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EVALUATION OF THE ANTIOXIDANT PROPERTIES AND GC-MSD ANALYSIS OF COMMERCIAL ESSENTIAL OILS FROM PLANTS OF THE Lamiaceae FAMILY

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Abstract

Plants of the Lamiaceae family have been used for thousands of years in cooking, as well as phyto- and aromatherapy. Their essential oils are characterized by high antioxidant and other types of biological activities. In our study, the phytochemical profile and quantification of the essential oil components of thyme, marjoram, and sage were analyzed by gas chromatography with mass-spectrometric detection (GC-MSD). The antioxidant properties of the samples were evaluated using total antioxidant parameters (total antioxidant capacity (TAC), ferric reducing power (FRP), antioxidant activity (AOA) towards 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), and total phenolics by Folin–Ciocalteu method). The obtained FRP was 46–321-fold lower than TAC, which is consistent with the contents of phenolics identified in the samples. Terpenes, isopropylmethylphenols, and eugenol turned out to be the major components of all essential oils and determined their TAC and AOA. The Folin–Ciocalteu method was applicable to the thyme essential oil only. Its FRP, which is based on the reaction of phenolic antioxidants with electrogenerated ferricyanide ions, agreed well with the total phenolic contents (329 ± 17 and 334 ± 15 mg of carvacrol per mL, respectively). The thyme essential oil had the highest antioxidant parameters, while sage showed the weakest antioxidant properties. Positive correlations (r = 0.8846–0.9964) of the antioxidant parameters were obtained.

Keywords: essential oils, total antioxidant capacity, ferric reducing power, total phenolics, coulometric titration, phytochemical profile, marjoram, thyme, sage

Introduction

The Lamiaceae family is one of the most representative in the plant kingdom. Owing to their essential oils, aromatic plants of this family, such as oregano, rosemary, thyme, and sage, are widely used in cooking, phyto- and aromatherapy [1], as well as for the extraction of various bioactive compounds. They also have potential as natural food preservatives and functional food additives, thus contributing to better human nutrition [1, 2]. Since essential oils are highly beneficial – they possess antioxidant, antimicrobial, antitumor, anti-inflammatory, antiviral, and other properties, their traditional application range is steadily expanding [3].

Of particular interest and practical utility are the antioxidant properties of essential oils. The latter are also very useful to characterize plant samples. However, it is important

to consider that the components of essential oils and their amounts are strongly affected by many factors: the type and geographic origin of the plant material, along with the conditions of its growth, harvesting, and storage, etc. [4–6]. These aspects thwart any efforts to unify the features of essential oils. Despite being heterogeneous, all essential oils contain terpenes (hydrocarbon and oxygenated mono- and sesquiterpenes, as well as diterpenes) [7, 8]. The presence of phenolic fragments and double bonds in the structure of terpenes enables them to react with reactive oxygen species, i.e., they exhibit antioxidant activity. Hence, essential oils exert a pronounced antioxidant effect due to the synergistic action of terpenes and some individual compounds [9, 10].

Therefore, it is helpful to evaluate the total antioxidant parameters of essential oils in order to characterize the plant sample in general because this approach takes into account the possible mutual influence of the sample components and the effects they cause.

This paper focuses on thyme, marjoram, and sage essential oils as the samples under investigation. Our overview of the available literature demonstrates that marjoram and sage have been less studied than thyme, oregano, basil, and rosemary. The antioxidant properties of the essential oils of these plants have been characterized using various approaches. In many works, to assess the phytochemical profiles and quantify individual antioxidants, the method of gas chromatography with mass spectrometric detection (GC-MSD) has been applied [5, 11–13]. The total antioxidant parameters have been measured following standard spectrophotometric protocols. Typical examples are summarized in Table 1.

The most commonly studied parameter is the antioxidant activity (AOA) towards stable radicals like 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) [11, 13–21] or peroxyl radicals obtained from 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$) [13, 18, 19, 21]. Oxygen-centered radicals are used less often [15, 21–23] despite they rely on the processes that are more similar to those in living systems. Their application is probably limited by time-consuming procedure complicated by the addition of many reagents to evaluate the hydroxyl radical-scavenging activity [15, 22] and the need to use fluorescent detection for peroxyl radicals [21, 23]. In the β-carotene/linoleic acid bleaching assay, the ability of antioxidants in essential oils to inhibit lipid peroxidation, which is similar to the processes that occur in living cell membranes in the presence of peroxyl radicals, is analyzed [25].

The ferric reducing properties of essential oils must be also taken into account to measure the reducing ability of antioxidants, but they apply only to certain antioxidants contributing to ferric reducing power (FRP) and ferric reducing antioxidant power (FRAP) [26]. Furthermore, the application of Fe^{2+} as a standard in FRP requires standardization because it is unstable and easily oxidized. Another disadvantage is that the results obtained are affected by the time needed to complete the analysis. The reaction between antioxidants and Fe^{3+} takes different amount of time (from several minutes to hours) and depends on the antioxidant nature [27]. For this reason, the resulting data can be controversial unless they reflect a complete reaction.

Recently, coulometric titration with electrogenerated titrants (bromine and ferricyanide ions) has been introduced to evaluate the antioxidant properties of essential oils [28]. Based on the reactivity of individual antioxidants, electrogenerated bromine has been successfully applied to estimate the total antioxidant capacity (TAC) in the presence of phenolics and terpenes [28].

Table 1

Spectrophotometric evaluation of total antioxidant parameters of the thyme, marjoram, and sage essential oils

Antioxidant parameter	Reagent	Plant	Units	Ref.
Antioxidant activity	DPPH$^{\bullet}$	*Thymus algeriensis*	IC_{50}, μg mL^{-1}	[11]
		Salvia officinalis L.		[13]
		Thymus capitatus		[14]
		Origanum majorana L. from Albania		[15]
		Salvia tomentosa Miller		[16]
		Origanum majorana L. from Nepal		[17]
		Thymus quinquecostatus Celak.		[18]
		Origanum majorana L. from Northwest Egypt	Inhibition percentage	[19]
		Origanum majorana, *Thymus satureioides*		[20]
		Thymus zygis and *Thymus hyemalis* from Spain	μmol TE* mL^{-1}	[21]
	$ABTS^{+\bullet}$	*Salvia officinalis* L.	IC_{50}, μg mL^{-1}	[13]
		Thymus quinquecostatus Celak.		[18]
		Origanum majorana, *Thymus satureioides*	μmol TE mg^{-1}	[19]
		Thymus zygis, *Thymus hyemalis* from Spain	μmol TE mL^{-1}	[21]
Hydroxyl radical-scavenging activity	$^{\bullet}OH$	*Origanum majorana* L. from Albania	IC_{50}, μg mL^{-1}	[15]
		Thymus caespititius, *T. camphoratus*, *T. capitellatus*, *T. carnosus*, *T. pulegioides*, *T. zygis* subsp. *zygis*, *T. zygis* subsp. *sylvestris*	Inhibition percentage	[22]
Oxygen radical absorption capacity	ROO$^{\bullet}$	*Thymus zygis*, *Thymus hyemalis* from Spain	μmol TE g^{-1}	[21]
		Thymus mastichina L. from Murcia (Spain)	mg TE	[23]
β-carotene/ linoleic acid bleaching assay	β-carotene/ linoleic acid mixture	*Salvia tomentosa* Miller	Inhibition percentage	[16]
		Origanum majorana L. from Northwest Egypt		[19]
		Origanum majorana, *Thymus satureioides*		[20]
Ferric reducing power	Potassium ferricyanide	*Salvia officinalis* L.	IC_{50}, μg mL^{-1}	[13]
		Thymus capitatus		[14]
		Origanum majorana L. from Nepal		[17]
		Origanum majorana, *Thymus satureioides*	Percentage vs. BHA	[20]
		Thymus zygis, *Thymus hyemalis* from Spain	μM AAE**	[21]
Ferric reducing antioxidant power	Fe^{3+}-2,4,6-tripyridyl-S-triazine complex	*Thymus caespititius*, *T. camphoratus*, *T. capitellatus*, *T. carnosus*, *T. pulegioides*, *T. zygis* subsp. *zygis*, *T. zygis* subsp. *sylvestris*	Inhibition percentage	[22]
		Thymus vulgaris, *Thymbra spicata*	μM of Fe^{+2}/g	[24]

* Trolox equivalent. ** Ascorbic acid equivalents.

