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CHEMICAL COMPOSITION, MINERAL CONTENT AND ANTIOXIDANT CAPACITY OF PHENOLIC EXTRACTS AND ESSENTIAL OILS OF *LAVANDULA STOECHAS* L.

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Abstract: *Lavandula stoechas* L. from the west of Algeria were analyzed for their chemical composition, mineral content, total phenol and flavonoid contents, and also the antioxidant activity of the essential oils and methanol extract. Many essential minerals including potassium, magnesium, sodium and trace elements, such as iron, zinc, copper and manganese were determined. The total phenol and flavonoid contents in *Lavandula stoechas* were determined spectrophotometrically as 9.74 mg ± 0.48 gallic acid equivalents / gram of plant, and 4.615 mg ± 0.18 rutin equivalents / gram of plant for polyphenols and flavonoids respectively. The essential oils extracted by water distillation leads to extract 1.36 % dw, its composition identified by the GC–MS analysis was characterized by high proportions of *α*-fenchone (39.0 %), camphor (18.5 %), bornyl acetate (7.79 %), viridiflorol (4.55 %), and myrtenyl acetate (3.32 %). Our results show, in all tests of antioxidant capacity, that methanol extract has power to scavenging free radicals and reducing metals, better than *Lavandula stoechas* essential oils.

Keywords: *cyclic voltammetry, GC–MS, Lavandula stoechas L., minerals, phenolic extract*

INTRODUCTION

Phenolic compounds and essential oils are important plant constituents for effective therapeutic activities because they are promising as sources of natural antioxidants [1]. These biomolecules contribute to the prevention of various human diseases (aging, cardiovascular disease, asthma, neurodegenerative diseases, malaria coronary, vascular diseases and the formation of tumors) by inhibiting oxidative reactions resulting of the action of free radicals on, for example, lipids or DNA [2, 3].
Lavandula stoechas (*L. steochas*) is a plant of the Lamiaceae family that is known as Halhale in Algeria, widespread on the coast and in the Tell and widely used in folk medicine in different parts of the world. *L. stoechas* is popular for its culinary use, but it is especially distinguished as an antiseptic in the same way as the lavender officinal. L. stoechas is used as bactericidal, tonic, antispasmodic, stimulant, sudorific stomachic and diuretic, it is effective for the stomach pains, the migraines and in external use to treat wounds, burns pelt. The essential oils of *Lavendula stoechas* L. are effective for relieving nervous headaches and to colic and chest ailments. Infusion of the flowers is advised to treat the affections of the respiratory ways [4, 5].
Natural products are used as raw materials in many fields, such as pharmaceutical, agronomic, food, sanitary, cosmetic, and perfume industries to replace synthetic products [4].
For this, the objectives of the present were to determine minerals, chemical composition and total phenolic content of *L. steochas*, to evaluate and to compare the antioxidant properties of the essential oil and methanol extract.

MATERIALS AND METHODS

Plant material

The plant *Lavandula stoechas* L. was collected from the natural forest of Tiaret (west of Algeria). The aerial parts of the plant were dried in the dark in a well-ventilated place at room temperature. The dried plant were ground using electric grinder (Moulinex, type A591, France) in order to get a fine powder, then it was stored in clean paper bags until its use.

Chemicals

The chemicals Folin-Coiocaltau's reagent, 2,2-diphenyl-1-picrylhydrazyl - DPPH (97 %), aluminum chloride (98 %), rutin (95 %), butylated hydroxyanisole - BHA (98.5 %), 2,2' azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) - ABTS (98 %) and ammonium sulfate (99 %) were purchased from Sigma-Aldrich (France). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium phosphate (98.5 %) , sodium monobasic phosphate (98 %), trichloroacetic acid - TCA (99 %), iron (III) chloride, potassium persulfate (99 %) and potassium ferricyanide (99 %) were purchased from Fluka Chemika (Germany). Sodium carbonate (99.5 %), gallic acid, and ascorbic acid (99.5 %) were purchased from BioChem (France). Hydrochloric acid (37 %) was purchased from Panreac (Barcelona Spain). Nitric acid was purchased from

Scharlau (Spain) and orthophosphoric acid (85 %) was purchased from Analar Normapur (France). All other chemicals were of analytical grade.

Spectrophotometric measurements

All determinations were performed in triplicate using an OPTIMA SP-3000 Nano UV-VIS spectrophotometer (Tokyo, Japan).

