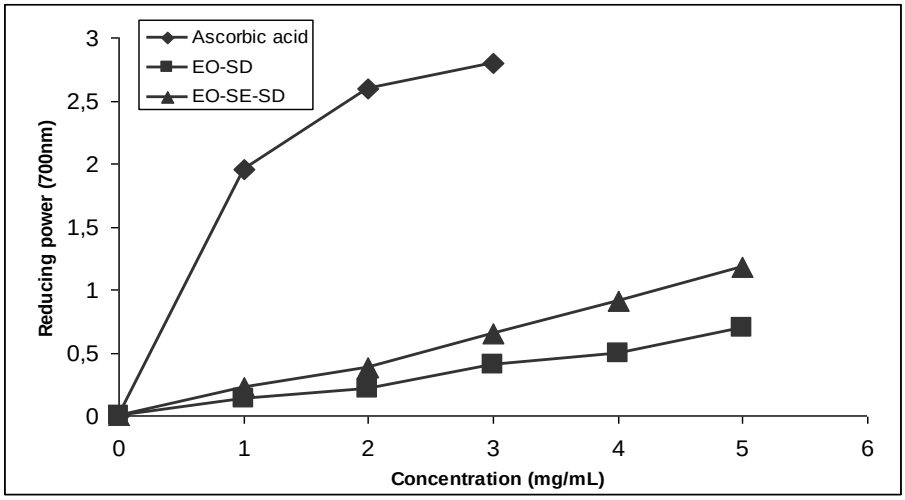

Figure 7: Ferric reducing activity of *Artemisia campestris* essential oil and ascorbic acid

activity carried out on *Escherichia coli, Klesbiella pneumoniae* and *Staphylococcus aureus* showed that the essential oil has a high activity and significant inhibitory power against *Escherichia coli.* In addition, *A.campestris* essential oil isolated by SE-SD showed reducing ability and exhibited antioxidant activity; it had the best reducing power compared to the essential oil isolated by SD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper

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