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LC-MS ANALYSIS AND ANTIOXIDANT ACTIVITY OF THE HYDRO-ALCOHOLIC EXTRACT OF *MELISSA OFFICINALIS* L. FROM ALGERIA

Brahim Ben Aicha [a,b], Rachid Rouabhi [a], Salim Gasmi [a*], Chawki Bensouici [c], Hichem Mohammedi [d], Imad Mennai [e]

[a]*Laboratory of Toxicology and Ecosystems Pathologies, Faculty of Exact Sciences and Nature and Life Sciences, Larbi Tebessi University, Tebessa 12002, Algeria*
[b]*Laboratory of Bioactive Molecules and Applications, Faculty of Exact Sciences and Nature and Life Sciences, Larbi Tebessi University, Tebessa 12002, Algeria*
[c]*Laboratory of Biochemistry, Biotechnology and Health Division, Centre for Research in Biotechnology, Ali Mendjli New City, UV 03 BP E73, Constantine 25000, Algeria*
[d]*Laboratory of Functional Organic Analysis, Faculty of Chemistry, University of Sciences and Technology Houari Boumediene, El Alia, PB 32, Bab Ezzouar, Algiers 16111, Algeria*
[e]*Research Unit, Valorisation of Natural Resources, Bioactive Molecules and Physicochemical and Biological Analyzes, University of Mentouri Brothers, 1 Constantine, Ain El-Bey Road, Constantine 25000, Algeria*
[*]*e-mail: biosalim@gmail.com / salim.gasmi@univ-tebessa.dz; phone: +213550861697*

Abstract. The present work focuses on evaluation of the chemical composition and antioxidant activity of the hydro-methanolic extract of *Melissa officinalis* L. from Algeria. The liquid chromatography-mass spectrometry analysis allowed the identification of six compounds: caffeic acid, caftaric acid, hydroxyjasmonic acid glucoside, caftaric acid glucoside, rosmarinic acid and sagerinic acid. The *in-vitro* antioxidant activity of the hydro-methanolic extract was evaluated by using four different methods including: radical scavenging assay (DPPH), scavenging activity (ABTS), cupric reducing antioxidant capacity (CUPRAC), and ferric reducing power assay. The extract exhibited a relatively strong antioxidant activity compared to the synthetic antioxidants. The highest radical scavenging activity was registered using DPPH and ABTS methods, IC_{50}= 20.53±2.64 μg/mL and 22.50±0.67 μg/mL, respectively. These results suggest that *Melissa officinalis* L. could be considered a potential source of natural antioxidants with potential interest in the agrochemical and pharmaceutical industries.

Keywords: hydro-methanolic extract, *Melissa officinalis* L., LC-MS analysis, antioxidant activity.

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Introduction

Over the past decade, there has been a significant interest towards herbal medicine in treating various diseases [1]. Pharmacological and therapeutic properties of medicinal plants have been traced to chemical compounds isolated from their crude extracts, with different physiological roles at low concentrations, ranging from cellular signal transduction to defence against pathogens [2,3]. Excessive reactive oxygen species (ROS) production leads to oxidative stress, which can cause cellular structure damage, including lipids and membranes, protein and deoxyribonucleic acid (DNA) [4]. Therefore, many studies focus on the potential use of natural and synthetic antioxidants in various *in vitro* and *in vivo* models of human pathologies that could be regarded as novel therapeutic agents in the treatment of diseases [3].

