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The Chemical Evaluation of Natural Extracts of Marjoram Essential Oils as Antioxidants

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Abstract. *Essential oils of Marjoram (Marjorana hortensis) has been evaluated for possible antioxidant activity. Essential oils were hydrodistilled and the chemical composition of volatile fraction was determined by GC. γ-Terpinene, sabinene, β-phellandrene, terpinen-4-ol and di (2-ethylhexyl) phthalate were the major constituents of Marjoram. The reducing power of essential oils of Marjoram was measured by the ferricyanide method. Different concentrations of Marjoram oil (MO) 25, 50, 75, 100 and 200 ppm were investigated. Higher absorbance at 700 nm of the reaction mixture indicated greater reducing power. The reducing power of an antioxidant is an important parameter reflecting one aspect of its antioxidant properties. The obtained results revealed that increasing the antioxidant capacity is MO in all tested concentrations. The antioxidant capacity of fat-soluble antioxidant was expressed as equivalents of α-tocopherol (nmol/g oil) using a molar absorption coefficient of α-tocopherol (ϵ= 4.0 × 103 M-1 Cm-1. The percent of H2O2 scavenging activity of the tested EOs increased in the MO at all tested concentrations. There was a significant difference among all EOs samples in the percent of H2O2 scavenging activity. a positive correlation has been observed between concentrations and antioxidant activity for 3 days' test period. There were significant differences among the tested EOs in their antioxidant activities. For 3 days, the antioxidant activity of the tested EOs decreased statistically in MO. BHT on the peroxide value of sunflower oil as mmol eq O2/kgoil in the seventh day*

Keywords: *Antioxidants; BHT; Essential oil; Marjoram; Reducing power*

1. INTROUDUCTION

However, ROS and free radicals are only beneficial when they are generated in the proper quantity, at the appropriate time, and in the appropriate location. Because they are highly reactive and target molecules that are very close to them relatively rapidly, they can be quite harmful elsewhere. As a result, ROS and free radicals interact with non-radicals to start harmful chain reactions such lipid peroxidation. They also harm other vital molecules, including as DNA, proteins, and carbohydrates.

Humans and other living things have evolved strong and sophisticated antioxidant systems to protect themselves from harm caused by free radicals and ROS. Antioxidants, a broad class of chemicals that shield important biological locations from oxidative damage, are parts of these systems. Usually, they work by eliminating or deactivating the chemical intermediates that give rise to free radicals.

Antioxidants come from the diet or are endogenously created by the body. They are categorized according to a number of factors, including their solubility in water or lipids, as well as their chemical and physical properties (small molecules, proteins, and enzymatic and non-enzymatic substances). It is crucial to remember that different antioxidants work in systems with substantial dependency and additive or synergistic

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effects rather than alone. These systems are highly specialized defense mechanisms against ROS and free radicals that have been generated by different parts of the body.

One major issue with food shelf life is the oxidative degradation of lipids. Food acceptance is reduced by lipid oxidation, which produces unwanted off tastes (Kerrihard et al. 2015).

Furthermore, when cooking or processing, lipid oxidation produces potentially hazardous compounds and subsequent reaction products, which reduces food safety and nutritional quality (Kasaai 2025).

Widely consumed by humans, plant phenolic and polyphenolic chemicals can be found in fruits, vegetables, soybeans, grains, tea, coffee, red wine, and natural herb extracts. In vitro, extracts from culinary herbs and medicinal plants have potent antioxidant activity. Plant-based phenolic chemicals are primarily responsible for this antioxidant action (Koleckar et al. 2008).

Aim of the Study

The goal of the current study is to identify the most promising natural antioxidant sources by analyzing the chemical makeup and antioxidant activity of the essential oils of marjoram (Majorana hortensis). The study's primary focus was on the chemopreventive, bioevaluative, and chemical evaluation of marjoram essential oils. The results collected have been categorized as follows:

a. The chemical composition of marjoram essential oils was investigated with GC-MS. The chemical evaluation of essential oils of marjoram will be tested by:
b. Reducing power has been determined by ferrecyanide method. All tested essential oils exhibited a reducing power, but to various degrees.
c. A positive correlation has been observed between concentrations and reducing power for the tested EOs.
d. Significant differences have been noticed among the tested EOs in their reducing power.
e. The highest reducing power has been noticed in BO followed by MO and then TO.

