The Influence of Temperature on the Kinetics of Antioxidant Activity and Phenolic Compounds in Extracts of *Origanum Majorana*

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Abstract:

The aromatic plant that goes by the name *Origanum majorana* is the source of an essential oil that is later used in the treatment of a broad range of conditions. In this specific piece of study, one of our goals is to investigate the pharmacological effects that are associated with the use of extracts from *Origanum majorana.* The quantity of phenolic compounds that were present was determined with the use of colorimetric methods. On a microplate with 96 wells, tests for antioxidant and antidiabetic activity were performed. The ethanol extract has the most total phenolic (296.34±5.28µg GAE/mg), followed by the chloroform and hexane extracts. The ethanol extract has the most flavonoids, flavonols, and condensed tannins. In contrast, the *Origanum majorana* extracts were tested as antioxidants utilizing DPPH and ABTS as artificial radicals. IC50 for the ethanol extract was 19.27±0.68mg/mL. IC_{50} values for hexane and chloroform extracts were 32.79±1.81mg/mL and 64.65±0.98mg/mL, respectively. IC50 values for ethanol and chloroform extracts were 18.02±3.81 and 22.41±2.1mg/mL, respectively. IC50 for hexane extract was

47.11±0.94mg/mL.The ethanolic extract of the *Origanum majorana* plant was evaluated in terms of total antioxidant activity by the DPPH test. The kinetics of changes in *Origanum majorana* samples heated at different temperatures (25, 35, 45, 50, and 60 °C) over one hour were studied. The results showed that increasing the treatment temperature and time increased all factors, including, the antioxidant activity and the variation of the rise in the antioxidant activity depending on the heating temperatures and time. The results showed that all extracts significantly displace the DPPH root, as it was found that heating the marjoram extract to 60 °C for 30 minutes is more effective than heating to 45 or 50°C.
Keywords: Origanum majorana, extracts, DPPH test, antioxidant.

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1. Introduction

Medicinal plants are important food sources and they have a therapeutic and medicinal benefit in addition to their high nutritional value, which is due to the fact that they include a source of life energy in the form of carbs, proteins, and fats. In point of fact, medicinal plants do play a part in the treatment of a wide variety of disorders due to the presence of chemical substances that have clear biological activity [1–3]. They have evolved to the point that they are now an indispensable component of conventional medical practices, which are the oldest and most adaptable for many therapies. According to estimates provided by the World Health Organization (WHO), more than 80% of people worldwide currently use herbal medicines in some capacity to provide primary medical care [4,5]. Traditional and folk medicine still record a remarkable presence, despite the great scientific development in the field of treatment with modern techniques, as well as the attempt to avoid the use of medicines containing chemical and unnatural ingredients, and the cost of traditional and folk treatment is low [6–8].

It is well known that members of the oregano (*Origanum majorana.*, Lamiaceae) family exhibit therapeutic characteristics (diaphoretic, carminative, antispasmodic, antiseptic, and tonic), and these properties are used in the traditional medical systems of many nations [9]. Because of its pungent aroma, it has found widespread use as a culinary herb, a flavoring component in food items and alcoholic drinks, and in the perfumery industry [10–12]. Moreover, it has been utilized in the pharmaceutical and cosmetics sectors. Oregano extracts have the most potent antioxidant activity out of all aromatic plants [13], and this is due to the nonvolatile components they contain. Oregano alcohol extracts have been investigated by many different teams of researchers [14,15]. It is widely agreed that the presence of rosmarinic and caffeic acid in the extracts is responsible for the antioxidant action that they have [15,16].