This behavior is defined by the properties of electrogenerated bromine: its ability to participate in oxidation reactions, electrophilic addition to multiple bonds, and electrophilic substitution in aromatic systems [29]. Electrogenerated ferricyanide ions react only with phenolic antioxidants and allow the evaluation of FRP reflecting the total phenolic contents [28, 30, 31]. Coulometric approaches are more simple compared to spectrophotometry and can be used with antioxidants of various nature and with different mechanisms of action. Another plus is that an electron acts as a titrant, thereby making the use of standard antioxidants unnecessary. The method is absolute and is not affected by sample dilution; the possibility of miniaturization and automation is favorable in routine analysis [32].

A noteworthy detail is that previous studies on the antioxidant properties of the essential oils of marjoram, thyme, and sage have been based on the samples from wild or cultivated plants of different chemotypes and geographical origin. Another aspect of the majority of these investigations is that the impact of the extraction methods on the properties (antioxidant, antibacterial, etc.) of the final product was studied. Commercial essential oils should step out of the shade and have their phytochemical profile and antioxidant properties thoroughly inspected. Furthermore, total antioxidant parameters could be considered as potential markers of the quality of essential oils.

The purpose of this paper is to characterize the commercial essential oils of marjoram, thyme, and sage by analyzing their phytochemical profiles, quantifying their composition with GC-MSD, as well as assessing the total antioxidant parameters (TAC, FRP, AOA towards $DPPH^{\bullet}$, and the total phenolic content) using the coulometric and spectrophotometric approaches. The relationship between the total antioxidant parameters and the phytochemical constituents of the essential oils is discussed.

1. Material and Methods

1.1. Samples and reagents. Commercially available essential oils of marjoram, thyme, and sage were studied. A tenfold dilution with ethanol was applied for the evaluation of antioxidant properties. Carvacrol (purity 98%) (Aldrich, Germany) was used as a standard for the evaluation of total phenolics. Its 100 mg L^{-1} stock solution was prepared by dissolving an accurately weighed portion in 5.0 mL of ethanol (rectificate). The exact dilution before measurements was used to get less concentrated solutions. A 0.10 mM solution of $DPPH^{\bullet}$ (Aldrich, Germany) was prepared in methanol (c.p.). The Folin–Ciocalteu reagent (Aldrich, Germany) was applied for the total phenolics determination. Other reagents were of chemical purity and used as received.

1.2. Phytochemical profile and analysis by GC-MSD. The identification and quantification of the essential oil components were performed by GC-MSD in the total ion current mode using a Crystal 5000.2 gas chromatograph with a quadrupole MSD and the Advanced Ion Source for the electron impact (EI) and chemical ionization (CI) (Chromatec, Russia), as well as a quartz capillary column CR–5MS ((5%-phenyl)-dimethylpolysiloxane phase, 30 m × 0.25 mm × 0.25 μm). Injection of 1 μL of the essential oil was applied in 1:100 split mode. GC measurements were performed under the following conditions: injector temperature 280 °C, interface temperature 270 °C, ionic source temperature 250 °C. The column temperature was initially 60 °C for 1 min, then gradually increased to 210 °C at 5 °C min^{-1}, raised again to 280 °C at 12 °C min^{-1}, and

kept at 280 °C for 40 min. Helium with a constant flow rate of 0.9 mL min^{-1} was used as a carrier gas. Mass spectra in the positive ions mode were recorded in the range of *m/z* 50–550 after EI ionization at 70 eV. In the case of low intensity (≤ 1% rel.) of molecular ion [M^+] peaks, the CI at 30 eV using methane as reagent gas (flow rate of 1.5 mL min^{-1}) was applied to register more intensive peaks of protonated molecules $[M + H]^+$. Mass spectra-based identification of the components was carried out using the following software: Chromatec Analytic (Chromatec, Russia); NIST MS Search Program V.2.3 (NIST, USA) and NIST 20 (NIST, Mass Spectra Libraries, USA); Wiley Registry of Mass Spectral Data, 12th ed. (Wiley Science Solutions, USA). In addition, the retention times and indices were compared with those reported in [33, 34] and presented in the databases mentioned above.

1.3. Evaluation of TAC and FRP. TAC and FRP were evaluated using coulometric titration of the samples with electrogenerated bromine and ferricyanide ions, respectively [28], using the coulometric analyzer Expert-006 (Econix-Expert, Russia) supplied with a glassy electrochemical cell with four electrodes. Two electrodes (working and auxiliary) formed a generating circuit. The working electrode was a platinum wire with 0.5 cm^2 surface area. The auxiliary electrode (platinum wire) was separated from the anodic compartment of the cell with the semipermeable membrane to avoid side reactions. The other two needle platinum electrodes were polarized with a potential of 200 mV and used as an indicator circuit. Electrogeneration of bromine and ferricyanide ions was carried out from a solution of 0.2 M KBr in 0.1 M H_2SO_4 and 0.1 M $K_4Fe(CN)_6$ in 2 M NaOH, respectively, at a current density of 5 mA cm^{-2}, providing 100% yield of the titrants. The volume of the solution in the electrochemical cell was 20 mL. Coulometric titration was carried out in the following way: the titrant was electrogenerated to the indicator current of 40 μA, an aliquot portion (10 μL) of the 10-fold diluted essential oil was added to the cell, and the timer was started simultaneously. The titration end point was registered at the moment when the indicator current reached the value of 40 μA. TAC and FRP were expressed as the quantity of electricity spent on the titration of the sample and recalculated per 1 mL of the essential oil.

1.4. AOA towards DPPH$^\bullet$. The standard procedure was applied for the estimation of AOA using DPPH$^\bullet$ as a reagent [35]. Briefly, 3 mL of 0.10 mM DPPH$^\bullet$ solution were mixed with 4 μL of the essential oil (10-fold diluted with ethanol) and incubated in the dark for 20 min. Then, the absorption was read at 515 nm using methanol containing 4 μL of the sample as a blank on the spectrophotometer PE-5300 (NPO Ecros, Russia). Control DPPH$^\bullet$ absorption was measured vs. methanol. The AOA of the essential oil was expressed as a relative decrease in the DPPH$^\bullet$ absorption.