2. REVIEW OF LITERATURE

Chemical Composition of Marjoram Essential Oils

Marjoram oil is mainly composed of γ-terpinene (14.1%), cis-sabinene hydrate (10.8%) and terpinen-4-ol (20.8%). while estragole (86.1%) is the major component of basil. (Baratta et al. 1998)**.**

Antioxidants

According to (Sharma et al. 2012), the antioxidant may work by lowering the concentration of oxygen in a specific area; scavenging initiating radicals, like hydroxyl radicals, to prevent the start of a first chain; binding metal ions in forms that will not yield the species that initiate lipid peroxidation, like hydroxyl radicals, ferryl radicals, or Fe2+/Fe3+/O2 complexes; dissolving peroxides by converting them into nonradical products, like alcohols; and/or chain-breaking, which scavenging intermediate radicals, like peroxyl and alkoxyl radicals, to stop further hydrogen abstraction.

ROS is frequently used to refer to all oxygen-derived species. (Sisein 2014) demonstrated that a free radical is any chemical species that may exist independently and has one or more unpaired electrons, with an unpaired electron being one that is alone in an orbital.

Synthetic Antioxidant

(Suh et al. 2005) investigated the artificial antioxidants used in food goods to slow down lipid oxidation, including propyl gallate (PG), tertiary-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT).

Researchers are very interested in finding naturally occurring antioxidants in plants as substitutes for manufactured antioxidants. The ability of natural antioxidants to shield cells and organisms against oxidative stress—which is thought to be a contributing factor to cancer, degenerative diseases, and aging—has been the subject of much research. (Kowalski et al. 2024).

According to (Carocho and Ferreira 2013), antioxidants can be physically categorized into two groups based on their solubility: 1- hydrophilic antioxidants, which include vitamin C and most polyphenolic chemicals, and 2- lipophilic antioxidants, which mostly include vitamin E and carotenoids.

The Antioxidant Activity of Some Essential Oils Extracted from Marjoram Plant

Using rat liver and egg yolk, (Baratta et al. 1998) investigated the antioxidant properties of marjoram essential oil both with and without the radical inducer 2,20-azobis (2-amidinopropane) dihydrochloride (ABAP). In the absence of ABAP, marjoram and rosemary oils showed an activity higher or comparable to that of α-tocopherol at the highest concentrations. The activity of basil oil slightly decreased. In the presence of ABAP, α-tocopherol was shown to be more effective while a small variation was observed with BHT. In both tissues, the activity of the oils decreased considerably, except for the activity of marjoram oil, which was slightly higher.

Instead, research shown how natural polyphenol antioxidants can reduce lipid oxidation in both cooked and fresh beef. When dried spices like caraway, wild marjoram, and marjoram were added, the amount of MDA in the meat samples decreased. However, the dried spices were less effective than the ethanol extracts of ginger, thyme, basil, and sage. By reducing the levels of MDA and peroxide value, the addition of an ethanolic spice extract to meat patties prepared with sodium salt decreased lipid oxidation. Using antioxidants from natural sources, like culinary herbs or other plant components, could extend the shelf life of beef products by up to six months. After the vitamins C, A, and E, naturally occurring, non-toxic plant compounds known as polyphenols are the most important antioxidants (Baâtour et al. 2012).

The antioxidative properties of the essential oil and various extracts made from Origanum acutidens have been evaluated (Sellami et al. 2009). The essential oil showed a moderate antioxidative capacity in studies with β-carotene/linoleic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH). The GC and GC-MS analyses of the oil revealed 38 components, with carvacrol being the main component. The antioxidative activity of the MeOH extracts was higher than that of butylated hydroxytoluene (BHT).

Applications of Natural Antioxidant in Foods

According to (Lee and Shibamoto 2002), lipid oxidation causes the production of unwanted off-flavors and reduces the acceptability of meals, making it a major concern with regard to food shelf life. Consuming foods high in antioxidants may be crucial for maintaining health and preventing disease, according to research by (Güllüce et al. 2003).