Historically, herbs from *Lamiaceae* family have been widely used for many common purposes especially in cooking and medicine [5]. They are known as natural sources of antioxidants due to their high polyphenol content [3,4]. *Melissa officinalis* (*Lamiaceae*) plants are known as a lemon balm growing widely in the Mediterranean region, northern Africa, western Asia and southwestern Siberia [6,7]. *M. officinalis* L. used in traditional medicine [7], was also the focus of several types of research that proved its beneficial effects such as anti-tumoral [8], anti-microbial [9], anti-bacterial [10], anti-inflammatory and antioxidant [10,11]. Many reports demonstrated that ethanolic extracts

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of *M. officinalis* L. cultivated in Iran present good antioxidant activity [3], similarly, the hydro-alcoholic extract of *M. officinalis* originally from Romania had a significant radical scavenging ability [11]. In addition, *M. officinalis* ethanolic extracts may have the potential for cancer chemoprevention [12]. According to Barros, L. *et al.*, rosmarinic acid a compound often identified in the extracts of *Lamiaceae* plants, could be used as a non-steroidal anti-inflammatory agent [13].

Previous studies showed that *M. officinalis* L. leaves contain several classes of constituents including polyphenolic compounds (rosmarinic acid, caffeic acid and protocatechuic acid), essential oils (geranial, neral, citronellal, geraniol, β-pinene, α-pinene, β-caryophyllene, germacrene D and ocimene), monoterpene aldehydes, sesquiterpenes, flavonoids (luteolin) and tannins [8,9,14]. Caftaric acid glucoside is a natural antioxidant that inhibits protein degradation and carbonylation induced by hydroxyl radicals, and restrains the oxidation of DNA [15]. Upon administration, caffeic acid acts as an antioxidant, prevents oxidative stress, thereby preventing DNA damage induced by free radicals [16]. Studies showed that cinnamic acids, such as caftaric acid, a naturally occurring phenolic compound possesses various biological activities, including [17,18].

The main purpose of the present work was to quantify the total phenolic and flavonoid contents in the hydro-alcoholic extract of *M. officinalis* L. and also to identify the main compounds of the extract using LC-MS analysis. The secondary purpose was to assess the antioxidant activity of *M. officinalis* L. aerial part extract by using four different methods: the DPPH radical scavenging assay, the ABTS scavenging activity, the cupric reducing antioxidant capacity and the ferric reducing power assay. In the end, an estimation of the correlation importance has been settled between polyphenols components and antioxidants activities.

Experimental

Generalities

Chemical compounds and reagents such as Folin-Ciocalteu reagent, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT); 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), neocuproine, ammonium acetate, phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, ascorbic acid, α-tocopherol, sodium carbonate, methanol, aluminum nitrate, potassium acetate, were purchased from Sigma (Germany). The measurements and calculations of the activity were carried out on a 96-well microplate reader, PerkinElmer Multimode Plate Reader EnSpire, USA.

The aerial parts of *M. officinalis* L. were collected from Betita, region of Tebessa, north-east of Algeria, in October 2018. A botanist (Prof. Souraya Hayoune) performed the authentication in the Faculty of Applied Biology, Tebessa, Algeria. The samples were air-dried at room temperature and ground into fine powder with an electrical grinder. The samples were packed instantly in polyethylene bags to avoid the decomposition of some bioactive compounds.

Methods

Extract preparation procedure: The *M. officinalis* L. extract was obtained from its powder by using methanol-water mixture (7:3, v:v) with shaking for 24 h at a rotational speed of 200 rpm. The ratio used in this study was 1:10 (m:v) (100 g sample with 1000 mL of solvent). The obtained methanol-soluble fraction was filtered and concentrated under reduced pressure at 60ºC using a rotary evaporator. The extract was kept in the dark at 4ºC after lyophilization.

LC-MS analysis

The high-performance liquid chromatography coupled with mass spectrometry (LC-MS) analysis was performed using an Agilent triple quadrupole 6401 LC-MS system equipped with a binary pump and diode array detector (DAD) system controlled by Mass Hunter workstation software. Mass spectra were registered using ESI negative ionization mode applying a capillary voltage of 3500 V, and a fragment or voltage of 135 V. The most important MS parameters were as follows: nebulization of nitrogen at a pressure of 25 psi at a gas flow rate 7 L/min, ion source temperature at 300°C. Separation was conducted on an SB-C18 column zorbax, 2.1×50 mm×18 μm (Phenomenex) using a mobile phase of acetonitrile: water (formic acid 0.1%) 50:50 (v:v) with a flow rate of 0.5 mL/min.