Origanum majorana L. is a species that belongs to the enormous plant family known as Lamiaceae, which is comprised of more than 230 distinct genera and almost 7000 different species [17]. A scented annual plant that develops a root structure that persists for years, *Majorana hortensis* is

the approved scientific name for the plant, despite the fact that it is more often referred to as sweet marjoram. It may also be referred to as "Garden majoram", which is its synonym. Greece, Cyprus, and Turkey are the plant's native habitats; however, it has been successfully grown in other countries as well, including Morocco, Egypt, Tunisia, and Algeria [18–21]. One of the most significant aspects that play a role in the level of antioxidant activity is temperature. In most cases, heating causes an acceleration of the initiation processes and, as a result, a reduction in the activity of the antioxidants that are already present or that have been added [22,23]. As food is heated, it may undergo a number of chemical reactions, one of which is known as the Maillard reaction [24]. This process takes place in foods that include free amino acids and sugars, and it results in the development of compounds known as Maillard reaction products (MRPs). These MRPs are browning agents that don't use enzymes and have a large amount of antioxidant action. Hence, subjecting some meals to heat treatment may result in an increase in both the antioxidant activity and brown color of certain foods [25]. During the heating process, a variety of physical and chemical variables may have an impact on the antioxidant properties of MRPs. The Maillard reaction involves the participation of carbon molecules such as ascorbic acid and polyphenols [26]. Nevertheless, in model systems, the production of MRPs during prolonged heating at 100 °C does not have an effect on browning in a direct manner [27].

The objective of this research was to assess the extent to which *Origanummajorana* can be used both as a source of bioactive substances and as a treatment for a number of different medical conditions. In this regard, the study was conducted on the antioxidant qualities of the aerial parts of *Origanum majorana*, harvested in the Algerian desert. Also, the corresponding kinetic parameters of these qualities were evaluated.

2. Materials and Methods

2.1. Chemicals

The chemicals employed in this study were: 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), hydrochloric acid, Folin–Ciocalteu reagent, ethanol, chloroform, n-hexane, aluminum chloride, aluminum nitrate, potassium persulfate, quercetin, vanillin, sodium carbonate, catechin, gallic acid, butylhydroxyanisole (BHA), and butylhydroxytoluene (BHT), bought from Sigma-Aldrich. All chemicals were of analytical grade, and were used without further purification.

2.2. Plant Material and extraction

In March of 2021, *Origanum majorana* was gathered fromHassi Khalifa region, which is located in the southeast coast of Algeria (in the coordinates 33°15′46″ North, and 7°22′33″ East). After the plant had been harvested, the aerial portions were washed and then allowed to dry at room temperature for 15 days while being shaded. Afterwards, the materials were ground into powder. The extraction process was conducted at room temperature for twenty-four hours by mixing 40 g of the resulting powder with 200 mL of solvent (ethanol, chloroform, and n-hexane). After that,

the mixture was filtered using filter paper, and thesolvent was evaporated. The obtained extract was kept in the dark at 4°C.Extractions were conducted in triplicate.

2.3. Phytochemistry

2.3.1. Total phenolic content (TPC)

Total phenolic content was determined using spectrophotometric analysis based on the Folin-Ciocalteu method as previously described [28–31]. After combining 20µL of plant extract solution (1 mg of extract in 1 mL of water), 100µL of Folin-Ciocalteu Reagent (diluted with water 1/10), and 75µL of Na_2CO_3 (7.5%), the mixture was incubated for 120 min at room temperature. then, the absorbance was measured at 760 nm. Gallic acid was used as a reference standard and the curve of absorbance versus concentration was represented by the equation: Y=0.0027X+0.0854 (R^2=0.997). Results were given as µgGAE/mg.

2.3.2. Flavonoids total content (TFC)

The TFC were determined using the aluminum nitrate colorimetric assay [32]. 50µL of extract solution (1mg/mL) was combined with 10µL of 10% aluminum nitrate, 130µL of ethanol, and 10µL of potassium acetate (1M). After waiting 40 minutes at room temperature, the absorbance was read at 415 nm. The flavonoid content was determined by using the quercetin calibration curve equation: Y=0.0034X+0.0226 (R^2=0.995), and the findings are provided as µg quercetin equivalent per milligram of extract (µg QE/mg).