Antioxidants are added to foods that contain fat to prevent the formation of toxic compounds and off-flavors caused by lipid oxidation. Previous research has demonstrated that lipid oxidation reduces food safety and nutritional quality by producing potentially toxic products and secondary reaction products during cooking or processing. The chicken flesh treated with a number of dry spices successfully prevented lipid oxidation. The best dry spices were caraway, marjoram, and wild marjoram. The same spices' antioxidant properties were stronger in ethanolic extracts. When applied to cooled and refrigerated pig patties that had previously been treated with NaCl, the ethanolic extract of spices (ginger, thyme, basil, and sage) dramatically reduced the rate of lipid peroxidation. (Amarowicz et al. 2009).

3. MATERIALS AND METHODS

This section includes the various experiments and procedures which were performed during the course of the present investigation.

Extraction of the marjoram oil

A marjoram herb was extracted by water distillation for 3 hours according to AOAC

Gas Chromatographic Analysis

The chromatographic separation of the components of essential oils has been carried out. 1µL of sample solution (1 µg/ml) in hexane was injected in HP 6890 GC. ZB wax capillary column, 30m length× 0.25mm I.D, 0.25µm film. Analysis also was carried out by using the following temperature program – 75-75/8min -initial temperature,75-200 -4°C/min,270-270/3min- Injector: 270°C , detector : FID C°270 . Calibration standard was made by the mono and Sesquiterpenoids authentic samples under the same conditions.

Antioxidant Activity Determination

a. Reducing Power

The following formula was used to calculate the reduction power of marjoram essential oils:

a. 1 ml of potassium ferricyanide 1% and 1 ml of phosphate buffer 0.2 M, pH = 6.6, were mixed with 0.4 ml of ethanolic solutions of each sample at varying concentrations (10, 20, 40, 60, 80 ppm for main components and 25, 50, 75, 100, 200 ppm for essential oils).
b. For 20 minutes, the reaction mixture was incubated at 50°C.
c. Following incubation, the mixture was centrifuged at 650× g for 10 minutes after 1 ml of 10% Trichloroacetic acid was added.
d. 4-The absorbance was measured using spectrophotometry at 700 nm after two milliliters of the supernatant were combined with two milliliters of purified water and 0.4 milliliters of 0.1% ferric chloride. Greater reducing power was shown by the reaction mixture's higher absorbance.

b. Assessment of Overall Antioxidant Capability in Terms of A-Tocopheroel Equivalents

The total antioxidant capacity of marjoram essential oils was determined using the following methodology: 0.5 ml of ethanolic solution of each sample with different concentrations (10, 20, 40, 60, and 80 ppm for their principal component) and 25 ml, 50, 75, and 100 ppm for essential oils were combined with 3.5 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate).

The tubes were sealed and then incubated in a water bath at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of each sample's aqueous solution was measured at 695 nm in relation to a blank.
A standard blank solution, consisting of 3.5 ml of reagent solution and 0.5 ml of ethanol, was incubated similarly to the other samples. α-tocopheroel equivalents are used to represent the antioxidant properties.

$A = Є\ C\ L$

Where

A= sample absorbance

Є= molar absorption coefficient of α-tocopherol $4\times1000\ M^{-1}cm^{-1}$

C= molar concentration of sample as equivalents of α-tocopherol

L= length of cuvette

4. RESULTS AND DISCUSSION

The chemical composition and the antioxidant activity of the essential oils. The present study was concerned mainly with the biochemical evaluation of Marjoram oil. Since the natural antioxidants research occurring in plants as alternatives to synthetic antioxidants is of great interest. The antioxidant activity of the essential oils and their major component were evaluated.

The Result of Chemical Composition of the Essential Oils of Marjoram

Table (1) presents the chemical composition of Marjoram oil. The different constituents. γ-terpinene (12.02%), α-terpinene (10.46%), sabinene (12.59%), terpinen-4-ol (10.62%), β- phellandrene (11.19%) and di-(2-ethylhexyl) phthalate (17.40%) are the major components of Marjoram oil.