Confirmation of the *melting point* values relative to recovered pure substance was done using a power compensated differential scanning calorimeter (DSC) SDT Q600 (T.A. Instruments, USA).

DPPH radical scavenging assay

The capacity of the *M. officinalis* L. extract to inhibit the DPPH free radical was evaluated by the method described by Mohammedi H. *et al.* [19]. A volume of 160 μL of DPPH solution

(0.06 mg/mL) prepared in methanol was mixed with 40 μL of the tested sample (extract, BHA, BHT, and α-tocopherol) in different concentrations. After incubation of the mixture in the dark for 30 min, the absorbance was measured at 517 nm. The percentage of DPPH radical-scavenging activity of the extract was calculated according to the Eq.(1).

$$DPPH \text{ scavenging effect (\%)} = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100 \quad (1)$$

where, $A_{Control}$ - the absorbance of blank;
A_{Sample} - the absorbance of the reference or the sample.

ABTS scavenging activity assay

The ABTS scavenging activity evaluation of *M. officinalis* L. extract was performed according to the method reported by Antolovich, M. *et al.* [20]. A volume of 160 μL of the diluted ABTS cation solution was added to 40 μL of the tested sample solution (extract or standards BHT and BHA) at different concentrations (0.0156-1 mg/mL). After incubation for 10 min, the absorbance of the tested samples was measured at 734 nm using a 96-well microplate reader, PerkinElmer Multimode Plate Reader EnSpire, USA, and the percentage of inhibition was calculated using the Eq.(2).

$$ABTS \text{ scavenging effect (\%)} = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100 \quad (2)$$

where, $A_{Control}$ - the absorbance of blank;
A_{Sample} - the absorbance of positive control or sample.

Cupric reducing antioxidant capacity

The determination of the reducing antioxidant capacity (CUPRAC) was assayed using the method described by Apak, R. *et al.* with minor modifications [17]. A volume of 50 μL of neocuproine solution (7.5 mM) and 60 μL of ammonium acetate buffer (1 M, pH 7.0) were mixed with 50 μL of Cu^{2+} solution (10 mM). The mixture was added to 40 μL of the sample solution *M. officinalis* L. extract at different concentrations. After 60 min, the absorbance was recorded at 450 nm against a reagent blank using a 96-well microplate reader. The results were given as EC_{50} (μg/mL) corresponding to the concentration indicating 50% absorbance intensity and those of the standards BHA and BHT.

Ferric reducing power assay

The reducing power was determined according to the method described by Oyaizu, M. *et al.* with some modifications [21]. A volume of 10 μL of the prepared sample at various concentrations was mixed with 40 μL of phosphate buffer (0.2 M, pH 6.6) and 50 μL of potassium ferricyanide (10 mg/mL). The mixture was then incubated at 50°C for 20 min. The solution was mixed with 50 μL of trichloroacetic acid solution (100 mg/mL), 10 μL of ferric chloride solution (1.0 g/L), and finally diluted with 40 μL of distilled water. For measuring the absorbance, a 96-well microplate reader was used at 700 nm. Ascorbic acid and α-tocopherol were used as standards.

The results were given as EC_{50} (μg/mL), corresponding to the effective concentration for which the absorbance at 700 nm is 0.5.

Determination of total phenolic content (TPC)

The total polyphenols were determined using the Folin-Ciocalteu reagent (FCR) method [22] and according to a microplate assay method described by Müller, L. *et al.* [23]. A volume of 20 μL of plant extract was added to 100 μL of diluted FCR (1:10); 4 min after, a volume of 75 μL of sodium carbonate (7.5%) was added. At room temperature, the solution was allowed to react for 2 h and the mixture was incubated. The absorbance was recorded at 765 nm. By replacing the extract with the solvent used (methanol), the blank was prepared in the same way. The TPC was expressed as microgram of gallic acid equivalent/mg (μgGAE/mg).

