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EXTRACTION OF SELECTED MINERALS FROM *Melissa officinalis* L. LEAVES BY USING TWO-LEVEL FACTORIAL DESIGN

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ABSTRACT. In this study, a two-level factorial design was used to evaluate the significant extraction parameters in achieving higher recovery yield of Cu, Fe, Mn, and Zn contents from *Melissa officinalis* leaves. The independent parameters were solid-to-solvent ratio, extraction temperature, and duration of extraction. The experimental data obtained were fitted to a first-order polynomial equation using multiple regression analysis and studied by appropriate statistical methods (ANOVA). The optimum extraction conditions were as follows: solid-to-solvent ratio 1:15 g/mL, extraction temperature 100°C, and extraction time 80 min. Under these conditions, the experimental concentrations were in close agreement with the value predicted by the model. The correlation coefficients of 0.9947, 0.9966, 0.9939, and 0.9957 observed between the predicted and actual values for the response variables are evidence that the regression model can represent the experimental data well. Minerals present in the extracts may be maximized when process conditions are carefully adjusted within the reported values.

Keywords: extraction, lemon balm, factorial design, minerals

INTRODUCTION

Tea owes its popularity to the variety of flavors and the healthy properties of this beverage associated with the content of many bioactive compounds. These compounds primarily include phenolics and flavonoids and may offer a protective effect against a wide range of diseases because of their pharmacological, biochemical properties, and antioxidant activities (BOUKADA and MEDDAH 2021; BOUKADA *et al*., 2022). The term 'tea' is currently used to describe not only black, green, red, yellow, or white tea but is also used in reference to herbal infusions obtained from plants other than *Camellia* spp. 'Teas' also denote mixes of dried fruit, herbs, seasonings, and various additions (SAMOLINSKA *et al*., 2017).

The consumers use these herbal infusions with medicinal properties such as hypocholesterolemic, hypotensive, anti-osteoporosis or diabetes preventing, also as alleviating stress, fatigue, insomnia, anxiety, nervousness or simply for their organoleptic properties "to give water a better taste". The water intake that involves the herbal infusion consumption contributes to body hydration including some minerals to maintain good health. Herbal infusions have low contents of energy and nutrients, even though, from a nutritional point of view, they can contribute to mineral intake. Determination of mineral elements in herbal infusions is important to judge their nutritional value and to prevent any probable ill effects (ALDARS-GARCIA *et al*., 2013).

Since mineral elements have important positive as well as negative roles in human organism it is important to determine them to understand their effect on human health. They are classified as macro- and microminerals. The macro minerals include calcium, phosphorus, sodium, potassium, and chloride, and of these, calcium and phosphorus are needed in large quantities. Microminerals include magnesium, manganese, zinc, iron, copper, molybdenum, selenium, iodine, cobalt, and chromium which are required by the body in minute quantity for normal metabolic activities (KADAN *et al*., 2003).

Both copper (Cu) and zinc (Zn), two essential trace minerals, perform important biochemical functions and are necessary for maintaining health throughout life. Copper is an essential constituent of some metalloenzymes. It is required in hemoglobin synthesis, and in the catalysis of metabolic oxidation. Symptoms of Cu deficiency in humans include bone demineralization, depressed growth, depigmentation, and gastro-intestinal disturbances, among others, while toxicity due to excessive intake has been reported to cause liver cirrhosis, dermatitis, and neurological disorders (SILVESTRE *et al*., 2000). Zinc is an essential constituent of many enzymes involved in several physiological functions, such as protein synthesis and energy metabolism. Zinc deficiency (resulting from poor diet, alcoholism, and malabsorption) causes dwarfism, hypogonadism, and dermatitis, while toxicity of Zn, due to excessive intake, may lead to electrolyte imbalance, nausea, anemia, and lethargy (MA and BETTS, 2000). Iron (Fe) deficiency anemia, for example, affects one third of the world population. On the other hand, excessive iron intake has been associated with an overall increased risk of colorectal cancer (SENESSE *et al*., 2004). Manganese (Mn) is an essential metal that, at excessive levels in the brain, produces extra pyramidal symptoms like those in patients with Parkinson's disease and decreased learning activity in school-aged children, and increased propensity for violence in adults (FINLEY, 2004).

Herbal materials are usually used in the form of aqueous extracts. For this reason, it is necessary to know the percentage of extraction of desirable but also hazardous and even toxic compounds. This is especially the case with herbal teas which are usually prepared as infusion or decoction hot or boiling water as an extraction medium. The transfer of the metal from the plant to the infusion medium depends on various factors such as the solvent/solid ration, time of contact, temperature, and particle size of the solid matrix (MILIĆ *et al*., 2014; MITIĆ *et al*., 2019). Thus, it is very important to optimize the extraction efficiency for each raw material. The traditional one-factor-at-a-time approach to the process optimization is time-consuming. Moreover, the interactions among various factors may be ignored, hence the chance of approaching a true optimum is very unlikely. Experimental design is unique, time and cost effective, and mostly uses statistical tool for optimizing the experimental parameters.

Melissa officinalis L. (commonly known as lemon balm, honey balm, balm mint, garden balm, or common balm) is a perennial herbaceous plant that belongs to the family Lamiaceae (mint family). It is found predominantly in the Mediterranean region of the world and elsewhere such as Central Asia, Iran, Europe, Serbia, America, and Africa (SHAKERI *et al*., 2016; ABDELLATIF *et al*., 2021). This plant species is reputed in folk medicine for memory enhancing effects, promoting long life, action against gastrointestinal disorders, rheumatism, Graves', Alzheimer's, thyroid diseases, flatulence, colic, anemia, nausea, vertigo, syncope, asthma, influenza, bronchitis, amenorrhea, cardiac disorders, epilepsy, insomnia, migraines, nervousness, malaise, depression, psychosis, hysteria, and wounds (PAPOTI *et al*., 2019). The bioactivity of its extracts is mainly attributed, as for any other plant formulation, to the comprised phenolic acids, flavonoids, terpenoids and minerals.