2.3.3. Flavonol content

The flavonol content of the extracts was determined using the aluminum trichloride colorimetric method. 50µL of extract solution (1mg/mL) was combined with 150µL of 5% sodium acetate solution and 50µL of 2% $AlCl_3$. After the sample had been kept in the dark for 2 hours, the absorbance was read at 440 nm. The estimation of flavonol content was made through the quercetin calibration curve equation: Y=0.0053X+0.0854 (R^2=0.978).

2.3.4. Condensed tannins content

Vanillin method was used to evaluate condensed tannins content in 96-well microplates [33,34]. 25 µLof extract solution (1mg/mL) was aliquoted into a microplate. Then 150µL of 4% vanillin solution in ethanol and 75 µL of 30% HCl were added. After 15 minutes, the absorbance was read at 500 nm using a microplate reader. Condensed tannins content (in catechin equivalents per mg of extract (µg CE/mg)) was obtained utilizing the catechin calibration curve equation: Y=0.0027X+0.0301 (R^2=0.993).

2.4. Antioxidant activity

2.4.1. DPPH free radical scavenging test

The ability of the extracts to stifle the effects of the free radical DPPH was evaluated according to the methodology previously described [29,35]. The experiment was carried out in a microplate with 96 wells by mixing 160µL of 1 mM DPPH solution in ethanol and 40µL of each extract. The mixture was incubated in dark for 30 min. Then, the absorbance was determined using a

microplate reader at a wavelength of 517 nm. The DPPH inhibition percentage was calculated following equation (1):

$$DPPH\ inhibition\ (\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

In this equation, A_0 represents the absorbance of the control, and A_1 represents the absorbance of the sample incubation. The IC50 value was calculated graphically from the plot of DPPH inhibition versus the concentration of the extract sample.

2.4.2. ABTS radical scavenging

The anti-radical activity was accomplished utilizing the ABTSassay. In a prior experiment, the radical ABTS+ was produced via the reaction of 7 mM ABTS with 2.45 mM $K_2S_2O_8$. After 16h, the obtained solution was diluted until obtaining an absorbance of 0.7 at 734 nm. After that, 40µL of each extract solution with different concentrations were combined with 160µL of diluted $ABTS^+$ solution. After waiting for ten minutes, the absorbance was read at 734 nm. The percentage of inhibition was determined using equation (1).

BHA and BHT were served as the standards for studying antioxidant activity.

2.5. Kinetics

The kinetic models of zero (Equation (2)), first (Equation (3)) and second (Equation (4)) order were utilized in order to derive the antioxidant activities of the *Origanum majorana* extracts [36].

$$[A] = [A]_0 - kt \quad (2)$$

$$\ln[A] = \ln[A]_0 - kt \quad (3)$$

$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt \quad (4)$$

Where, k (min^{-1}) represents the reaction constant; t (min) represents the time; $[A]_0$ and [A] represent the starting and final concentrations, respectively.

Using equation (5), we were able to analyze the impact that temperature had on k. (Arrhenius equation). The activation energy (Ea) required for the generation of each parameter was calculated by doing a linear regression on the logarithmic temperature dependence of the k curve.

$$\ln k = \ln k_0 - \frac{E_a}{RT} \quad (5)$$

where, k signifies the reaction constant, Ea (kJ mol^{-1}) represents the activation energy, R (J/mol K) stands for the universal gas constant, T (K) stands for the temperature, and T (K) defines the temperature.

Excel software was used to create regression models for each of the parameters at different temperatures. The models were obtained by plotting the [A], ln [A], and 1/[A] versus the time.

2.6. Statistical analysis

All tests gave three-measure mean SD. The values were compared using Minitab 17's ANOVA and Tukey tests. $p<0.05$ was judged significant.

3. Results and Discussion

3.1. Contents of phenolic compounds

The quantities of phenolic compounds of *Origanum majorana* extracts obtained using different solvent were measured and results were illustrated in Table 1.