Determination of total flavonoid content

The content of flavonoids in the mixture depends on the formation of a complex between Al^{+3} and the flavonoids. The method proposed by Topçu, G. *et al.* was used with some modifications for the determination of a 96-well microplate [24]. A volume of 50 μL of the tested sample was mixed to 130 μL (methanol). A volume of 10 μL aluminium nitrate (10%) and 10 μL potassium acetate (1 M) were added. The mixture was vigorously stirred and incubated for 40 min; then the absorbance was measured at 340 nm. A blank sample was prepared by replacing the reagents with methanol (180 μL methanol). Total flavonoids were calculated using the standard quercetin compound (12.5, 25, 50, 75 and 100 μg/mL). All values were expressed as microgram of quercetin equivalent/mg (μgQE/mg).

Statistical analysis

All data on biological activity tests were the average of triplicate analyses. Data were recorded as the mean ±standard deviation. Significant differences between means were determined using Student's *t*-test; p values <0.05.

Results and discussion

Chemical composition of M. officinalis L. extract

Six phenolic constituents have been identified in *M. officinalis* L. extract LC-MS analysis (Figure 1). Identification of every single compound was mainly based on their retention time, mass spectrum, and MS^2 fragmentation profiles with standards analysed under the same experimental conditions and/or with published data in Figure S1 (see Supplementary material). The identified compounds, their retention times and mass spectral data are summarized in Table 1.