1.5. Total phenolics determination. Total phenolic contents were evaluated by the Folin–Ciocalteu method [36] with slight modifications. 0.5 mL of the 10 000-fold diluted thyme essential oil and 1000-fold diluted marjoram and sage essential oils or the standard solution of carvacrol (10, 25, 50, 75, and 100 mg L^{-1}) were placed in a 5.0 mL volumetric flask. Then, 2.5 mL of the diluted Folin–Ciocalteu reagent (1:10 (v/v)) were added and thoroughly mixed. After 4 min, 2.5 mL of 7.5% Na_2CO_3 solution were added, mixed, and incubated for 1 h. The absorbance of the solution was measured at 765 nm in a 0.5 cm cuvette. The blank solution contained all the reagents excluding

essential oil, which was replaced with 0.5 mL of ethanol. Total phe-nolics were expressed in carvacrol equivalents recalculated per 1 mL of the essential oil. Carvacrol calibration graph parameters (Equation 1) were used:

$$A\ [\text{a.u.}] = (0.007 \pm 0.005) + (32.3 \pm 0.8)\cdot 10^{-4}\, c_{\text{carvacrol}}\ [\text{mg L}^{-1}]. \quad (1)$$

1.6. Statistical and correlation analysis. The antioxidant parameters were evaluated as an average value of five (for coulometric titration) or three (for spectrophotometry) parallel measurements. GC-MSD was run in three replications. Statistical treatment of the data obtained was performed at a significance level of 5%. The results were presented as an average value ± coverage interval. A random error was reflected by the relative standard deviation (RSD).

Correlation analysis was performed in the OriginPro 8.1 software (OriginLab, USA).

2. Results and Discussion

2.1. Phytochemical profile of the essential oils. The phytochemical profile of the essential oils was studied by GC-MSD (Figs. 1–3). Identification and quantification data are summarized in Table 2. Components with ω > 0.04% are shown.

The terpene components were relatively similar for the marjoram, thyme, and sage essential oils (Table 2). The major terpenes (ω > 2%) for the marjoram essential oils were terpinene-4-ol (24.7% and 28.0%), isoterpinene (15.53% and 17.5%), γ-terpinene (14.2% and 2.42%), linalyl acetate (8.82% and 10%), α-terpineol (8.35% and 9.5%), β-caryophyllene (4.6% and 4.89%), *o*- or *p*-cymene (5.27% and 4.16%, respectively), α-pinene (3.45% and 3.5%), α-phellandrene (2.98% and 3.3%), and *cis*-sabinene hydrate (1.72% and 2.32%). Limonene (2.49%) was found only in marjoram sample 2. *o*-Cymene (12.0%), β-caryophyllene (4.26%), γ-terpinene (4.1%), linalool (3.3%), and β-myrcene (2.01%) were the major terpenes of the thyme essential oil. Lower levels of α-terpinene (1.86%), terpinen-4-ol (1.67%), and α-pinene (1.66%) were also found. Sage was characterized by the high contents of eucalyptol (19.5%), camphor (17.6%), borneol (9.6%), thujone (6.0%), α-pinene (5.92%), isoborneol (5.8%), linalyl anthranilate (5.7%), camphene (5.2%), β-pinene (4.4%), linalool (3.5%), β-caryophyllene (2.82%), α-terpineol (2.48%), β-myrcene (2.3%) *p*-cymene (2.3%), and α-humulene (2.01%). Other terpenes were present in amounts less than 2%.

The essential oils from Lamiaceae plants are a rich source of natural phenolics (oxygenated terpenes), which is confirmed by the GS-MS data for the essential oils of the marjoram, thyme, and sage samples. Isopropylmethylphenols were the major phenolics. Their contents varied significantly depending on the plant material. The highest contents of both carvacrol and thymol were found in the thyme essential oil (61.5% and 1.50%, respectively). The marjoram essential oils contained carvacrol (0.18% and 0.20%) and trace thymol. Only trace thymol was identified in sage. Furthermore, the thyme essential oil contained eugenol (0.080%), and the marjoram essential oils contained anethole (0.22% and 0.23% in samples 1 and 2, respectively).

In general, the major components identified are similar to those reported for the marjoram [12, 15, 17, 37–41] and thyme [1, 21, 22, 41–45] essential oils. The slight differences were observed in minor components.

The major component of the marjoram essential oils turned out to be terpinene-4-ol. This fits very well with most studies [12, 15, 17, 37–40, 46–48], in which its content