Therefore, the objectives of the present work are: 1) to study the effect of extraction time, temperature, and water to plant ratio on the microelement concentrations in *M. officinalis* infusions, and 2) to find out the optimum conditions for microelements extraction using two-level factorial design. The data obtained were used to establish the thermodynamics of the extraction process.

MATERIALS AND METHODS

Plant material

The samples were collected in the second half of May from the location of Niška Banja, Serbia. The herb materials were dried immediately after harvesting and then packed in paper bags. Before being used, the plant material was comminuted by a hammer mill.

Reagents

Multi-element standard solution for ICP (North Kingstown, RI, U.S.A.) of about 20.00±0.10 mg/L was used as a stock solution for calibration. Nitric acid (65%) and perchloric acid (Merck, Darmstadt, Germany) were used for the complete mineralization of analyzed samples.

Extraction experimental design

The maceration procedure was employed for the extraction of minerals from *M. officinalis* leaves. Plant samples (2 g) were extracted by a different volume of water, at a different temperature and a different extraction time. The extraction process was carried out using a bath thermostat. After the filtration (Whatman No.1, USA), the extracts were stored in the flask.

A full factorial design was used for this study, based on two levels of the three variables (Table 1). The extraction variables considered were solid-to-solvent ratio (1:15 and 1:30 g/mL), extraction temperature (40 and 100) and extraction time (20 and 80 min). All other parameters were kept constant. Using the coded levels, the natural levels were calculated and outlined in Table 1, comprising eight experimental runs and different extraction conditions.

Table 1. Parameters and levels used in the 2^3 factorial design study.

	Levels	
Parameters	**(-)**	**(+)**
Water/raw material ratio, V/m (x_1)	15	30
Extraction temperature, ºC (x_2)	40	100
Extraction time, min (x_3)	20	80
Extraction method	Maceration	

Initial content of mineral in M. officinalis (q_o)

The *M. officinalis* leaves samples were digested in a solution containing HNO_3:$HClO_4$ (3:1, v/v). The samples were heated on a heating block at 200ºC to evaporate the samples to dryness. The residue was taken up in 25 mL of 1 mol/L HCl.

Determination of minerals using the ICP-OES method

The overall analysis was conducted with an iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, United Kingdom) with combined an Echelle optical design and a charge injection device (CID) solid-state detector, was used under the operating conditions as follows: flush pump rate – 100 rpm; analysis pump rate – 50 rpm; RF power – 1150 W; nebulizer gas flow – 0.7 Lmin^{-1}; coolant gas flow – 12 Lmin^{-1}; auxiliary gas flow – 0.5 Lmin^{-1}; plasma view – axial; time of rinse – 30 s; measurement in three repetitions.

The precise method was optimized for each mineral. The choice of wavelength was performed based on relative intensity of the signal, as a measure of sensitivity, defects in response to the standards, and the extent of interference in the real sample.

The chosen analytical wavelengths (nm) were as follows: Cu (324.754), Fe (259.940), Mn (257.610), Cr (283.563), Ni (231.604) and Zn (213.856). To construct a calibration graph, which gives the dependence of relative intensity of the signal on the concentration of the analyte, blank samples (*deionized water*) and two concentrations of the standard were recorded.

Thermodynamic parameters were calculated using the following equations:

$$K = \frac{q_{ex}}{q_o - q_{ex}} \tag{1}$$

$$ln\frac{K_{100}}{K_{40}} = \Delta H^o \frac{T_{100} - T_{40}}{R(T_{100} \cdot T_{40})} \tag{2}$$

$$\Delta G^o = -RTlnK \tag{3}$$

$$\Delta G^o = \Delta H^o - T\Delta S^o \tag{4}$$

where q_{ex} is the mineral content in extracts and q_o is the mineral content in the crude plant material (both in the mg/kg of the dry plant material).

The values of Gibbs free energy change (ΔG^o) (kJ/mol) gives an idea of the spontaneity of the extraction process. A negative or positive value of ΔG^o is an indicator of spontaneous or nonspontaneous extraction process. Similarly, enthalpy change (ΔH^o) (kJ/mol) postulates whether the reaction is exothermic or endothermic having value negative and positive respectively. Disorder or randomness of an extraction process was calculated from values of ΔS^o. A reaction of values of ΔS^o higher than zero is indicative of erreversible reaction.

Extraction coefficient, *EC*, is defined by the equation:

$$EC = 100\frac{q_{ex}}{q_0} \tag{5}$$

Statistical analysis

To determine if there exists a relationship between the independent variables and the dependent variables, the data collected were subjected to regression analysis. Regression analysis is used to model a response factor (y) as a mathematical function of three continuous factors. Each response (y) was represented by a mathematical equation. The response was then expressed as linear first-order regression equation according to equation (6):

$$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 \tag{6}$$

where y is the predicted response used to relate to the independent variable; x_1, x_2, x_3 are independent variables (factors); b_o is constant regression coefficient, *bij* and *bijk* (*i,j,k* = 1,2,3) are the regression coefficients of 2- and 3-factor interaction, respectively and $x_1, x_2,$ and x_3 are the factors (independent variables).

The statistical significance of the terms in the regression equations was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response using a computer program. The adequacy of model was checked accounting for R^2, adjusted-R^2 and coefficient of variation (CV) and the significance of the regression coefficient was checchecked by t-test, F-test and p-value.

RESULTS AND DISCUSSION

The influence of liquid/solid ratio, temperature, and time on the extraction yield of minerals from *M. officinalis* leaf was investigated. The range of each extraction variable for the increased concentrations was determined using a one-factor-at-a-time experiment. For temperature 100°C was the upper limit because of the boiling point of water. The lower limit of temperature 40°C was selected to observe the extraction results at lower temperature. The upper limit of time was chosen as 80 min because the extraction time bigger than 80 min did not affect the mineral content. Also, solvent to solid ratio bigger than 30 mL/g did not affect the mineral content significantly. A 2^3 full factorial design was performed to evaluate the importance of the selected extraction experimental variables. The experimental design has eight simplified experimental sets with two replication extraction procedures.