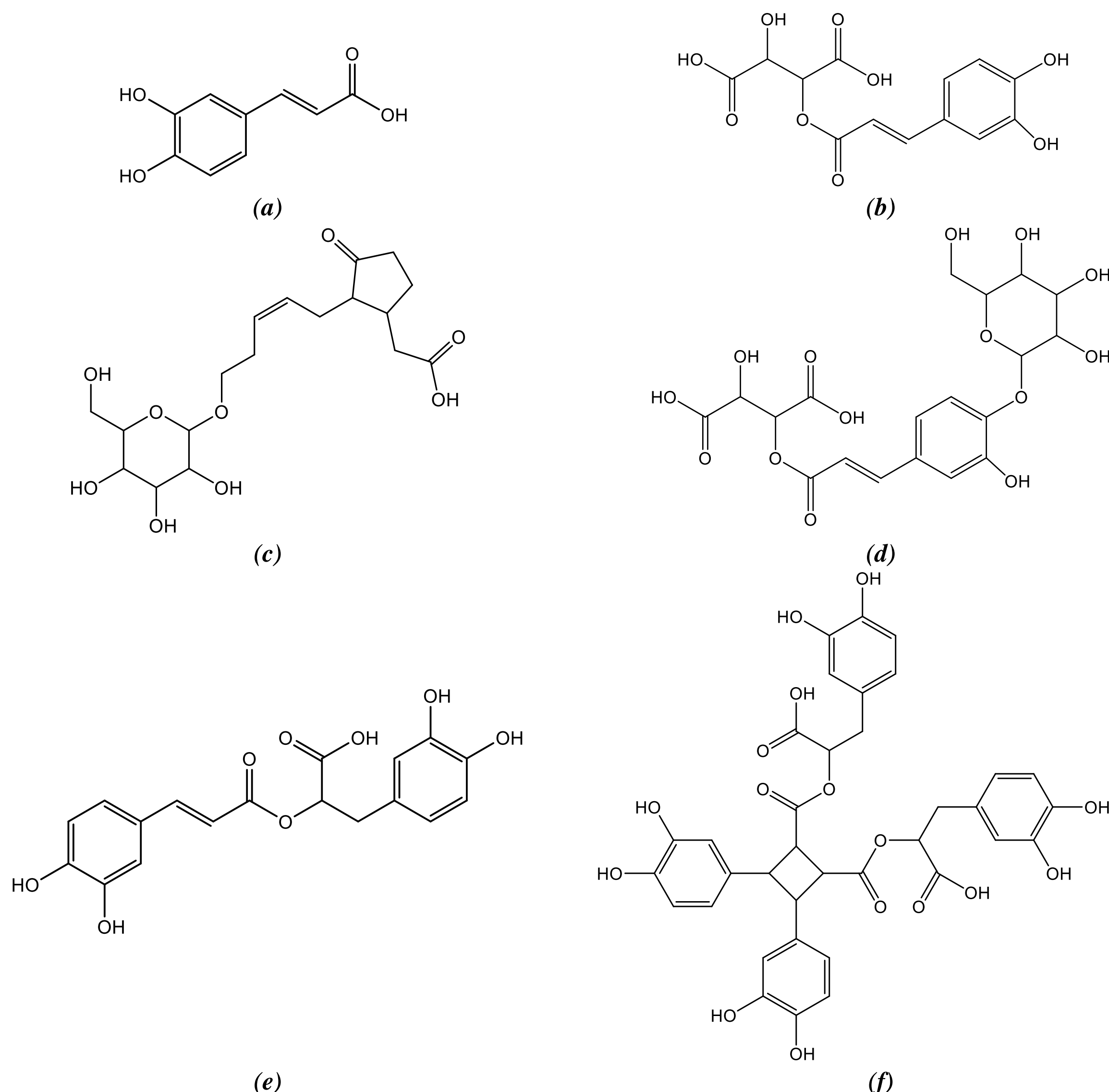

Figure 1. The chemical structures of the identified compounds in *M. officinalis* L. extract: (*a*) caffeic acid, (*b*) caftaric acid, (*c*) hydroxyjasmonic acid glucoside, (*d*) caftaric acid glucoside, (*e*) rosmarinic acid and (*f*) sagerinic acid.

Table 1

The ESI-LC-MS analysis of some phenolic constituents from *M. officinalis* L. hydro-methanolic extract.

	Compounds	*Rt* (min)*	*Molecular formula*	*Molecular ion $[M-H]^-$ (m/z)*	*MS^2 (m/z)*	*Ionization mode*	*Reference*
1	Caffeic acid	40.528	$C_9H_7O_4$	179	135, 175	N	[25]
2	Caftaric acid	40.663	$C_{13}H_{11}O_9$	311	135	N	[25]
3	Hydroxyjasmonic acid glucoside	40.958	$C_{18}H_{27}O_9$	387	179	N	[25]
4	Caftaric acid glucoside	41.927	$C_{13}H_{12}O_9$	473	225, 310.9	N	[13]
5	Rosmarinic acid	43.215	$C_{18}H_{16}O_8$	359	161	N	[13]
6	Sagerinic acid	43.326	$C_{36}H_{31}O_{16}$	719	359	N	[13]

Rt - retention time.*

As shown in Table 1, a total of six phenolic compounds were identified. However, several lines of evidence have been determined. The identification was based on the available literature for these compounds [13,25].