Table 1. The chemical composition of marjoram essential oil by gas chromatography

compounds	Marjoram oil
α-Pinene	_
Camphene	_
β- Pinene	_
Sabinene	12.59
Myrcene	0.15
α-phellandrene	1.98
D,L- Limonene	
α-terpinene	10.46
P-Cymene	_
β-phellandrene	11.19

γ- Terpinene	12.02
Cis sabinenehydrate	1.83
Fenchone	_
α-terpinolene	1.26
Trans sabinenehydrate	3.74
Linalool	_
Pulegone	_
1-Borneol+ α-Terpineol	_
terpinen-4-ol	10.62
Carvone+ Citral	_
Dihydrocarveol	_
Geraniol	_
2-Piperitone	_
Eugenol	_
Thymol	_
Carvacrol	_
trans caryophllene	0.65
di-(2-ethylhexyl)phthalate	17.40
α- Thujene	4.10

The Result of the Antioxidant Activity of Marjoram

a. Reducing Power

The reducing power of essential oils of Marjoram was measured by the ferricyanide method. Different concentrations of Marjoram oil (MO) 25, 50, 75, 100 and 200 ppm were investigated. Higher absorbance at 700 nm of the reaction mixture indicated greater reducing power.

Data presented in Table (2). Demonstrate the reducing power of MO at the forementioned concentrations.

Table 2. Reducing power of Marjoram Essential oils as absorbance at 700 nm.

Conc. (ppm)	Marjoram
	absorbance at 700 nm
25	0.16
50	0.26
75	0.37
100	0.45
200	0.91

The obtained results reveal that the highest reducing power has been noticed in MO. The reducing power of an antioxidant is an important parameter reflecting one aspect of its antioxidant properties. A useful way of viewing the interactions among various

antioxidants is to take into account oxidation-reduction potential. Antioxidants can be described as redox reactions. (Kerrihard et al. 2015).

(Huang, Ou, and Prior 2005) demonstrated that the single electron transfer reaction is the foundation of the ferric reducing antioxidant capacity (FRAP). Fe (III) and a single electron donor (ArOH) undergo a single electron reaction in the FRAP method, as indicated by equation (1).

The following requirements must be fulfilled in order for the suggested mechanism to measure the total decreasing power accurately. (1) Under the reaction conditions (thermodynamically), only antioxidants have the ability to decrease Fe (III). (2) In order for the reaction to be finished in a brief assay time (such as 20 minutes for the FRAP assay), the reaction rate needs to be rapid enough. (3) The maximal absorption of Fe (II) occurs at 700 nm, where the oxidized antioxidant ArOH+ and its byproducts of the secondary reaction should not be absorbed. Meeting these requirements is extremely challenging. Since Fe(III)/Fe(II) has a standard redox potential of 0.77 V, any molecule with a lower redox potential can reduce Fe(III) to Fe(II), which raises the FRAP values unnecessarily. For instance, the reducing power cannot be accurately evaluated due to the extremely slow reaction of glutathione with Fe (III).

The Antioxidant Capacity of the Essential Oils of Marjoram by Phosphomolybdenium Method

The antioxidant capacities of the essential oils of Marjoram by phosphomolybdenium method were determined and expressed as equivalents of α-tocopherol (nmol/g oil). The antioxidant capacities of tested essential oils have been measured at concentration levels of 25, 50, 75 and 100 ppm.

Table 3. The antioxidant capacity of thyme, Basil and Marjoram Essential oils by phosphomolybdenium methods as equivalents of α-tocopherol (nmol/g oil)

Conc. (ppm)	Marjoram
	equivalents of α- tocopherol (nmol/g oil)
25	102.5
50	250
75	460
100	640

The obtained results revealed that increasing the antioxidant capacity is MO in all tested concentrations. The antioxidant capacity of fat-soluble antioxidant was expressed as equivalents of α-tocopherol (nmol/g oil) using a molar absorption coefficient of α-tocopherol ($\epsilon = 4.0 \times 10^3\ M^{-1}\ Cm^{-1}$). (Huang et al. 2005).

According to (Jayaprakasha, Jagan Mohan Rao, and Sakariah 2003), the phosphomolybdenium method of determining antioxidant capacity was based on electron transfer, which may be used to explain the mechanism of the antioxidant activity. The assay's foundation was the antioxidant's reduction of Mo (VI) to Mo (V), which was followed by the development of a green phosphate/Mo (V) complex at an acidic pH. At 695 nm, the absorbance of this compound was measured. Mo (VI) plus e- Mo (V).