Table 1. Total phenolics, flavonols, flavonoids, and condensed tannins contents of *Origanum majorana* extracts.

Extracts	Total phenolics (µg GAE/mg)	Flavonoids (µg QE/mg)	Flavonol (µg QE/mg)	Condensed tannin (µg CE/mg)
Ethanol	296.34±5.28	248.71±6.85	121.32±3.58	192.56±4.69
Chloroform	82.41±1.2	20.82±4.89	102.63±4.71	130.13±1.42
n-hexane	23.67±7.12	19.47±1.4	36.04±4.2	30.74±0.87

* Mean ± SD (n=3).

Total phenolics in the ethanol extract was found to be the greatest (296.34±5.28µg GAE/mg of extract), followed by the chloroform extract (82.41±1.2µg GAE/mg), and then the hexane extract (23.67±7.12µg GAE/mg). In a similar vein, the flavonoids, flavonols, and condensed tannins found in the ethanol extract were found to be in the highest concentrations.

3.3. Antioxidant activity

The capacity of extracts from *Origanum majorana* to serve as antioxidants was evaluated using DPPH and ABTS methods, and the results are presented in table 2 in the form of IC50 values.

Table 2.IC50 (µg/mL) values of antioxidant capacity of *Origanum majorana* extracts.

	Ethanol extract	Chloro-form ex-tract	n-hexane extract	BHA	BHT
IC50-DPPH (µg/mL)	19.27±0.68	64.65±0.98	32.79±1.81	7.90±0.52	21.14±0.89
IC50-ABTS (µg/mL)	18.02±3.81	22.41±2.10	47.11±0.94	1.-78±0.20	1.30±0.34

The data are the means standard deviations of three measurements. . The values are evaluated using ANOVA, and then the tukey test is performed ($p<0.05$).

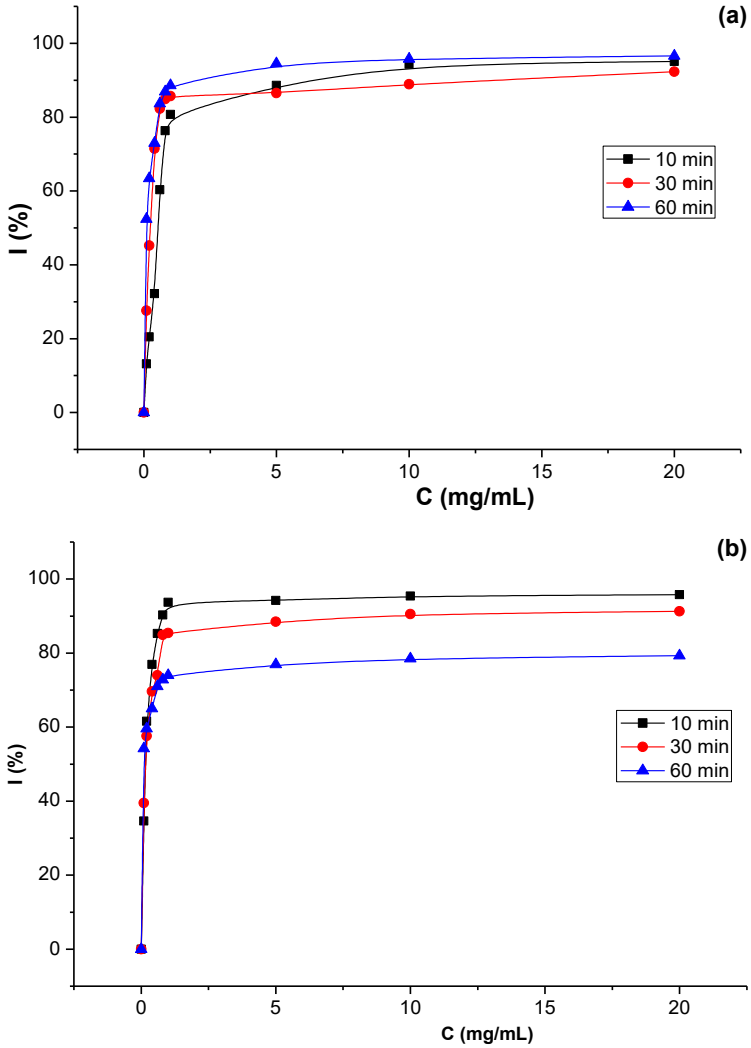
The ethanol extract was found to have the highest level of DPPH scavenging activity, with an IC50 value of 19.27±0.68 µg/mL. This was followed by the hexane extract, which had an IC50 value of 32.79±1.81µg/mL, and the chloroform extract, which had an IC50 value of 64.65±0.98µg/mL. The ethanol and chloroform extracts were the most effective at capturing the radical ABTS, with IC50 values of 18.02±3.81 and 22.41±2.1µg/mL, respectively. The hexane