Compound **1** was identified as caffeic acid, it exhibited a $[M-H]^-$ ion at m/z 179 (Figure S1*(a)*), lead to three peaks at 161, 135 and 121 (m/z) corresponding to the deprotonated form of 3-(4-hydroxyphenyl)acrylic acid, 4-vinylbenzene-1,2-diol and 3,4-dihydroxyphenyl methine respectively (Figure S1*(a)*) based on the fragmentation pattern found in the literature [26]. It is shown in Figure S1*(d)* a molecular ion $[M-H]^-$ at m/z 312, yielding prominent ion at m/z 179. Compound **2** (caftaric acid) was identified according to their mass spectrum, showing a molecular ion $[M-H]^-$ at m/z 311 and characteristic fragment ion at m/z 179 corresponds to the deprotonated form of 2-(formyloxy)-3-hydroxysuccinic acid (Figure S1*(b)*) [25]. In addition, this compound showed two peaks 135 and 149 (m/z) in its mass sprectrum corresponding to deprotonated form of 4-vinylbenzene-1,2-diol and 2,3-dihydroxysuccinic acid, respectively [27]. Compound **3** (hydroxyjasmonic acid glucoside) was identified according to its mass spectrum, showing a molecular ion $[M-H]^-$ at m/z 387 and a characteristic fragment ion at m/z 179 corresponding to deprotonated 6-(hydroxymethyl)tetrahydro-2*H*-pyran-2,3,4,5-tetraol (Figure S1*(c)*), the compound **3** is derived from jasmonic acid [25]. Moreover, this compound exhibited a $[M-C_2H_3O_2-OH]^-$ ion at m/z 310 (Figure S1*(c)*) and is in line with similar results previously reported [13,25]. Figure S1*(d)* shows a molecular ion $[M-H]^-$ at m/z 473 yielding product ions at m/z 179 and 310.9 corresponding to deprotonated glucose and caftaric acid, respectively, and based on the previously reported results, the compound **4** was identified as caftaric acid glucoside (caftaric acid derivative) [13]. Compound **5** was identified as rosmarinic acid, it exhibited a molecular ion $[M-H]^-$ ion at m/z 359, leading to three peaks at 197, 179 and 161 (m/z) corresponding to the deprotonated form of 3-(3,4-dihydroxyphenyl)lactic and caffeic acids and their dehydrated forms, respectively (Figure S1*(e)*). These results were in agreement with the fragmentation scheme proposed by Moller, J. *et al*. [28]. Compound **6** was identified as sagerinic acid based on the fragmentation pattern found in the literature where dimerization had occurred by a union of the olefinic moieties and supported by prominent parent ion at m/z 719 consistent with $[M-H]^-$ and a base peak at m/z 359 corresponding to $[M/2-H]^{-2}$ (Figure S1*(f)*) [29,30].

Antioxidant activity

The antioxidant properties of *M. officinalis* L. are associated with the high content of phenolics and flavonoids compounds that may contribute to the plant's antioxidant activities [31].

In the present study, the antioxidant activities of *M. officinalis* L. were determined using four different tests: DPPH radical scavenging activity, ABTS scavenging activity, cupric reducing and ferric reducing power. The results of antioxidant activities are given in Table 2.

The DPPH free radical-scavenging by antioxidants is due to their hydrogen-donating ability [32]. As shown in Table 2, the extracts were proved to have a significant antioxidant activity as they require a low concentration in order to inhibit 50% of DPPH (20.53±2.64 μg/mL). Comparing with other results, Caleja, C. *et al.* working on *M. officinalis* and using the same solvent have reported a lower antioxidant activity (IC_{50}= 79±2 μg/mL) [33]. On the other hand, the obtained results in this study are similar to those reported by Lin, J. *et al.* and Mabrouki, H. *et al.* (IC_{50}= 36.15±1.71 μg/mL; 18.16±0.64 μg/mL, respectively) [31,34]. Another method to assess the antiradical activity of the extract of *M. officinalis*, the ABTS decolourization assay, is applicable for both hydrophilic and lipophilic antioxidants to measure the loss of colour when an antioxidant is added to the blue-green chromophore $ABTS^+$ (2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) [35]. *M. officinalis* extract reduces $ABTS^+$ to ABTS and decolorizes it. Table 2 depicts the result of the ABTS test of *M. officinalis* methanolic extract aerial parts, a strong exhibited activity (IC_{50}= 22.50±0.67 μg/mL) compared with the result for the *M. officinalis* infusion reported by Gayoso, L. *et al.* (IC_{50}= 491.67±16.67 μg/mL) [36]. The obtained results appear to be better correlated with the finding of Dastmalchi, K. and Jafarpour, M. with an IC_{50} of 8.60±1.48 - 12.7±0.4 μg/mL respectively, for the ethanol extract of *M. officinalis* [3,14].