Percent of H_2O_2 scavenging activity of the essential oils of Marjoram

At concentrations of 25, 50, 75, 100, 200, and 300 parts per million, the essential oils of marjoram were tested for their percentage of H2O2 scavenging activity. Tested EOs exhibited concentration-dependent hydrogen peroxide (H2O2) scavenging, as seen in Table (4). In the first ten minutes of the assay, the H2O2 concentration in the systems containing EOs samples fell extremely precipitously.

Table 4. The percent of H2O2 scavenging activity of the essential oils of Marjoram

Conc. (ppm)	Thyme	Marjoram	Basil
	% H_2O_2 scavenging activity		
25	9.4	9.45	25.73
50	19.71	14.78	44.72
75	34.8	21.03	67.28
100	48.87	29.31	89.8
200	87.26	60.36	100
300	100	90.51	100

At every measured concentration, the tested EOs' percentage of H2O2 scavenging activity rose in the MO. The percentage of H2O2 scavenging activity varied significantly across all EO samples. (Wettasinghe and Shahidi 1999) could be used to describe the mechanism of H2O2 scavenging activity. The following reaction could lead to hydrogen peroxide breaking down into water:

The equation is H2O2 + 2H+ + 2e- 2H2O.

The conversion of H2O2 to H2O may be accelerated by the phenolic chemicals found in marjoram essential oils since they are effective electron donors. However, highly reactive hydroxyl radicals (OH) can be produced by H2O2 when iron ions are present.

The Antioxidant Activity of the Essential Oils of Marjoram in Linoleic Acid System

The antioxidant activity of the essential oils of Marjoram WAS determined in linoleic acid system and compared with BHT (synthetic antioxidant). Different concentrations used in determinations 250, 500, 750 and 1000 ppm for the tested EOs and 200 ppm for BHT.

Table 5. The antioxidant activity of the essential oils of Marjoram in linolic acid system compared with BHT activity.

Periods (Days)	Conc. (ppm)	Marjoram	BHT (200 ppm)
		% antioxidant activity	
	250	13.64	46.59
	500	18.18	
1	750	30.68	
	1000 250	27.27 21.24	69.03
	500	26.55	
2	750	38.94	
	1000 250	46.02 33.33	87.23
	500	36.88	
3	750	42.55	
	1000	54.61	

Table (5) showed that, a positive correlation has been observed between concentrations and antioxidant activity for 3 days test period. There were significant differences among the tested EOs in their antioxidant activities. For 3 days, the antioxidant activity of the tested EOs decreased statistically in MO. the obtained results in the first day have shown that the antioxidant activity of MO was the high antioxidant activity. the antioxidant activity of MO in the first day was 13.64, 18.18, 30.68 and 27.27 % at corresponding concentrations. In the second day, the antioxidant activity of MO was increased to 21.24, 26.55, 38.94 and 46.02 % at corresponding concentrations. in the third day, the antioxidant activity of MO was increased to 33.33, 36.88, 42.55 and 54.61 % at corresponding concentrations. the antioxidant activity of MO showed the lowest activity for 3 days test period at all tested concentrations. the antioxidant activity of MO was increased with the test period. This increasing of antioxidant activity of MO was not related to increase of antioxidant activity but because of the decomposition of peroxide, so decrease in the absorbance of MO samples treatments compared with control

(Sultana, Anwar, and Przybylski 2007) showed that the inhibition of Linoleic acid oxidation was used to assess the antioxidant activity. Lenoleic acid is a polyunsaturated fatty acid that, when oxidized, produces peroxides that convert Fe2+ to Fe3+, which then combines with thiocyanate ions to form a complex that absorbs most at 500 nm. There is less antioxidant activity when the absorbance is higher since there are more peroxides produced during the process.