extract was the least effective, with an IC50 value of 47.11±0.94µg/mL. In addition to this, the extracts shown a powerful capacity to ensnare the radical ABTS.

3.4. The kinetics of ethanolic extract of the Origanum majorana

3.4.1. Antioxidant capacity at temperatures 45, 50, and 60°C

We prepared several different concentrations of the concentrated extract in ethanol and put them in the incubator at a temperatures of 45, 50, and 60°*C* for 10, 30 and 60 minutes. We take 0.5 mL from each concentration and add 1 mL of (DPPH) to it, homogenize the solution and leave it for 30 minutes in the dark, after which the absorbance reading is done in the UV-Visible device at wavelength λ = 517 nm(Figure 1).



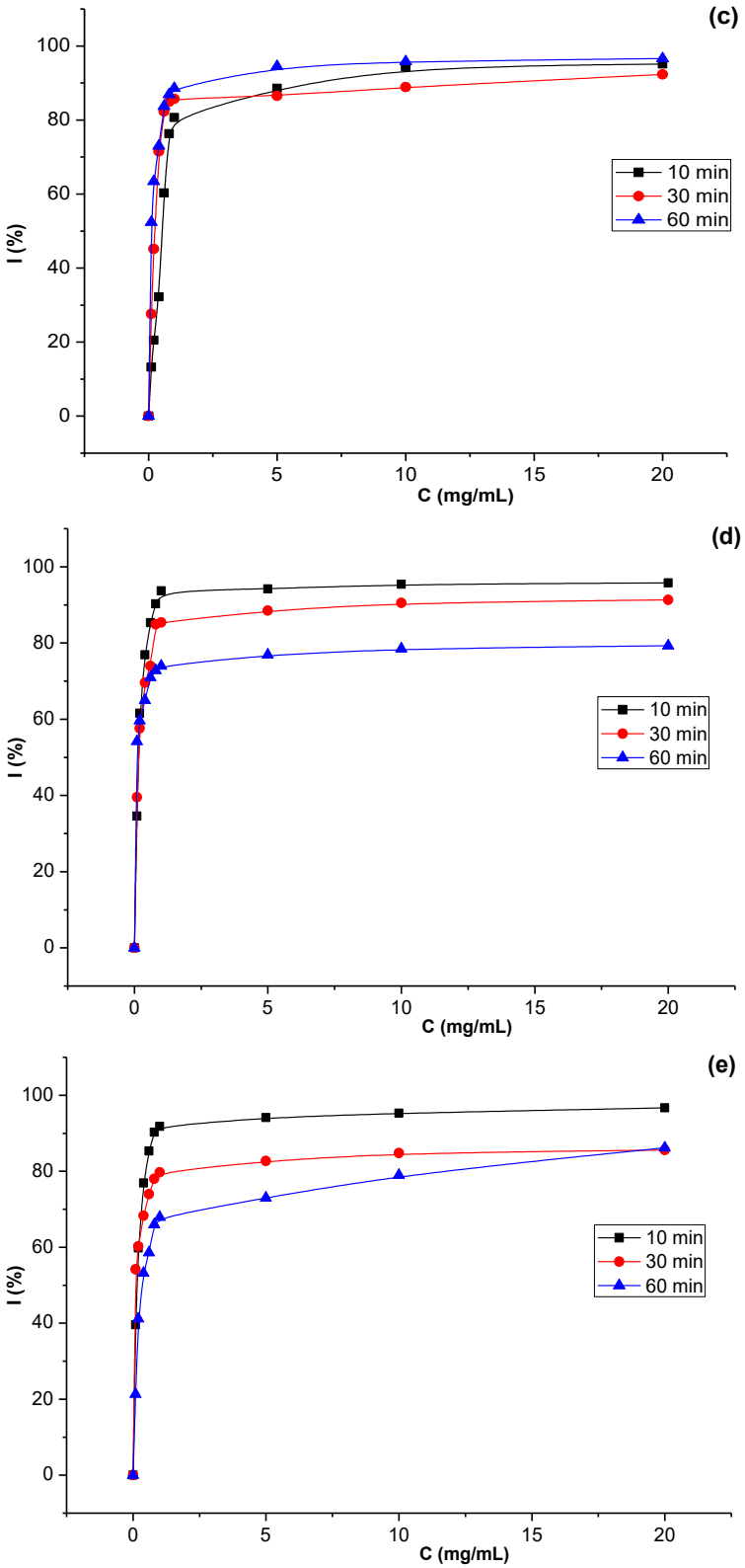


Figure 1. The percent DPPH radical inhibition of extract at different temperatures: (a) 25°C; (b) 35°C; (c) 45°C; (d) 50°C; (e) 60°C

3.4.2. Determine optimal conditions

The aim of this study is to determine the optimal time and temperature for the extract of the marjoram plant to be more effective that is, it has a high ability to inhibit the largest percentage of free radical DPPH, and that is, it has a minimum value of IC50. The following table shows a summary of the results that we obtained after drawing the curves .

In order to provide an explanation for the phenomena of antioxidant activity and TPC, kinetic models were applied to the data and fitted to them. Table 3 presents the results of the parameter fitting process in the form of numerical values. The zero-order kinetic model was able to describe both TPC at all temperatures, which was in line with the findings of earlier investigations.