The results showed an association between the reducing power of a bioactive compound and the antioxidant activity (Table 2), this result may significantly reflect its potential antioxidant activity [35]. The ferric reducing power method represents the ability of antioxidants in the extracts to transform Fe^{3+} into Fe^{2+}. The sum of Fe^{2+} was accompanied by measurement of Perl's Prussian blue at 700 nm absorption [37].

Table 2

Comparative analysis of antioxidant activity of *M. officinalis* L. extract.

Extract and standards	*Antioxidant activity*			
	DPPH[a]	*ATBS*[a]	*CUPRAC*[b]	*Ferric reducing power*[b]
M. officinalis L	20.53±2.64	22.50±0.67	29.18±2.61	24.09±0.50
BHA	6.14±0.41	1.29±0.30	5.35±0.71	nt
BHT	12.99±0.41	1.81±0.10	8.97±0.94	nt
α-Tocopherol	13.02±0.17	nt	nt	34.93±2.38
Ascorbic acid	nt	nt	nt	6.77±1.15

Values expressed are means ±S.D. of three parallel measurements;
[a]*Values expressed in IC_{50} (μg/mL);*
[b]*Values expressed in EC_{50} (μg/mL);*
nt: not tested.

Table 2 presents the ferric reducing power $A_{0.5}$ value of *M. officinalis* extract compared to the standards *α*-tocopherol and ascorbic acid. Our results have shown that *M. officinalis* had effective ferric reducing power (EC_{50}= 24.09±0.50 μg/mL) as compared to *α*-tocopherol (EC_{50}= 34.93±2.38 μg/mL). The obtained reducing power was higher than that of the ethanolic extract of *M. officinalis* previously reported (EC_{50}= 49±1 μg/mL) [33].

The CUPRAC method is based on the reduction of Cu^{2+} to Cu^{+} at neutral pH by reductants (antioxidants) present in a sample, using the copper(II)-neocuproine reagent as the chromogenic oxidant [38]. The results of the CUPRAC essay of *M. officinalis* extract is presented in Table 2. The *M. officinalis* extract exhibited strong antioxidant activity (EC_{50}= 29.18±2.61 μg/mL), comparable to that reported by Bendjabeur, S. *et al.* (EC_{50}= 19.40±0.78 μg/mL) [39]. Also, a higher activity than the infusion of *M. officinalis* was reported by Sentkowska, A. *et al.* (267 mgGAE/g and 2.62 mmolTRE/g) [16].

Total phenolic content

The total phenolic content (expressed as gallic acid equivalent in mg/g material, mgGAE/g) of *M. officinalis* L. extract is presented in Table 3.

Table 3

Total phenolic compounds and flavonoids contents in the leaves of *M. officinalis* L. extract (1 mg/mL).

Compounds	*Content*
Total phenolic content, (mgGAE/g)	118.62±6.57
Total flavonoid content, (mgQE/g)	52.77±2.50

Values were expressed as means ±S.D. (n= 3).

After the quantitative evaluation and the phytochemical analysis, by using LC-MS, of the hydro-alcoholic extract of *M. officinalis*, leaves showed the existence of main phenols and flavonoids. The extraction yield was 13.37%; the total phenolic content was 118.62±6.57 mgGAE/g. The obtained result was higher than those reported in the same species from Egypt and India (71.02±4.43 and 48.66±3.03 mgGAE/g, respectively) [40,41]. However, samples from Brazil and Taiwan were characterized by an equal higher content of phenols (177±13 and 175.15±11.02 mgGAE/g, respectively) [34,42].

These differences in phenols content can be attributable to a wide range of factors, such as genotypical differences, geographical and climate conditions, harvest time, and cultural traditions [26,38].