The Antioxidant Activity of Marjoram Oil and BHT On Sunflower Oil Oxidation

a. Effect of tested EOs and BHT on the Peroxide Value of Sunflower Oil

Since hydroperoxides are the main byproducts of lipid oxidation and are converted to peroxides, it appeared logical to measure the peroxide content in the sunflower oil samples in order to assess the degree of oxidation. The peroxide value was determined for all treatments and expressed as mmol eq O_2/kg oil (6). Data presented in Table (6) described that there was a significant increase in the mean daily level of peroxides formed during the oxidation period for 4 days in control treatment and for 5 days in all other treatments**.**

Table 6. The effect of Marjoram oil and BHT on the peroxide value of sunflower oil as mmol eq O2/kgoil

Period (Days)	Conc. (ppm)	Marjoram value	BHT (200ppm) O_2/Kg Oil	Control
Zero time	250	1.06	1.06	1.06
	500	1.06		
	750	1.06		
	1000	1.06		
1	250	1.83	1.25	1.54
	500	1.49		
	750	1.4		
	1000	1.35		
2	250	1.82	1.3	2.12
	500	1.49		
	750	1.4		
	1000	1.3		
3	250	3.67	2.22	4.1
	500	3.28		
	750	3.04		
	1000	2.75		
4	250	8.14	4.05	13.7
	500	9.2		
	750	9.07		
	1000	8.72		
5	250	14.18	5.98	8.49

	500	13.04		
	750	12.45		
	1000	11.48		
6	250	9.07	4.63	4.44
	500	8.49		
	750	8.10		
	1000	6.56		
7	250	3.36	2.95	2.97
	500	3.76		
	750	5.07		
	1000	4.63		

Table (6) Presented that, BHT on the peroxide value of sunflower oil as mmol eq O_2/kgoil in the seventh day

In the first day the POV decreasing was MO (250 ppm)> control >MO (500 ppm) . In the second day, POV of all treatments was increased MO (250ppm) .in the third day POV decreasing was control > MO (250 ppm) > MO (500 ppm) > MO . POV in the forth day was increased with very fast rate in all treatments especially in control (>MO (500ppm) > MO (750 ppm) . In the fifth day, POV was increased with very fast rate in all treatments but decreased sharply in control. This decreasing in POV of control because of the decomposition of peroxides rate in control to the secondary oxidation products was higher than the formation of peroxides, when the increasing of POV of all other treatments because The POV was > MO (500 ppm) > MO (750 ppm) > BO (1000 ppm) > TO (500 ppm) MO (1000). POV of all treatment was decreased in the sixth day (MO (500 ppm) > MO (750 ppm) . POV decreasing was continued in the seventh day with different degrees indicated that thE, POV of MO (750 ppm) MO (1000 ppm) MO (500 ppm) MO (250 ppm).

All treatments were decreased the peroxide value compared with control treatment. There was a positive correlation between decreasing of the peroxide value and concentrations of all tested EOs. The order of the peroxide value increasing was BHT > MO > control.

As shown in Table (6) at zero time, peroxide value was 1.06 mmol eq O_2/kg oil for all treatments. In the first, second, third and forth day peroxide value was increased at different levels in all treatment, the highest increase in the peroxide value was observed in control treatment while the lowest increase in the peroxide value was observed in 1000 ppm TO and BHT treatments. As known, POV was increased to maximum value then POV was decreased sharply. This because of POV measured the peroxides content of samples.

When, peroxides were a primary products of lipid oxidation and it isn't stable compounds. Peroxides were decomposed to stable compounds (secondary products of lipid oxidation).

5. CONCLUSION

a. The ferricyanide method was used to test the reducing power of Marjoram essential oils. Investigations were conducted with several quantities of Marjoram oil (MO): 25, 50, 75, 100, and 200 ppm. Greater reducing power was demonstrated by the reaction mixture's higher absorbance at 700 nm.
b. An essential metric that represents one facet of an antioxidant's characteristics is its reducing power.
c. 3. Increasing the antioxidant capacity is MO in all tested concentrations, according to the results obtained. The molar absorption coefficient of α-tocopherol (ε = 4.0 × 103 M-1 Cm-1) was used to express the antioxidant capacity of fat-soluble antioxidants as equivalents of α-tocopherol (nmol/g oil).
d. At all tested concentrations, the evaluated EOs' percentage of H2O2 scavenging activity rose in the MO. The percentage of H2O2 scavenging activity varied significantly across all EO samples.
e. During the three-day test period, a positive connection between concentrations and antioxidant activity was noted. The antioxidant activity of the investigated EOs varied significantly from one another. The antioxidant activity of the evaluated EOs dropped significantly during the course of three days in MO5-BHT on the sunflower oil peroxide value as measured by mmol eq O2/kgoil on the seventh day.

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