Table 3. Temperature-dependent kinetic parameters for antioxidant activity and total phenolic content in *Origanum majorana* extract.

	tempera-tures	zero-order		First -order		second-order		Ea
	(°C)	K (mol.L^{-1}.min^{-1})	R^2	K (min^{-1})	R^2	K (mol^{-1}.L.min^{-1})	R^2	(kJ/mol)
Total phenolics content (TPC)	45	0.0062	0.943	0.329	0.982	-0.0073	0.970	32.17
	50	0.0091	0.972	0.357	0.994	-0.0061	0.972	
	60	0.0183	0.991	0.378	0.973	-0.0053	0.987	
Total Antioxidant capacity	45	0.217	0.962	1.511	0.981	0.845	0.993	41.63
	50	0.641	0.983	1.567	0.965	0.891	0.986	
	60	0.936	0.992	1.642	0.993	0.962	0.991	

The kinetics of changes in extract samples heated at different temperatures (45, 50, 60 °C) over one hour were studied by calculating the IC50 for each sample. Factors including, antioxidant activity and varying the rise in antioxidant activity depending on heating temperatures and time, heating marjoram extract to 60 °C for 30 minutes was found to be more effective than heating to 45 or 50 °C, followed by 50 °C. Corresponding to a time of 10, 30 and 60 minutes. The findings of the current research point to the possibility that the antioxidant activity of *Origanum majorana* is due to the presence of phenolic components. According to the results of Koca and Karadeniz [37], which are in line with those of the current research, there is a direct correlation between the

antioxidant qualities of blueberries and the quantity of phenolic and anthocyanin chemicals that they contain (a type of fruit that is found in the Black Sea area). Because of their antioxidant qualities, phenolic compounds are very useful molecules. These features make it easier to get rid of potentially harmful free radicals and stop the transformation of hydroperoxides into free radicals [38].