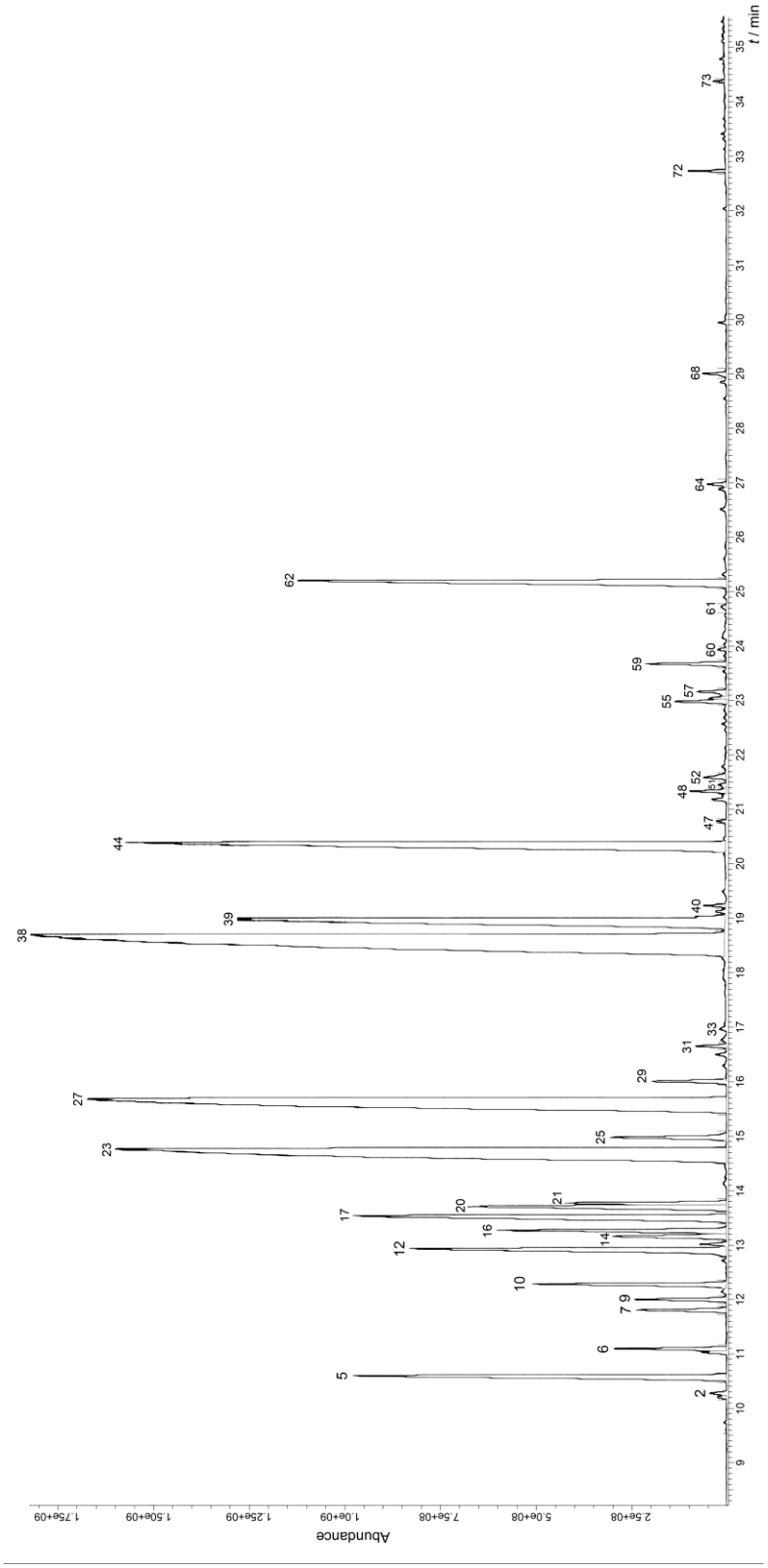

Fig. 1. Chromatogram of the marjoram essential oil

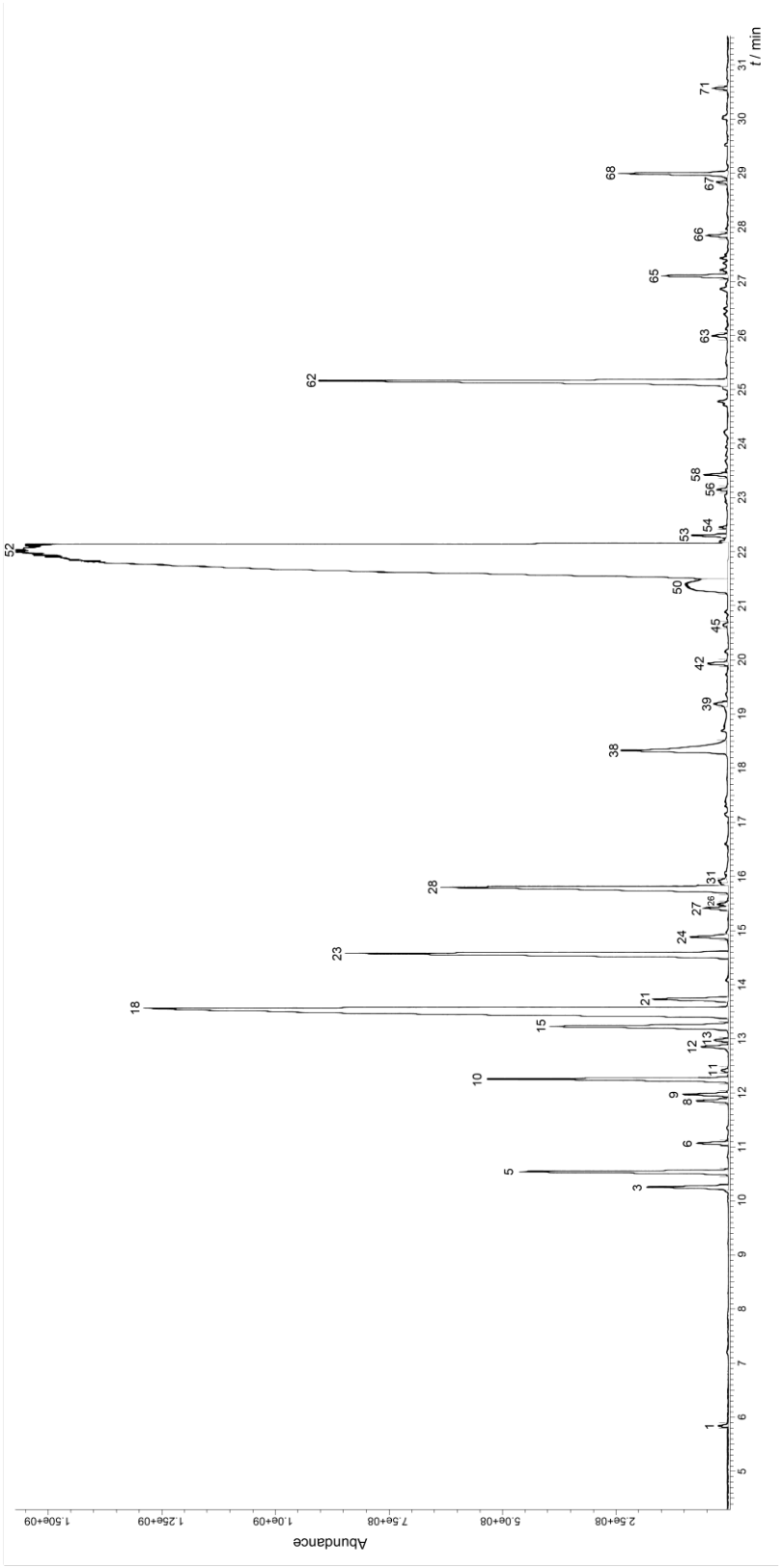

Fig. 2. Chromatograms of the thyme essential oil

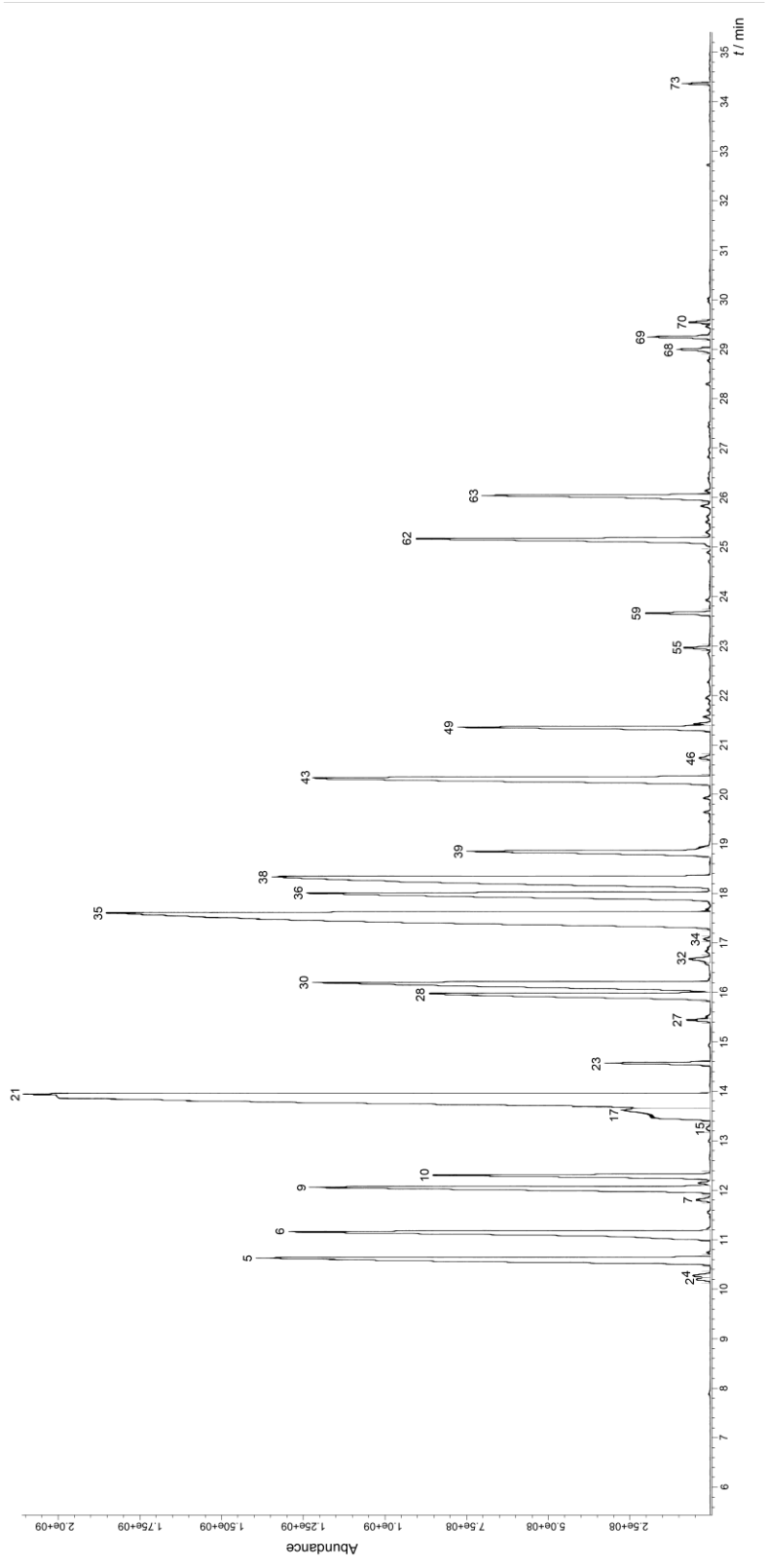

Fig. 3. Chromatograms of the sage essential oil

Table 2

GC-MS identification and quantification of the marjoram, thyme and sage essential oils (n = 3, p = 0.95)

No.*	t_R (min)**	RI***	Component	*m/z* and peak relative intensity (%)****	ω (%)			
					Marjoram 1	Marjoram 2	Thyme	Sage
1	5.84	764	Methyl 3-methyl-butanoate	117 ([M + H]$^+$, 42%), 74 (100%), 43 (30%)			0.070 ± 0.004	
2	10.20	833	4-Ethenyl-1,5,5-trimethylcyclopentene	136 ([M$^+$], 28%), 93 (100%), 121 (58%)	0.060 ± 0.005			0.11 ± 0.04
3	10.26	926 ± 2	β-Thujene	137 ([M + H]$^+$, 55%), 93 (100%), 77 (36%)		0.13 ± 0.06		
	10.25						0.65 ± 0.08	
4	10.27	930	3-Thujene	136 ([M$^+$], 11%), 93 (100%), 91 (52%)				0.16 ± 0.03
5	10.60	933 ± 7	α-Pinene	137 ([M + H]$^+$, 18%), 93 (100%), 91 (41%)	3.45 ± 0.01			
	10.58					3.5 ± 0.1		
	10.53						1.66 ± 0.05	
	10.63							5.92 ± 0.06
6	11.09	946 ± 8	Camphene	137 ([M + H]$^+$, 15%), 93 (100%), 121 (58%)	0.70 ± 0.05			
	11.08					0.49 ± 0.02		
	11.07						0.25 ± 0.06	
	11.17							5.2 ± 0.1
7	11.80	967 ± 2	Sabinene	136 ([M$^+$], 18%), 93 (100%), 91 (40%)	0.59 ± 0.06			
	11.79					0.65 ± 0.08		
	11.81							0.12 ± 0.03
8	11.85	971	Oct-1-en-3-ol	129 ([M + H]$^+$, 31%), 57 (100%), 43 (21%)			0.25 ± 0.09	
9	12.00	974 ± 5	β-Pinene	137 ([M + H]$^+$, 38%), 41 (61%), 77 (28%)	0.60 ± 0.08			
	11.99					0.60 ± 0.06		
	11.97						0.36 ± 0.08	
	12.06							4.4 ± 0.1
10	12.28	983 ± 3	β-Myrcene	137 ([M + H]$^+$, 86%), 69 (80%), 39 (30%)	1.39 ± 0.06			
	12.27					1.7 ± 0.2		
	12.25						2.01 ± 0.06	
	12.30							2.3 ± 0.1
11	12.41	987	Octan-3-ol	131 ([M + H]$^+$, 44%), 59 (100%), 55 (69%)			0.060 ± 0.004	
12	12.93	998 ± 8	α-Phellandrene	136 ([M$^+$],16%), 93 (100%), 91 (33%)	2.98 ± 0.04			
	12.91					3.3 ± 0.4		
	12.85						0.23 ± 0.09	

13	12.97	1010	(Z)-β-ocimene	137 ([M + H]$^+$, 22%), 93 (100%), 91 (50%)			0.12 ± 0.06	