Total flavonoid content

As shown in Table 3, the total of flavonoid content in *M. officinalis* L. extract was 52.77 mgQE/g extract. The obtained value was higher than that determined in hydro-alcoholic extract from Brazil (26±3 mgQE/g extract) [42]. Most previous studies on various plants have shown that absolute methanol is recommended for flavonoid extraction [41]. These flavonoid compounds have a large scale of chemical and biological activities. Indeed, their antioxidant activity strength is due to its compound formula, depending on chemical structures and hydroxyl groups' number and position [43].

Correlation between antioxidant activities and phytochemical compounds

A significant correlation between the IC_{50} values and the antioxidant activities for all methods was attested. Wherefore, the correlation coefficients between the antioxidant capacities and the total phenolic content for extract were determined (Table 4).

Several studies showed that plant extracts' antioxidant activity is highly related to the total phenolics and not to an individual phenolic compound [43]. This study highlights a significant correlation of total phenolic content with antioxidants activities (Table 4).

Table 4

Correlation coefficients (r) for relationships between assays.

	TPC[a]	*DPPH*[b]	*ABTS*[c]	*CUPRAC*[d]	*Ferric reducing power*[e]
TPC[a]	1	/	/	/	/
DPPH[b]	-0.98955	1	/	/	/
ABTS[c]	-0.97103	0.92646	1	/	/
CUPRAC[d]	-0.96097	0.91106	0.99923	1	/
Ferric reducing power	-0.96045	0.91036	0.99916	0.99999	1

[a]Total phenolic content; [b]Concentration of test compound required to produce 50% inhibition of DPPH radical;
[c]Concentration of test compound required to produce 50% inhibition of ABTS radical;
[d]Coefficient of CUPRAC;
[e]Coefficient of ferric reducing power.

The correlation coefficient between total phenolic content and DPPH radical scavenging activity (r= -0.98955) was the highest, compared with that of the total phenolic content with the other tests ABTS activity (r= -0.97103, -0.96097 and -0.96045, respectively for ABTS activity, CUPRAC assay and ferric reducing power). Among all antioxidants activities, the highest correlation was found between the ferric reducing power and CUPRAC activity (r= 0.99999). Meanwhile, a positive correlation was obtained between DPPH radical scavenging activity and ABTS activity value (r= 0.92646), DPPH radical scavenging activity, and CUPRAC assay (r= 0.91106) and DPPH radical scavenging activity and ferric reducing power (r= 0.91036). This study's highly significant correlations support the hypothesis that phenolic compounds have a great contribution to the total antioxidant capacity of the examined plant species following the findings of Piluzza, G. *et al.* [44].

Conclusions

LC-MS analysis and antioxidant activity studies of *Melissa officinalis* L. aerial parts were performed. As a result of this study, six constituents were identified in the hydro-alcoholic extract: caffeic acid, caftaric acid, hydroxyjasmonic acid hexoside, caftaric acid hexoside, rosmarinic acid and sagerinic acid. This extract is considered a potential source of natural antioxidants, which is sustained by the strong antioxidant activity that was determined in this study (DPPH, IC_{50}= 0.53±2.64 µg/mL, ABST, IC_{50}= 22.50±0.67 µg/mL; CUPRAC, EC_{50}= 29.18±2.61 µg/mL and ferric reducing power, EC_{50}= 24.09±0.50 µg/mL).

The content of phenols was 118.62±6.57 mgGAE/g and that of flavonoids was 52.77±2.50 mgQE/g. A significant correlation between the total phenolic content and the antioxidants activities was observed.

The obtained results show that *M. officinalis* extract has a natural antioxidant power more potent *in vitro* and rich in polyphenols and flavonoids. However, further studies are necessary to isolate these bioactive molecules and individually evaluate their antioxidant activity.

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Supplementary information

Supplementary data are available free of charge at http://cjm.asm.md as PDF file.

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