According to some reports, there is a direct connection between total polyphenol content (measured using the Folin–Ciocalteu method) and antioxidant activity (measured using the trolox equivalent antioxidant capacity assay, the diphenyl picryl hydrazyl assay, and the antioxidant potential of iron regeneration assay) [39]. Researchers from all around the world have looked into the phenolic chemicals and tried to figure out how antioxidant activity is related to them.

There was also an evaluation of first- and second-order models, however these models had lower correlation coefficients. On the other hand, first-order kinetic models worked well for TPC at 45, 50, and 60 °C, respectively, increases in antioxidant activity with treatment time were fitted to second-order, first-order, and zero-order reaction kinetic models, respectively. The effect of the activity of different reactants in the Maillard reaction was brought to light by the various kinetic models that were used for antioxidant activity at various temperatures of heating. This may be because of differences in the temperature sensitivity of the various steps that comprise the Maillard reaction [40]. There has not been a single research that reports the progression of antioxidant activity during the process of heat treatment of extract [41,42], on the other hand, found that the antioxidant capacity of mulberry fruit extract that had been subjected to treatment at temperatures ranging from 80–100 °C matched both first- and zero-order models. Nevertheless, the authors who were previously cited supported the latter model since it is compatible with the findings obtained in the current investigation when the temperature was set to 60 °C. The data presented in Table 3 demonstrates a clear rise in kinetic constants with an increase in temperature. This finding lends credence to the hypothesis that an increase in treatment temperature led to increases in total antioxidant activity as well as TPC.

4. Conclusions

This research, which was carried out in Algeria for the very first time, gave us the opportunity to characterize the pharmacological characteristics of the organic extracts of *Origanum majorana*, a naturally occurring plant that is native to the area of Hassi Khalifa in Algeria. It is necessary to conduct an analysis of the bioactivity of this species as well as a phytochemical screening. It is only possible to speculate that the high enzyme inhibitory effect of *Origanum majorana* could be related to its rich phenolic contents, which are considered to be the major contributors to a wide range of its antioxidant properties. As of right now, there is no report on the bioactivity and phytochemistry of *Origanum majorana*. The results of these tests were analyzed and compared and were shown to be effective antioxidant activities in a number of in vitro tests, including the DPPH free radical scavenging activity and the ABTS radical scavenging activity. In addition, it

was shown that both extracts had high antioxidant activity as well as inhibitory effects of the metabolic enzymes that were identified. The ethanolic extract of the marjoram plant that grows in our region (Hassi Khalifa) and is used in Algerian folk medicine, especially in our region, was evaluated in terms of total antioxidant activity by means of the DPPH test. The kinetics of changes in extract samples heated at different temperatures (45, 50, 60 °C) over a period of one hour was studied. Increasing the treatment temperature and time increased all factors including, the antioxidant activity and varied the rise in antioxidant activity depending on the heating temperatures and time. The results showed that all the extracts significantly displace the DPPH root. It was found that heating the marjoram extract to 60 °C for 30 minutes was more effective than heating to 45 or 50 °C.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, S.NN. and Y.M.; methodology, F.L.; software, S.NN.; validation, ML.B., F.L. and MS.N.; formal analysis, Y.M.; investigation, F.L.; resources, ML.B.; data curation, Y.M.; writing—original draft preparation, MS.N.; writing—review and editing, ML.B.; visualization, X.X.; supervision, S.NN.; project administration, ML.B.; funding acquisition, MS.N. All authors have read and agreed to the published version of the manuscript.

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