Mineral content determination by atomic absorption–emission spectrometry

Experimental protocol previously described by Garcia-Garcia *et al.* [6] was followed to determine the concentration of minerals in *L. stoechas*. Approximately 1 gram of dried powder of *L. stoechas* was treated overnight with 5 mL of HNO_3 at room temperature in Pyrex tube. Next, the content of the tube was heated from 40 °C to 130 °C with an increased program of 20 °C / 120 minutes, using a multi-place digestion block (Block Digest 20, Selecta, Barcelona, Spain) until the red-orange fumes of HNO_3 disappeared. After cooling at room temperature, the contents of the tube were diluted with deionized water of very high purity and were stored at 4 °C until the mineral content determination. Quantification of Ca, Mg, K, Na, Cu, Fe, Mn and Zn in a previously mineralized sample was performed with a Unicam Solaar atomic absorption spectrophotometer (Model 969, Unicam Ltd., Cambridge, UK).

Essential oil isolation

The extraction of *L. stoechas* oils was obtained by hydrodistillation of 200 g of the dry plant in a Clevenger-type apparatus for 4 hours [4]. The oil collected was stored at 4 ºC until its analyses.

Chromatographic analyses

The volatile compounds extracted from *L. stoechas* were analyzed by GC-MS method and were identified on a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu GC-MS QP-5050A mass spectrometer detector. The GC-MS system was equipped with a TRACSIL Meta.X5 column, 95 % dimethyl-polysiloxane and 5 % diphenyl-polysiloxane (Teknokroma S. Coop. C. Ltd, Barcelona, Spain; 30 m × 0.25 mm × 0.25 µm film thickness). The chromatographic conditions were those previously reported by Calín-Sánchez *et al.* [7]. Identification was based on three analytical methods the retention indexes, retention time of authentic standards, and mass spectra (authentic chemicals and Wiley 229 spectral library) [8]. After the identification, each volatile compound was quantified (relative abundance %) in a gas chromatograph GC (model 2010, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector (FID); the same column and similar chromatographic conditions to those previously reported for GC/MS were used.
Identification was considered tentative when it was based on only mass spectral data. The volatile studies were conducted in triplicate.

Polyphenol extraction

A quantity of 1 gram of the air-dried plant powder was macerated in a hydroalcoholic mixture (methanol/water 80/20: v/v) for 24 hours, in the dark at room temperature with continuous stirring. The macerate was filtered through filter paper to obtain a pure extract. Moreover, the solvent was evaporated under vacuum, at a temperature of 40 °C. After treatment with hexane in order to remove any traces of nonpolar compounds (pigments, lipids, etc.), the phenolic compounds were then extracted by ethyl acetate in the presence of ammonium sulfate (20 %) and orthophosphoric acid (2 %). After three successive extractions, the organic phase obtained is evaporated under vacuum at 40 °C. The residue thus obtained was dissolved in 10 mL methanol and stored in the refrigerator until spectrophotometric analysis [9].

Total phenolic content

Benhammou *et al.* [10] have been described the Folin-Ciocalteu method for quantifying phenolic compounds. The total phenolic content in our extract of *L. stoechas* was quantified by the Folin-Ciocalteu reagent; for this propose, 200 µL of the diluted extract was mixed with 1 mL of Folin–Ciocalteu reagent (10 %). After 5 min, 800 µL of saturated sodium carbonate solution (7.5 %) was added. The mixture was incubated in the dark at room temperature for 30 minutes and absorbance was measured at 765 nm. Gallic acid was used under the same conditions of the test as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE / g DW). All experiments were carried out in triplicate, and gallic acid equivalent values were reported as X ± SD of triplicates.

Total flavonoid content

For the determination of the total flavonoid content, the Dowd method as adapted by Mechraoui *et al.* [11] is incorporated using rutin as the standard. For it, 1 mL of aluminum trichloride (2 %) in ethanol was mixed with the same volume of the diluted extract. Absorption readings at 430 nm were taken after 15 minutes against a blank sample consisting of a 1 mL methanol with 1 mL $AlCl_3$.The same procedure was also applied to the standard solutions of rutin to obtain a standard curve as a control. The concentrations of flavonoids compound expressed as mg rutin equivalent per gram of dry weight (mg RE / g DW) were calculated according to the standard rutin graph. All experiments were carried out in triplicate, and rutin equivalent values were reported as X ± SD of triplicates.