Linear regression modelling

In regression analysis, model building is the process of developing a probabilistic model that best describes the relationship between the dependent and independent variables. In this study, effects of solid-to-solvent ratio (x_1), extraction temperature (x_2) and extraction time (x_3) and their interactions each at two levels on the Cu, Fe, Mn and Zn contents of *M. officinalis* leaf extracts were investigated.

Table 2. Experimental matrix and values of the observed responses
for four minerals in *Melissa officinalis*.

	Design matrix			**Cu**		**Fe**		**Mn**		**Zn**	
No	x_1 (mL/g)	x_2 (°C)	x_3 (min)	C_{obs} (g/L)	C_{prd} (g/L)	C_{obs} (g/L)	C_{prd} (g/L)	C_{obs} (g/L)	C_{prd} (g/L)	C_{obs} (g/L)	C_{prd} (g/L)
1	(15)-1	(40)-1	(20)-1	0.0300	0.0289	0.0124	0.0126	0.0109	0.0108	0.0345	0.0344
2	(30)+1	(40)-1	(20)-1	0.0192	0.0201	0.0050	0.0050	0.0055	0.0053	0.0244	0.0243
3	(15)-1	(100)+1	(20)-1	0.1443	0.1441	0.1349	0.1348	0.2728	0.2729	0.1365	0.1363
4	(30)+1	(100)+1	(20)-1	0.1162	0.1161	0.0570	0.0571	0.1313	0.1315	0.1122	0.1120
5	(15)-1	(40)-1	(80)+1	0.0510	0.0521	0.0169	0.0170	0.0150	0.0149	0.0706	0.0705
6	(30)+1	(40)-1	(80)+1	0.0443	0.0433	0.0523	0.0524	0.0088	0.0089	0.0423	0.0422
7	(15)-1	(100)+1	(80)+1	0.1829	0.1828	0.2350	0.2352	0.4805	0.4803	0.2863	0.2861
8	(30)+1	(100)+1	(80)+1	0.1552	0.1549	0.0823	0.0824	0.2410	0.1408	0.2051	0.2052

Observed response data from the experimental runs (Table 2) were used to develop models. The four (4) response variables (Cu, Fe, Mn, and Zn) were correlated with the independent variables using the first-order polynomial as represented by equation (6). The coefficients with one factor (x_1, x_2 and x_3) represent the sole effects of that particular factor, while the coefficients with two factors (x_1x_2, x_1x_3 , and x_2x_3) and those with three factors ($x_1x_2x_3$) represent the interaction between the two or three factors, respectively (Table 3). A positive value of the regression terms indicates a synergistic effect, while negative sign indicates an antagonistic effect (DANBABA *et al*., 2015).

Table 3. Regression coefficients of the predicted linear first-order model for the responses for four minerals in *Melissa officinalis*.

Regression coefficient	**Cu**	**Fe**	**Mn**	**Zn**
b_0	0.0923	0.0745	0.1457	0.1140
b_1	-0.0092	-0.0253	-0.0491	-0.0180
b_2	0.0567	0.0528	0.1357	0.0710
b_3	0.0155	0.0222	0.0406	0.0371
b_{12}	-0.0048	-0.323	-0.0462	-0.0084
b_{13}	0.0006	-0.0040	-0.0123	-0.0094
b_{23}	0.0039	0.0092	0.0387	0.0236
b_{123}	-0.0004	-0.0147	-0.0122	-0.0048

For the increased Cu concentration in extracts, solid-to-solvent ratio, extraction temperature and extraction time were found very significant (t > 2.31, Table 4, Fig. 1; p < 0.05, Table 5). The effect of extraction temperature was the major contributing variable of 62.24%, followed by extraction time (17.01%), and solid-to-solvent ratio of 10.10% (Table 4). On the two-way interaction, it is worth to mention how the combination of the solid-to-solvent ratio and extraction temperature (x_1x_2) and combination of the extraction temperature and extraction time (x_2x_3) affected Cu concentration. The three-way interaction ($x_1x_2x_3$) had no significant influence (p > 0.05) on the extraction yield of Cu. Pareto chart estimates the effect of each variable on the response in decreasing order whereby the t-value scale provides an accurate measure of the relative effects. Any variable or interaction that falls below the t-value limit of 2.31 is insignificant. In Fig. 1-A, the Pareto plot showed that extraction temperature was the most contributing factor in attaining highest Cu concentration in extract from *M. officinalis* leaf. Additionally, the line plot showed a significant gradient of the slope (Fig. 1-B, C and D).

Table 4. Percentage contribution (%) of extraction variables and t-values for the response for four minerals in *Melissa officinalis*.

Extraction variables	**Percentage contribution (%)**				**t-value for the responses**			
	Cu	**Fe**	**Mn**	**Zn**	**Cu**	**Fe**	**Mn**	**Zn**
A	10.10	15.76	14.67	10.45	30.66	25.87	12.59	39.96
B	62.24	32.90	40.53	41.21	189.16	55.89	34.78	157.86
C	17.01	13.83	12.13	21.53	51.50	23.25	10.41	63.41
AB	5.27	20.12	13.80	4.87	15.96	32.22	11.49	18.64
AC	0.66	2.49	3.67	5.45	1.87	2.90	3.16	20.86
BC	4.28	9.73	11.56	13.70	13.08	9.73	9.91	52.41
ABC	0.44	9.16	3.64	2.78	1.54	15.55	3.12	2.77

Similarly, for Fe, from Table 4 and Fig. 2, it is clearly that solid-to-solvent ratio, extraction temperature, and extraction time were significant (p < 0.05), contributing 15.76, 32.90, and 13.83%, respectively. Extraction temperature was highly significant in obtaining a higher concentration of Fe in extracts using water extraction solvent. The effect of interaction variables of extraction temperature with solvent-to-solid ratio contributed 20.12%, while with extraction time, it was 9.73%. Fe concentration in extracts increases as the extraction temperature and extraction time increased. More so, the Pareto chart showed that extraction temperature was the most contributing factor to attain higher concentration of Fe (Fig. 2-A), while the interaction variables of solid-to-solvent ratio with extraction time was light significant (similar the t-value limit).