14	13.15	1024 ± 2	Isocineole	154 ([M$^+$], 21%), 43 (100%), 111 (73%)	0.94 ± 0.03			
	13.16					1.84 ± 0.05		
15	13.23	1030 ± 2	α-Terpinene	136 ([M$^+$], 43%), 121 (100%), 93 (85%)			1.83 ± 0.05	
	13.24							0.050 ± 0.003
16	13.27	1033	*cis*-Sabinene hydrate	154 ([M$^+$], 5.3%), 43 (100%), 93 (93%)	1.72 ± 0.04	2.32 ± 0.08		
17	13.53	1050 ± 7	*p*-Cymene	134 ([M$^+$], 25%), 119 (100%), 91 (16%)	4.16 ± 0.05			
	13.62							2.3 ± 0.2
18	13.53	1060 ± 3	*o*-Cymene	134 ([M$^+$], 26%), 119 (100%), 91 (39%)		5.27 ± 0.05		
	13.56						12.0 ± 0.3	
19	13.70	1065	Limonene	136 ([M$^+$], 16%), 68 (100%), 93 (50%)		2.49 ± 0.04		
20	13.71	1067	2-Bornene	136 ([M$^+$], 32%), 93 (100%), 121 (90%)	2.34 ± 0.06			
21	13.73	1072 ± 12	Eucalyptol	154 ([M$^+$], 36%), 43 (100%), 81 (65%)			0.70 ± 0.04	
	13.77				1.02 ± 0.04	1.70 ± 0.06		
	13.88							19.5 ± 0.4
22	14.11	1085	(E)-β-Ocimene	137 ([M + H]$^+$, 28%), 93 (100%), 41 (36%)		0.070 ± 0.004		
23	14.57	1090 ± 14	γ-Terpinene	136 ([M$^+$], 36%), 93 (100%), 91 (37%)			4.1 ± 0.1	0.66 ± 0.08
	14.73				14.2 ± 0.1			
	14.76					2.42 ± 0.01		
24	14.88	1105	*trans*-Sabinene hydrate	155 ([M + H]$^+$, 37%), 93 (100%), 43 (50%)			0.33 ± 0.06	
25	14.97	1108 ± 2	4-Thujanol	155 ([M + H]$^+$, 43%), 93 (80%), 43 (75%)	0.87 ± 0.08			
	14.96					0.92 ± 0.06		
26	15.48	1111	*m*-Cymenene	132 ([M$^+$], 100%), 117 (96%), 115 (67%)			0.080 ± 0.003	
27	15.41	1113 ± 20	Isoterpinene	136 ([M$^+$], 82%), 93 (100%), 121 (76%)			0.18 ± 0.05	
	15.44							0.15 ± 0.04
	15.66					17.5 ± 0.2		
	15.68				15.53 ± 0.06			
28	15.80	1135 ± 12	Linalool	155 ([M + H]$^+$, 18%), 71 (58%), 93 (42%)			3.3 ± 0.3	
	15.97							3.5 ± 0.1
29	16.01	1148 ± 2	*trans*-Sabinene hydroxide	155 ([M + H]$^+$, 30%), 43 (50%), 91 (44%)	0.42 ± 0.07			
30	16.19	1155 ± 2	Thujone	153 ([M + H]$^+$, 33%), 110 (100%), 81 (88%)				6.0 ± 0.5
31	15.92	1160 ± 20	*cis*-*p*-Menth-2-en-1-ol	155 ([M + H]$^+$, 37%), 43 (100%), 139 (51%)			0.090 ± 0.004	
	16.64					0.22 ± 0.08		
	16.65				0.19 ± 0.05			