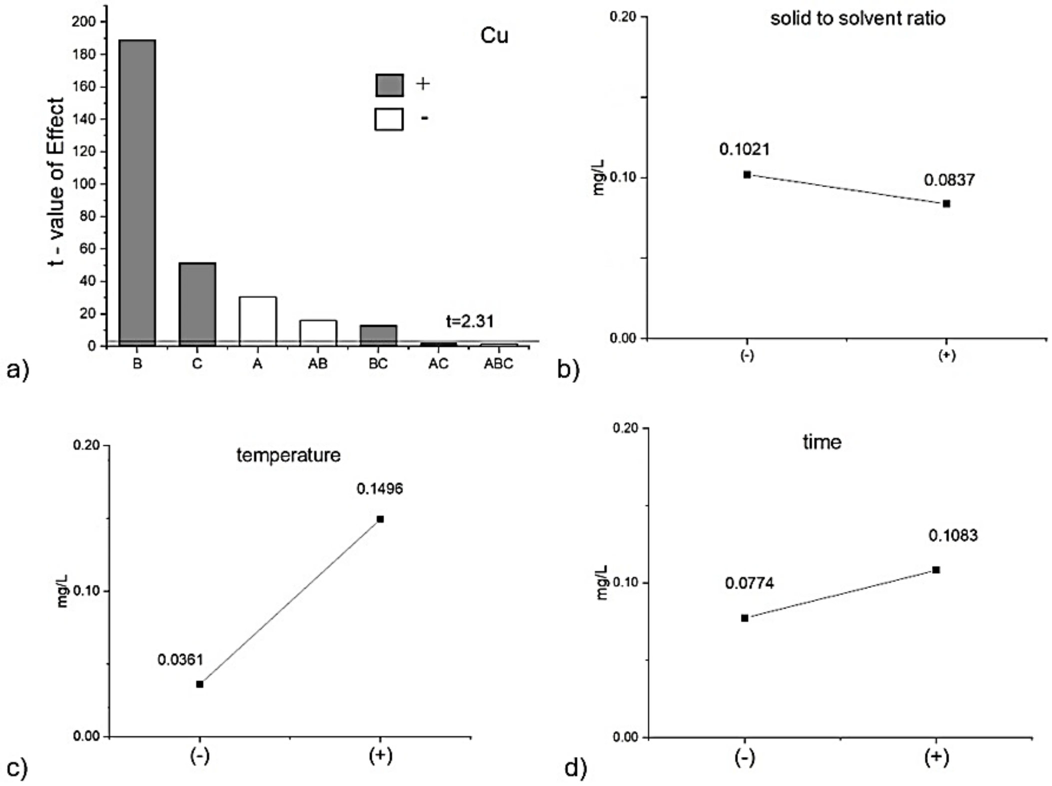

Figure 1. Pareto plot for Cu concentration.

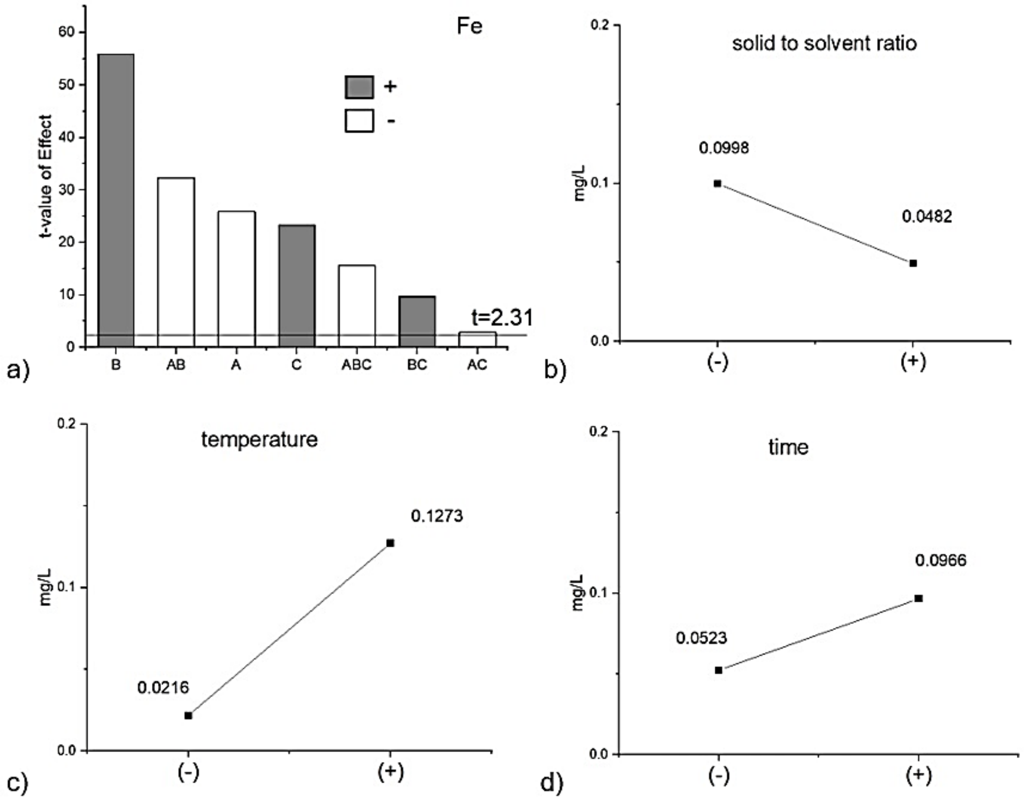

Figure 2. Pareto plot for Fe concentration.

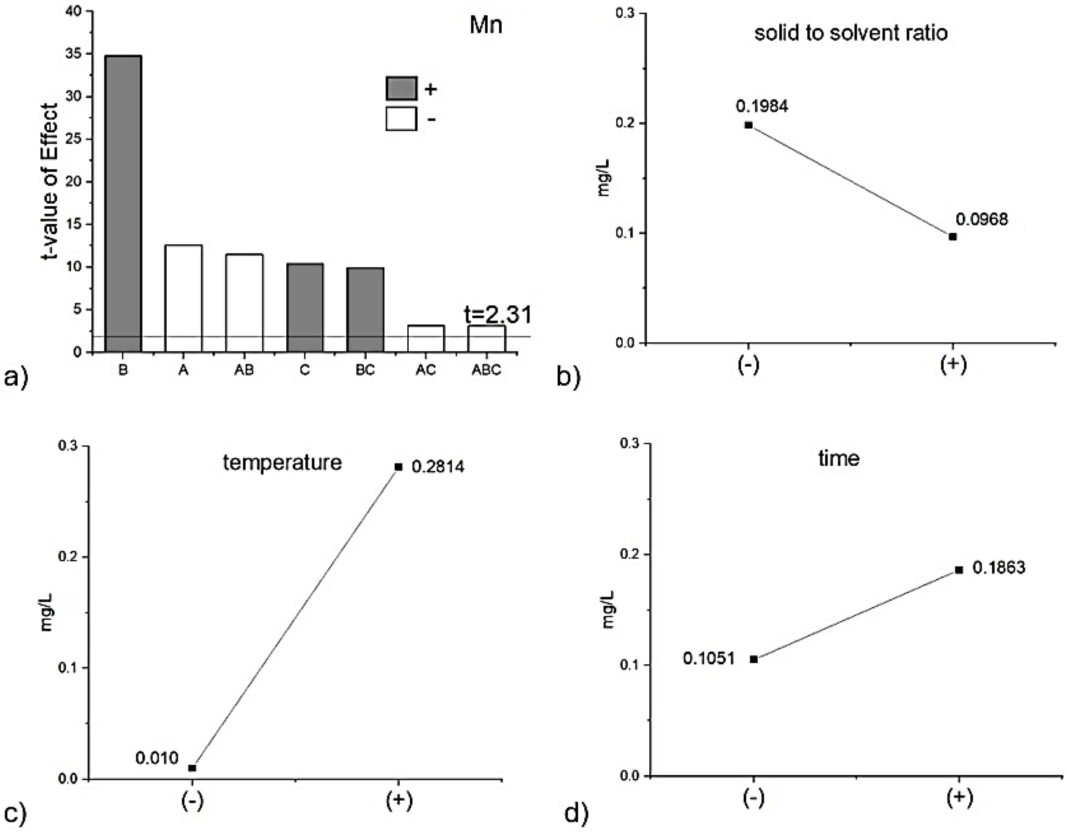

Figure 3. Pareto plot for Mn concentration.

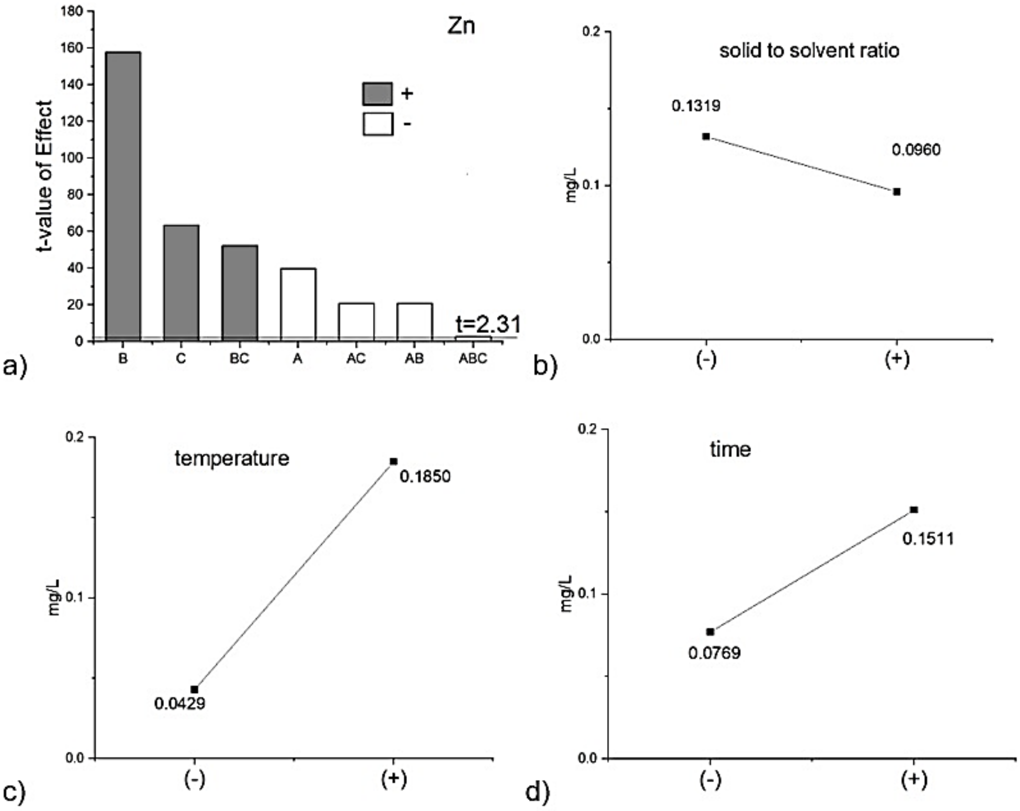

Figure 4. Pareto plot for Zn concentration.

Table 5. Results of the analysis of variance (ANOVA) for four minerals in *Melissa officinalis*.