32	16.67	1182	Fenchol	155 ([M + H]$^+$, 66%), 81 (100%), 80 (53%)				0.22 ± 0.06
33	16.95	1195	β-Terpineol	155 ([M + H]$^+$,42%), 43 (100%), 71 (53%)	0.070 ± 0.004	0.20 ± 0.03		
34	17.07	1200	1,2-Dihydro-linalool	157 ([M + H]$^+$, 31%), 73 (100%), 41 (58%)				0.050 ± 0.003
35	17.60	1238	Camphor	153 ([M + H]$^+$, 30%), 95 (100%), 41 (79%)				17.6 ± 0.6
36	18.00	1262	Isoborneol	155 ([M + H]$^+$, 55%), 95 (100%), 41 (20%)				5.8 ± 0.2
37	18.33	1270	Borneol	155 ([M + H]$^+$, 40%), 95 (100%), 110 (22%)				9.6 ± 0.4
38	18.33	1273 ± 20	Terpinen-4-ol	154 ([M$^+$],15%), 71 (100%), 111 (50%)			1.67 ± 0.06	
	18.64					28.0 ± 0.5		
	18.71				24.7 ± 0.2			
39	18.85	1295 ± 18	α-Terpineol	155 ([M + H]$^+$, 32%), 121 (73%), 136 (44%)				2.48 ± 0.08
	18.96				8.35 ± 0.09			
	18.98						0.14 ± 0.05	
	19.19					9.5 ± 0.2		
40	19.22	1305 ± 2	*trans*-Piperitol	155 ([M + H]$^+$, 25%), 84 (100%), 41 (27%)		0.14 ± 0.06		
	19.23				0.12 ± 0.08			
41	19.48	1315	α-Fenchyl acetate	197 ([M$^+$], 20%), 81 (100%), 43 (74%)		0.090 ± 0.005		
42	19.93	1333	Carvacrol methyl ether	164 ([M$^+$], 31%), 149 (100%), 91 (25%)			0.18 ± 0.03	
43	20.30	1362	Linalyl anthranilate	274 ([M + H]$^+$, 63%), 137 (100%), 119 (70%)				5.7 ± 0.1
44	20.38	1370 ± 2	Linalyl acetate	197 ([M + H]$^+$,16%), 80 (69%), 55 (23%)		10.0 ± 0.2		
	20.39				8.82 ± 0.05			
45	20.64	1388	β-Citral	153 ([M + H]$^+$, 40%), 41 (100%), 69 (85%)			0.050 ± 0.002	
46	20.74	1392	α-Citral	153 ([M + H]$^+$, 18%), 41 (100%), 69 (96%)				0.090 ± 0.003
47	20.78	1395	Dihydrolinalool	157 ([M + H]$^+$, 31%), 73 (100%), 41 (58%)	0.050 ± 0.003			
48	21.32	1412 ± 4	Anethole	148 ([M$^+$], 100%), 147 (54%), 117 (33%)		0.23 ± 0.04		
	21.34				0.22 ± 0.05			
49	21.36	1418	Bornyl acetate	197 ([M + H]$^+$, 43%), 95 (100%), 43 (70%)				1.99 ± 0.09
50	21.39	1422	Thymol	150 ([M$^+$], 25%), 135 (100%), 91 (16%)			1.50 ± 0.06	
51	21.44	1425 ± 2	*trans*-Ascaridol glycol	171 ([M + H]$^+$, 17%), 109 (100%), 127 (55%)		0.10 ± 0.03		
	21.45				0.070 ± 0.008			
52	21.58	1430 ± 20	Carvacrol	150 ([M$^+$], 27%), 135 (100%), 150 (27%)		0.20 ± 0.02		
	21.59				0.18 ± 0.06			
	21.89						61.5 ± 0.4	
53	22.30	1463	2-Methyl-5-(pro-pan-2-ylidene)cyclo-hexane-1,4-diol	171 ([M + H]$^+$, 56%), 74 (100%), 109 (43%)			0.22 ± 0.06	

54	22.45	1476	*trans*-3-*p*-Men-then-1,2-diol	170 ([M^+], 15%), 112 (100%), 97 (74%)			0.050 ± 0.003	
55	22.96	1494 ± 4	α-Terpinyl acetate	197 ([M + H]$^+$, 37%), 121 (92%), 68 (31%)				0.16 ± 0.04
	22.97					0.36 ± 0.05		
	22.98				0.34 ± 0.04			
56	23.15	1508	Eugenol	164 ([M^+], 100%), 103 (36%), 77 (35%)			0.080 ± 0.004	
57	23.16	1510	Nerol acetate	197 ([M + H]$^+$, 32%), 69 (100%), 93 (51%)	0.21 ± 0.06			
58	23.42	1531	Carvacryl acetate	192 ([M^+], 7%), 135 (100%), 150 (61%)			0.17 ± 0.02	
59	23.66	1547 ± 4	Geraniol acetate	197 ([M + H]$^+$, 31%), 69 (100%), 43 (62%)		0.58 ± 0.08		0.42 ± 0.05
	23.68				0.51 ± 0.05			
60	23.93	1578	Cadina-3,5-diene	204 ([M^+], 25%), 161 (100%), 105 (66%)	0.090 ± 0.006			
61	24.72	1621	Isocaryophyllene	205 ([M + H]$^+$, 16%), 41 (100%), 93 (78%)	0.050 ± 0.002			
62	25.16	1655 ± 4	β-Caryophyllene	205 ([M + H]$^+$, 72 %), 93 (100 %), 91 (85 %)			4.26 ± 0.04	
	25.17							2.82 ± 0.08
	25.18					4.89 ± 0.04		
	25.19				4.6 ± 0.1			
63	25.99	1701 ± 5	α-Humulene	205 ([M + H]$^+$, 20%), 93 (100%), 80 (28%)			0.13 ± 0.06	
	26.04							2.01 ± 0.06
64	26.95	1747 ± 4	Bicyclogermacrene	205 ([M + H]$^+$, 41%), 93 (100%), 107 (57%)		0.10 ± 0.06		
	26.97				0.10 ± 0.04			
65	27.10	1763	β-Bisabolene	204 ([M^+], 25%), 69 (100%), 93 (69%)			0.51 ± 0.05	
66	27.84	1788	*trans*-α-Bisabo-lene	205 ([M + H]$^+$, 15%), 93 (100%), 119 (35%)			0.17 ± 0.06	
67	28.83	1826	Spathulenol	221 ([M + H]$^+$, 48%), 43 (100%), 41 (63%)		0.070 ± 0.004	0.090 ± 0.004	
68	28.99	1835 ± 2	β-Caryophyllene oxide	221 ([M + H]$^+$, 27%), 43 (100%), 41 (93%)		0.28 ± 0.08	0.88 ± 0.07	0.23 ± 0.04
	29.00				0.15 ± 0.05			
69	29.24	1861	Viridiflorol	223 ([M + H]$^+$, 24%),109 (100%), 43 (76%)				0.37 ± 0.05
70	29.54	1899	Humulene-1,2-epoxide	221 ([M + H]$^+$, 17%), 109 (100%), 67 (84%)				0.13 ± 0.04
71	30.57	1946	14-Hydroxy-caryophyllene	220 ([M^+], 5%), 91 (100%), 41 (92%)			0.090 ± 0.005	
72	32.71	1967 ± 2	Isopropyl myristate	271 ([M + H]$^+$, 14%), 102 (67%), 55 (39%)		0.090 ± 0.003		
	32.72				0.16 ± 0.08			
73	34.36	1992 ± 2	*m*-Camphorene	273 ([M + H]$^+$, 35%), 69 (100%), 91 (26%)		0.050 ± 0.003		0.11 ± 0.06
	34.37				0.050 ± 0.003			