Mineral	SOV	A	B	C	AB	AC	BC	ABC	Error	Total
Cu	SS[1]	0.000672	0.025776	0.001913	0.000183	0.000003	0.000124	0.000002	0.000152	0.028825
	Df[2]	1	1	1	1	1	1	1	8	15
	MS[3]	0.000672	0.025776	0.001913	0.000183	0.000003	0.000124	0.000002	0.000019	1.001917
	F-value	35.368	1356.6	100.68	9.6317	0.1579	6.5263	0.1052		
	p-value	0.000343	<0.00001[4]	<0.00001	0.014589	0.701498[5]	0.033928	0.753995		
Fe	SS	0.005131	0.022324	0.003925	0.008359	0.000128	0.000677	0.001729	0.000144	0.042417
	Df	1	1	1	1	1	1	1	8	15
	MS	0.005131	0.022324	0.003925	0.008359	0.000128	0.000677	0.001729	0.000018	0.002828
	F-value	285.05	1240.2	218.05	464.38	7.1111	37.611	96.055		
	p-value	<0.00001	<0.00001	<0.00001	<0.00001	0.028509	0.000279	<0.00001		
Mn	SS	0.019266	0.147262	0.013187	0.017057	0.001220	0.012012	0.001181	0.001296	0.212481
	Df	1	1	1	1	1	1	1	8	15
	MS	0.01966	0.147262	0.013187	0.017057	0.001220	0.012012	0.001181	0.00162	0.014165
	F-value	118.92	909.02	81.401	105.29	7.5308	74.148	7.2901		
	p-value	<0.00001	<0.00001	0.000018	<0.00001	0.025283	0.000026	0.027073		
Zn	SS	0.002588	0.040413	0.011011	0.000563	0.000705	0.004451	0.000187	0.000256	0.060174
	Df	1	1	1	1	1	1	1	8	15
	MS	0.002588	0.040413	0.011011	0.000563	0.000705	0.004451	0.000187	0.000032	0.004012
	F-value	80.875	1262.9	344.09	17.593	22.031	139.09	5.8437		
	p-value	0.000019	<0.00001	<0.00001	0.00302	0.001554	<0.00001	0.042022		

SOV Source of variation; [1]Sum of squares; [2]Degree of freedom; [3]Mean of square; [4] $p < 0.00001$ highly significant;[5] $p \geq 0.05$ not significant

The effect of extraction parameters on the Mn concentration is shown in Tables 4 and 5. It can be clearly seen that the recovery yield of Mn was greatly affected by the solid-to-solvent ratio, extraction temperature, and extraction time ($p < 0.05$). The contribution of each extraction parameters was in the order, viz, extraction temperature > solid-to-solvent ratio > interaction of solid-to-solvent ratio with extraction temperature > extraction time > interaction extraction temperature with extraction time (Fig. 3). In addition, it was observed that the increase in extraction temperature from 40 through 100ºC resulted in an increased concentration of Mn from 0.0101 to 0.2814 mg/L (Fig. 3-C).

In the same vein, the effect of extraction parameters was studied for the maximum recovery of Zn (Tables 4 and 5). The contributing factors were in the order of extraction temperature (41.21%) > extraction time (21.53%) > interaction extraction temperature with extraction time (13.70%) > solid-to-solvent ratio (10.45%) > interaction solid-to-solvent ratio with extraction time (5.45%) and three-way interaction (2.78%), respectively (Table 5). The Pareto chart confirmed the highest significance of extraction temperature in attaining higher concentration of Zn in extracts from *M. officinalis* leaf (Fig. 4-A). Likewise, the line plots in Fig. 4-C reflected the significant gradient of the slope; extraction temperature has the prominent slope and solid-to-solvent ratio the least (Fig. 4-B). In addition, all interactions between the extraction parameters also contributed to the higher recovery of Zn.

Analysis of variance (ANOVA)

The analysis of variance (ANOVA) for the response variables (concentration of Cu, Fe, Mn, and Zn) are presented in Table 5. The statistical significance of all three factors and their possible two- and three-way interaction for the mineral concentrations in extracts were also evaluated for their F- and p-values. The statistical impact of a factor is greater if its F-value is higher. The value of $p < 0.05$ indicates the significance of the factors and their interaction. To simplify the linear regression model, all factors, and interactions, which were assessed to be statistically insignificant with the significance level of 0.05, were omitted; the simplified regression equations are given in Table 6.

Table 6. First-order linear regression models for the responses for *Melissa officinalis*.

Response variables	First-order linear models	R^2 (%)	R^2_{adj} (%)	CV (%)
Cu	$0.0923 - 0.0092x_1 + 0.0567x_2 + 0.0155x_3 - 0.0048x_1x_2 + 0.0039x_2x_3$	99.47	99.01	4.70
Fe	$0.0745 - 0.0253x_1 + 0.0528x_2 + 0.0222x_3 - 0.0323x_1x_2 - 0.0040x_1x_3 + 0.0092x_2x_3 - 0.0147x_1x_2x_3$	99.66	99.36	5.69
Mn	$0.1457 - 0.0491x_1 + 0.1357x_2 + 0.0406x_3 - 0.0462x_1x_2 - 0.0123x_1x_3 + 0.0387x_2x_3 - 0.0122x_1x_2x_3$	99.39	98.86	8.73
Zn	$0.1140 - 0.0180x_1 + 0.0710x_2 + 0.0371x_3 - 0.0084x_1x_2 - 0.0094x_1x_3 + 0.0236x_2x_3 - 0.0048x_1x_2x_3$	99.57	99.20	4.96

Validation of regression models

It is also necessary that the developed regression models (Table 6) provide an adequate approximation in real systems. In this study numerical method was used for the validation. The numerical method uses the coefficient of determination (R^2) and adjusted R^2 (R^2_{adj}). Also, the coefficient of variation (CV) was calculated to check the model adequacy (Table 6).

The coefficient of determination, R^2, is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for a good, fitted model, R^2 should not be less than 80% (KOOCHEKI *et al*., 2009). When R^2 approaches the unity, signifies the suitability of fitting the empirical model to the actual data. The lower value of R^2 shows the inappropriateness of the model to explain the relation between variables. Our results showed that the R^2 values for these response variables were higher than 0.80, indicating the regression models were suitable to explain the behavior. The R^2 values for Cu, Fe, Mn, and Zn were found to be 0.9947, 0.9966, 0.9939 and 0.9957, respectively.

It should be noted that adding a variable to the model will always increase R^2, regardless of whether the additional variable is statistically significant or not. Thus, a large value of R^2 does not always imply the adequacy of the model. For this reason, it is more appropriate to use an adj-R^2 of over 90% to evaluate the model adequacy (KOOCHEKI *et al*., 2009). The adj-R^2 values were found to be higher than 0.98 for all the responses. Higher adj-R^2 indicated that insignificant terms have not been included in the model.

Moreover, the coefficient of variation (CV) describes the extent to which the data were dispersed. Generally, the coefficient of variation (CV) should not be greater than 10% (KOOCHEKI *et al*., 2009). Our results showed that the coefficients of variation were less than 10% for all the responses (Table 6), representing a better precision and reliability of the conducted experiments.

Mineral content and extraction coefficient

The contents of Cu, Fe, Mn, and Zn in this study were all lower than 44 mg/kg. Based on their values, they could be arranged in order: Fe (43.355mg/kg) > Mn (21.722 mg/kg) > Zn (12.339 mg/kg) > Cu (7.313 mg/kg) (Table 7). Our previous research demonstrated that the Cu, Fe, Mn, and Zn contents in *Salvia officinalis* leaves are 5.063, 62.832, 3.346 and 10.735 mg/kg, respectively (MITIĆ *et al*., 2019). This is confirmed by investigations conducted by other authors. PETROVIĆ *et al*. (2015) in *Matricaria chammomilla* determined a mineral level in the range of 4.7–77, 1.9–7.4, 7.8–42.8 and 12.7–18.0 mg/kg for Cu, Fe, Mn, and Zn, respectively. OLIVIER *et al*. (2012) reported the averages of the mineral content of Cu, Fe, Mn, and Zn in leaves and infusions of traditional and herbal teas to be 4.7, 5.8, 181.4 and 12.7 mg/kg, respecttively. Contents of microelements in medicinal plants are influenced by genetically determined properties of a plant as well as by external factors, including geographic location, soil type and profile, fertilization, availability of water, pollution by pesticides or dusts, and gases.

The extraction coefficient of each element was calculated based on the content of the element in the raw material and its concentration in the herbal infusion. The extraction coefficient depends on the temperature. Generally, the extraction coefficient increased with increasing the extraction temperature because of the enhanced solubilities of the minerals at higher temperatures. The highest percentage of extraction into the infusion was determined for Cu, Zn and Mn (over 62, 58 and 55%). The weakest transfer into the herbal tea was noted for Fe (on average less than 8%). Zn and Cu were the most soluble elements. The least soluble was Fe, which agrees with the results of ALDARS-GARCIA *et al*. (2013) and SULAIMAN *et al*. (2013).

Table 7. Mineral contents, extraction coefficients and thermodynamic parameters of the mineral extraction processes for *Melissa officinalis*.

Extractive substance	**q_o^{a}**	**t**	**EC**	**K**	**ΔH°**	**ΔG°**	**ΔS°**
	mg/kg	(°C)	(%)		(kJ/mol)	(kJ/mol)	(J/K mol)
Cu	7.313	40 100	17.41 62.52	0.211 1.668	33.43	4.05 -1.58	93.87
Fe	43.355	40 100	0.97 13.55	0.010 0.157	44.56	11.98 5.74	104.07
Mn	21.722	40 100	1.73 55.30	0.018 1.237	68.45	10.45 -0.66	185.29
Zn	12.339	40 100	14.30 58.00	0.167 1.381	34.18	4.66 -1.00	94.33

Thermodynamic analysis

Table 7 shows the values of the equilibrium constant and other thermodynamic parameters for the extraction of minerals (Cu, Fe, Mn and Zn) from *M. officinalis* leaves by using water as a solution.

The enthalpy values for the extraction process were in the range of 33.43–68.45 kJ/mol for the different minerals. The enthalpy in this report, was comparatively higher than those of minerals from *S. officinalis* studied previously (MITIĆ *et al*., 2019), which were in the range 4.89–18.85 kJ/mol. This could be attributed to the morphology of the leaf which could influence mineral extraction. The positive enthalpy change indicated that the extraction process was endothermic in nature and as such required external energy sources during the extraction (TOPALLAR and GEÇGEL, 2000; AMIN *et al*., 2010; SULAIMAN *et al*., 2013).

The positive values of the entropy change for the extraction process of minerals from *M. officinalis* leaves using the water solution (93.87–185.25 K/Jmol) were an indication that the process was irreversible, thus in line with the findings of MILIĆ *et al*. 2014. During the extraction, the molecules of extractable substances, including the minerals, are extracted from the solid plant material, thus increasing the entropy of the suspension (AMARANTE *et al*., 2014).

The negative values of ΔG^o were obtained for Cu, Mn, and Zn at temperature 100°C, indicating the viability and spontaneous nature of the process under the investigated conditions. For these minerals at 20°C and for Fe at 20 and 100°C, the positive values indicate that the energy consumed in the process for the disorganization of mineral molecules in the solvent medium (ΔH^o) is greater than the energy consumed for the reorganization of molecules in the medium ($T\Delta S^o$) and the process is not spontaneous, i.e. under these conditions the process is not thermodynamically favorable.

CONCLUSION

The maceration extraction process was optimized by 2^3 full experimental design, and under optimal conditions (extraction temperature 100°C; water/raw material ratio 15 ml/g; extraction time 80 min), 0.1829, 0.2350, 0.4805, and 0.2863 g/L Cu, Fe, Mn, and Zn contents were obtained from *M. officinalis* leaves. The results show that the variations of enthalpy (ΔH^o in the range 33.43–68.45 kJ/mol) and entropy ((ΔS^o in the range 93.87–185.29 J/Kmol) were positive; indicating that the process was endothermic and irreversible while Gibbs free energy was negative at temperature 373.15 K ((ΔG^o in the range (-0.60)– (-1.58) kJ/mol) indicating that extraction was spontaneous and thermodynamically favorable.

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References:

[1] Abdellatif, F., Akram, M., Begaa, S., Messaoudi, M., Benarfa, A., Egbuna, C., Ouakouak, H., Hassani, A., Sawicka, B., Elbossaty W.F., Simal-Gandara, J. (2021): Minerals, essential oils, and biological properties of *Melissa officinalis* L. *Plants* **10** (6): 1-16. doi: 10.3390/plants10061066

[2] Aldars-Garcia, L., Zapata-Revilla, M.A., Tenorio-Sanz, M.D. (2013): Characterization and study of the essential mineral components of Spanish commercial herbal products and their infusions. *Journal of Food and Nutrition Research* **52** (3): 172–180.