[*] peak number; [**] retention time; [***] retention indice; [****] [M^+] (EI at 70 eV) and [M + H]$^+$ (CI at 30 eV).

varies in a wide range (21.3–38.4%). Isoterpinene (terpinolene) has been identified in two experiments [17, 41]: their results show that its contents vary significantly (2.5% [17] and 17.1% [41]). These values correspond to those found in our marjoram essential oil samples (15.53 and 17.50%). The detected contents of γ-terpinene, linalyl acetate, and α-terpineol are comparable with the earlier ones [17, 41]. Other studies demonstrate either significantly lower [49] or higher [50] amounts of linalyl acetate. β-Caryophyllene contents in our samples were approximately twice as high as those in [17, 41]. Another characteristic component was sabinene hydrate (both *cis*- and *trans*-isomers), the contents of which were significantly lower than in [12, 15, 17, 37, 39, 40, 51]. According to [41, 52], carvacrol is usually present in trace amounts or absent. However, we found 0.20% carvacrol in the marjoram essential oils.

Carvacrol was the primary component of the thyme essential oil samples. This obviously differs from the data in [21, 41, 43–45] suggesting the prevalence of thymol. Interestingly, in the essential oils extracted from Portuguese thyme species (*Thymus caespititius* Brot., *Thymus zygis* Loefl. ex L. subsp. *sylvestris*, and *Thymus zygis* Loefl. ex L. subsp. *zygis*), carvacrol ranked as a major component. Its content range was 31.8–61.9% [22], and these values are close to our data. *o*-Cymene prevailed among the terpenes in our samples, while other researchers [21, 41, 44, 45, 53] distinguished *p*-cymene as the main terpene. β-Caryophyllene and γ-terpinene contents were in line with those given for the essential oils of *Thymus vulgaris* L. obtained by hydrodistillation using a Dering-type apparatus [41] and commercial samples [44].

The principal components of the sage essential oil were consistent with the reports on the essential oils of *Salvia officinalis* growing in Sudan [54] and other sage plants at various phenological stages [13, 55]. Eucalyptol as a major component and its contents are also in line with these results. The content of camphor was similar to that for the essential oils from Algeria [56].

Generally, the phytochemical profile of essential oils and the content of their main components are determined by a number of factors, such as variations in the chemotypes of plant species [57], place of their origin, seasonal climate variations, as well as the conditions and method of essential oil production [4, 5, 58, 59].

Thus, the identified components of the essential oils are indicative of their antioxidant properties.

2.2. Total antioxidant parameters of the essential oils. TAC and FRP were evaluated based on the reactions of the essential oil components with electrogenerated bromine and ferricyanide ions, respectively (Fig. 4). In our data, TAC was significantly higher than FRP (46–321-fold difference), which is due to the presence of hydrocarbon terpenes that are reactive towards electrogenerated bromine but do not undergo oxidation by ferricyanide ions [28].

The thyme essential oil had the highest TAC and FRP values (1540 ± 20 and 4.8 ± 0.2 C mL^{-1}, respectively) among the studied samples, which is consistent with the phytochemical profile of this sample. Carvacrol was the major contributor to both TAC and FRP. Terpenes (β-caryophyllene, linalool, β-myrcene, α- and β-pinenes, camphene) defined the TAC value of the thyme essential oil. Thymol was also found to be reactive towards both titrants and had an impact on TAC and FRP.

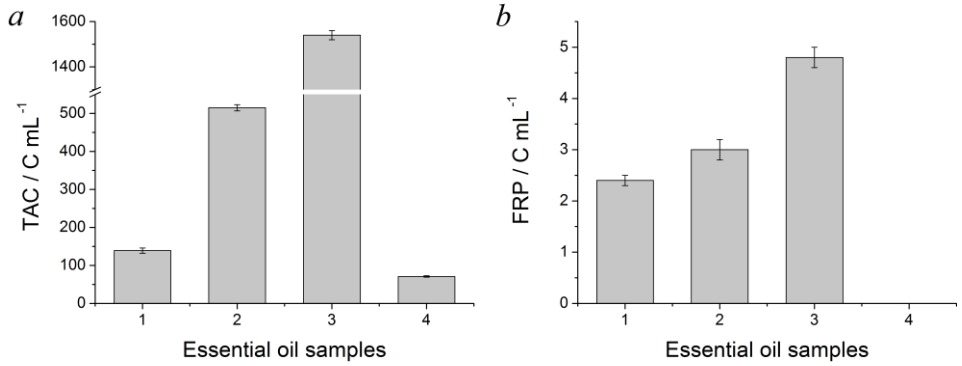

Fig. 4. Total antioxidant parameters of the marjoram (samples 1 and 2), thyme (sample 3), and sage (sample 4) essential oils based on the coulometric titration data: (*a*) TAC, (*b*) FRP

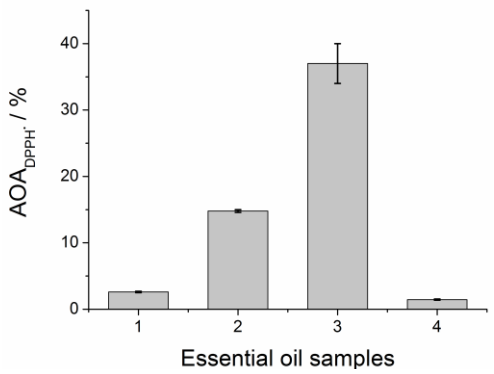

Fig. 5. AOA towards DPPH$^{\bullet}$ of the marjoram (samples 1 and 2), thyme (sample 3), and sage (sample 4) essential oils

Statistically significant differences in TAC and FRP were observed for the marjoram essential oil samples. Both these parameters changed in a similar way. The difference in FRP can be explained by the carvacrol contents in the samples. The trace amounts of thymol contained in the samples also contributed to FRP.

The essential oil of sage was characterized by the lowest TAC value because its major terpenes (eucalyptol, camphor, borneol, thujone, and isoborneol) do not react with electrogenerated bromine. The absence or trace contents of thymol resulted in a low TAC value as well. This is confirmed by the zero value of FRP for the sage essential oil.

The TAC and FRP values agree well with the phytochemical profile of the essential oils and the contents of hydrocarbon and oxygenated terpenes.

The DPPH$^{\bullet}$ test was carried out as a standard procedure to describe the ability of the essential oils under study to react with free radicals. All samples showed AOA towards DPPH$^{\bullet}$ (Fig. 5).