[3] Amarante, R., Oliveira, P.M., Schwantes, F., Moron-Villarreges, J.A. (2014): Oli Extraction from Castor Cake Using Ethanol: Kinetics and Thermodynamics. *Industrial and Engineering Chemistry Research* **53** (16): 6824–6829. doi:10.1021/ie500508n

[4] Amin, Sh.K., Hawash, S., El Diwani, G., El Eafei. S. (2010): Kinetics and thermody namics of oil extraction from *Jatropha curcas* in aqueous acidic hexane solutions. *Journal of American Science* **6** (11): 293–300.

[5] Boukada, F., Meddah. B., (2021): Flavonoids from Aerial Parts of Algerian *Ajuga iva* (L.) Schreb.: The HPLC-UV Analysis and Antioxidant Capacity. *Kragujevac journal of Science* **43**: 23–34. doi: 10.5937/KgJSci2143023B

[6] Boukada, F., Sitayeb, S., Khadem, H., Meddah. B., Zohra Soltani, F. (2022): Chemical composition, antioxidant and antibacterial activity of *Adiantum capillus-veneris* L. extracts from Algeria. *Kragujevac journal of Science* **44**: 91–101. doi: 10.5937/KgJSci 2244091B

[7] Danbaba, N., Nkama, I., Badau, M.N. (2015): Application of Response Surface Methodology (RSM) and Central Composite Design (CCD) to optimize minerals composition of rice-cowpea composite blends during extrusion cooking. *International Journal of Food Science and Nutrition Engineering* **5** (1): 40–52. doi: 10.5923/j.food. 20150501.06

[8] Finley, J.W. (2004): Does environmental exposure to manganese pose a health risk to healthy adults? *Nutrition Reviews* **62** (4): 148–153. doi: 10.1111/j.1753-4887.2004.tb000 37.x

[9] Kadan, R.S., Bryant, R.J., Pepperman, A. (2003): Functional properties of extruded rice flours. *Journal of Food Science* **68** (5): 1669−1672. doi:10.1111/j.1365-2621.2003. tb12311.x

[10] Koocheki, A., Taherian, A.R., Razavi, S., Bostan, A. (2009): Response surface methodology for optimization of extraction yield, viscosity, and hue and emulsion stability of mucilage extracted from *Lepidium perfoliatum* seeds. *Food Hydrocolloids* **23** (8): 2369−2379. doi: 10.1016/j.foodhyd.2009.06.014

[11] MA, J., BETTS, N.M. (2000): Zinc and copper intakes and their major food sources for older adults in the 1994–96 Continuing Survey of Food Intakes by Individuals (CSFII). *Journal of Nutrition* **130** (11): 2838–2843. doi: 10.1093/jn/130.11.2838

[12] MILIĆ, P., RAJKOVIĆ, K., BEKRIĆ, D., STAMENKOVIĆ, O., VELJKOVIĆ, V. (2014): The kinetic and thermodynamic analysis of ultrasound-extraction of minerals from aerial parts of white lady's bedstraw (*Galium mollugo* L.). *Chemical Engineering Research and Design* **92** (7): 1399–1409. doi: 10.1016/j.cherd.2013.10.024

[13] MITIĆ, M., TOŠIĆ, S., PAVLOVIĆ, A., MAŠKOVIĆ, P., KOSTIĆ, D., MITIĆ, J., STEVANOVIĆ, V. (2019): Optimization of the extraction process of minerals from *Salvia officinalis* L. using factorial design methodology. *Microchemical Journal* **145**: 1224–1230. doi: 10.1016/j.microc.2018.12.047

[14] OLIVIER, J., SYMINGTON, E.A., RAMPEDI, I.T., VAN EEDEN, T.S. (2012): Comparison of the mineral composition of leaves and infusions of traditional and herbal teas. *South African Journal of Science* **108** (1): 1–7. doi: 10.4102/sajs.v108i1/2.623

[15] PAPOTI, V.T., TOTOMIS, N., ATMATZIDOU, A., ZINOVIADOU, K., ANDROULAKI, A., PETRIDIS, D., RITZOULIS, C. (2019): Phytochemical content of *Melissa officinalis* L. herbal preparations appropriate for consumption. *Processes* **7** (2) 1-16. doi: 10.3390/pr7020088

[16] PETROVIĆ, S., SAVIĆ, S., DIMITRIJEVIĆ, M., PETRONIJEVIĆ, Z. (2015): The determination of macro and microelements in chamomile teas (*Matricaria chammomilla* L.). *Advanced Technologies* **4** (2): 37–42. doi: 10.5937/savteh1502037P

[17] SAMOLINSKA, W., KICZOROWSKA, B., KWIECIEN, M., RUSINEK-PRYSTUPA, E. (2017): Determination of minerals in herbal infusions promoting weight loss. *Biological Trace Element Research* **175**: 495–502. doi: 10.1007/s12011-016-0790-4

[18] SENESSE, P., MEANCE, S., COTTET, V., FAIVRE, J., BOUTRON-RUAULT, M.C. (2004): High dietary iron and copper and risk of colorectal cancer: a case-control study in Burgundy, France. *Nutrition and Cancer* **49** (1): 66–71. doi: 10.1207/s15327914nc 4901_9

[19] SHAKERI, A., SAHEBKAR, A., JAVADI, B. (2016): *Melissa officinalis* L. – A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology* **188**: 204–228. doi: 10.1016/j.jep.2016.05.010

[20] SILVESTRE, M.D., LAGARDA, M.J., FARRE, R., MARTINEZ-COSTA, C., BRINES, J. (2000): Copper, iron and zinc determinations in human milk using FAAS with microwave digestion. *Food Chemistry* **68** (1): 95–99. doi: 10.1016/S0308-8146(99)00160-0

[21] SULAIMAN, S., AZIZ, A.R., AROUA, M. K. (2013): Optimization and modeling of extraction of solid coconut waste oil. *Journal of Food Engineering* **114** (2): 228–234. doi: 10.1016/j.jfoodeng.2012.08.025

[22] TOPALLAR, H., GEÇGEL, U. (2000): Kinetics and thermodynamics of oil extraction from sunflower seeds in the presence of aqueous acidic hexane solutions. *Turkish Journal of Chemistry* **24** (3): 247–253.