The average values of the investigated parameters differ significantly among the essential oils from Lamiaceae plants that were analyzed. The data obtained are in line with TAC and FRP. The highest inhibition of DPPH$^{\bullet}$ was observed for the thyme essential oil containing the largest amounts of phenolics (carvacrol and thymol), which are the major contributors to AOA. The sage essential oil had the lowest AOA value and contained almost no phenolics (only trace thymol was identified by GC-MSD),

which is an indirect proof of the above finding. The marjoram essential oils demonstrate a 5.7-fold difference in the AOA values, even though their carvacrol contents were comparable. This confirms that other components of the studied essential oils also react with $DPPH^{\bullet}$. The analysis of the resulting phytochemical profile of the essential oils under consideration supports the conclusion that only terpenes can influence the AOA parameter. This assumption complies with the published data on the reactivity of terpenes towards $DPPH^{\bullet}$ [60–62]. For example, limonene, β-myrcene [60], spathulenol [61], and other monoterpenes [62] show AOA in reactions with $DPPH^{\bullet}$. Furthermore, the $DPPH^{\bullet}$ inhibition values are significantly lower than those for phenolic compounds because the H-atom transfer proceeds more easily from the H–O bond than from the H–C bonds (the dissociation energies are 364 kJ mol^{-1} for the allylic C–H bond, 410 kJ mol^{-1} for the alkylic C–H bond, and 452 kJ mol^{-1} for the vinylic C–H bond [62] vs. 243–314 kJ mol^{-1} for the O–H bonds in natural phenolics [63]). Therefore, it is impossible to apply the parameter IC_{50}, which corresponds to the concentration of terpene that causes 50% inhibition of $DPPH^{\bullet}$, because this value cannot be reached even at the highest concentration that this method allows [64]. In this case, relative $DPPH^{\bullet}$ inhibition is usually used. Thus, it is more informative for the studied essential oils to apply AOA towards $DPPH^{\bullet}$ expressed as a percentage of inhibited $DPPH^{\bullet}$.

A similar trend in AOA towards $DPPH^{\bullet}$ has been detected for the thyme, marjoram, and sage essential oils according to [6, 41, 45]. AOA values are also considered with regard to the contribution of phenolics (mostly thymol and carvacrol) as the major components [41]. As known [65], high levels of phenolic constituents remarkably accelerate the reaction with $DPPH^{\bullet}$. The AOA values of the marjoram essential oils in our study were significantly lower than those reported for the *Origanum majorana* L. leaves essential oil from northwest Egypt [19], which can be attributed to the high contents of sabinene and terpinenes.

The total phenolic content of the studied essential oils was measured using the Folin–Ciocalteu method. After a 10 000-fold dilution, the only essential oil which could be studied was that of thyme, while the essential oils of marjoram and sage became turbid after the addition of the photometric reagents. This is caused by the chemical composition of these essential oils. The phenolic content was negligible as compared to terpenes, which are insoluble in water media.

The phenolics of the thyme essential oil were mainly carvacrol (61.5%), thymol (1.50%), and trace eugenol, all being well-soluble in ethanol used in our study for sample dilution and not affected by water media used in subsequent determination of the total phenolic contents.

The total phenolic contents were expressed as equivalents of carvacrol, a major component of the thyme essential oil in our samples. Its average value was 334 ± 15 mg mL^{-1} with the RSD of 1.9%, which agrees well with the FRP value of 329 ± 17 mg mL^{-1} (RSD = 4.2%) obtained by coulometric titration and recalculated as carvacrol equivalents using its stoichiometric coefficient in the reaction with ferricyanide ions.

Gallic acid is usually used as a standard in the determination of the total phenolic content [20, 66–68]. In our opinion, this approach is not useful because the studied essential oils do not contain gallic acid and its reaction with the Folin–Ciocalteu reagent differs from that of carvacrol and/or thymol.

Table 3

Correlation of the essential oils' antioxidant parameters

Antioxidant parameter	r^*	
	AOA_{DPPH}. (%)	TAC (C mL^{-1})
TAC (C mL^{-1})	0.9964	–
FRP (C mL^{-1})	0.8846	0.8846

* r_{crit} = 0.950.

The antioxidant parameters obtained by coulometric titration and spectrophotometry were compared. Positive correlations were revealed, and the corresponding *r*-values are given in Table 3.

The correlation is significant ($p < 0.01$ and $r > r_{crit}$) in the case of TAC vs. AOA_{DPPH}.. Other parameters showed statistically insignificant correlations at $p > 0.1$ and $r < r_{crit}$. However, the strong correlations of TAC with FRP and FRP with AOA_{DPPH}. were seen from the Chaddock scale [69] based on *r*-values.

The correlation data obtained testify that TAC is the most informative and comparable with AOA_{DPPH}.. It is applicable to a wide range of antioxidants in the essential oils of Lamiaceae plants.

Conclusions

The phytochemical profile and total antioxidant parameters of the commercial essential oils from the most commonly used Lamiaceae plants (thyme, marjoram, and sage) were studied. The basic composition of the samples was similar to that from previous works. Their antioxidant effects were determined by phenolic constituents. Terpenes also showed a noticeable yet less pronounced antioxidant effect in the electron transfer reactions with electrogenerated bromine and $DPPH^{\bullet}$. The synergetic effect of hydrocarbon and oxygenated terpenes defined the antioxidant properties of thyme and marjoram. Sage, which does not contain oxygenated terpenes (phenolics), turned out to show weak antioxidant properties. Based on the total antioxidant parameters, essential oils can be characterized in general with respect to the mutual effects of their phytochemical constituents. Therefore, antioxidant parameters can be considered as markers for the primary screening of the essential oils from Lamiaceae plants.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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Оценка антиоксидантных свойств и ГХ-МСД анализ коммерческих эфирных масел из растений семейства Lamiaceae

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Аннотация

Растения семейства Lamiaceae уже много тысячелетий широко используются в кулинарии, а также фито- и ароматерапии. Их эфирные масла обладают высокой антиоксидантной и другими видами биологической активности. Изучен фитохимический профиль и компонентный состав эфирных масел тимьяна, майорана и шалфея методом газовой хроматографии с масс-спектрометрическим детектированием (ГХ-МСД). Антиоксидантные свойства всех образцов оценивали по суммарным антиоксидантным параметрам (интегральной антиоксидантной емкости (АОЕ), железовосстанавливающей способности (ЖВС), антиоксидантной активности (АОА) по отношению к 2,2-дифенил-1-пикрилгидразилу (ДФПГ$^{\bullet}$) и общему содержанию фенольных соединений по методу Фолина – Чокальтеу). Полученные значения ЖВС были в 46–321 раз меньше, чем АОЕ, что согласуется с содержанием фенольных соединений в образцах. Выявлено, что основными компонентами исследуемых эфирных масел являются терпены, изопропилметилфенолы и эвгенол, вносящие вклад в АОЕ и АОА. Метод Фолина – Чокальтеу оказался применим только к эфирному маслу тимьяна. Его ЖВС, основанная на реакции фенольных антиоксидантов с электрогенерированными феррицианид-ионами, хорошо согласуется с общим содержанием фенолов (329 ± 17 и 334 ± 15 мг карвакрола на мл соответственно). Эфирное масло тимьяна характеризовалось наиболее высокими антиоксидантными показателями, а шалфея – самыми низкими. По результатам проведенного анализа установлены положительные корреляции (r = 0.8846–0.9964) антиоксидантных параметров.

Ключевые слова: эфирные масла, интегральная антиоксидантная емкость, железовосстанавливающая способность, общее содержание фенольных соединений, кулонометрическое титрование, фитохимический профиль, майоран, тимьян, шалфей

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