Antioxidant capacity

There are many methods for evaluating the antioxidant potential of a pure product or a mixture, cyclic voltammetry and other usually used methods such DPPH radical scavenging activity, Ferric Reducing Antioxidant Power (FRAP) and $ABTS^{\bullet+}$ decolorization method were chosen to determine the antioxidant potential of phenolic and essential oil of *L. steochas*. A standard curve was prepared using different concentrations of Trolox. The results were corrected for dilution and expressed in

Trolox equivalent antioxidant capacity (TEAC) for ABTS and FRAP assay and in Inhibition capacity (IC_{50} %) for DPPH assay. There are many methods for evaluating the antioxidant potential of a pure product or a mixture, cyclic voltammetry and other usually used methods such as % for DPPH assay. All determinations were performed in triplicate and were compared to gallic acid, vitamin C (VC) and BHA as standards.

Cyclic voltammetry

In order to characterize the oxidation-reduction properties of our extracts, we choose the cyclic voltammetry. The electrochemical measurements are carried out in a glass cell containing sample and electrolyte solution consisting of potassium phosphate buffer (0.2 M, *p*H 7.2) and three electrodes: the working electrode is made of graphite, against electrode in platinum and the reference electrode saturated with KCl. The working electrode is cleaned after each measurement with distilled water. During electrochemical voltammetric measurements of phenolic extracts and essential oil or standards, a potential is applied in solution ranging from -200 to 800 mV, and a constant sweep rate: 100 $mV \cdot s^{-1}$ with a VoltaLab potentiostat (model PGZ 402, France) [12].

DPPH radical scavenging assay

To evaluate the antioxidant activity of *L. stoechas* volatile compounds, and phenolic extract, the DPPH radical scavenging method described by Brand-Williams *et al.* [13] was applied with some modifications. For this method, 1950 µL of a methanol solution of DPPH (60 µM) was added to 50 µL of each extract solution in methanol at different concentrations. The mixture was stirred vigorously and incubated for 30 minutes in the dark and at room temperature. The absorbance was measured at 517 nm in a spectrophotometer. The inhibition was calculated using the following equation:

$$I\,(\%) = 100 \text{ x } (A_0 - A_1)/A_0. \quad (1)$$

where: I represents the inhibition, A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard, respectively. The percent inhibition as a function of the concentration was plotted, and then from this curve obtained the concentration of the sample required for a 50 % inhibition was determined and expressed as IC_{50}.

ABTS radical-scavenging assay

The free radical-scavenging activity was determined by ABTS radical cation decolorization assay defined by Re *et al.* [14]. The radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution 7 mM with 2.45 mM potassium persulfate (final concentration) and kept in the dark at room temperature for 12–16 h before use. The radical was stored in the dark at room temperature it was stable in this form for more than 48 hours. To realized this test, the ABTS solution was diluted with ethanol to an absorbance of 0.700 (± 0.02) at 734 nm. A reagent blank reading was taken (A_0). After addition of 1980 µL of diluted $ABTS^{\bullet+}$ solution to 20 µL of antioxidant compounds or Trolox standards in ethanol, the absorbance reading was taken at 30 °C exactly 1 minute after initial mixing and up to 10 minutes. Trolox standards and selected extracts were plotted as the percentage inhibition of the absorbance of the ABTS solution as a function of the concentration of antioxidant [15]. The results obtained were recalculated to Trolox concentration and were described as "Trolox Equivalent Antioxidant Capacity" (TEAC) that is the Trolox concentration necessary

for obtaining the same radical $ABTS^{+}$ inhibition of a solution 1 $mg \cdot mL^{-1}$ of the extract under investigation [16].

Ferric reducing power

The reducing power of the extracts was determined according to the ferric reducing antioxidant potency (FRAP) assay of Oyaizu (1986) [17] with a slight modification. This method is based on the direct reduction of $Fe^{3+}(CN)^{-}_{6}$ to $Fe^{2+}(CN)^{-}_{6}$ and was determined by measuring absorbance resulting from the formation of the blue complex following the addition of excess ferric ions (Fe^{3+}) [18]. For this purpose, 25 µL of different concentrations of studied extracts were mixed with 0.5 mL of (0.2 M, *p*H 6.6) phosphate buffer and 0.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1 %). The mixture was incubated at 50 °C for 20 minutes. After this incubation, the reaction mixture was acidified with 0.5 mL of trichloroacetic acid (10 %). Finally, the mixture was mixed with 1.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1 %). The absorbance of this mixture was measured at 700 nm using a UV spectrophotometer. Decreased absorbance indicates ferric reducing power capability of the sample [19]. The standard curve was prepared using different concentrations of Trolox. The results were corrected for dilution, and expressed in TEAC.

RESULTS AND DISCUSSION

Minerals content

Mineral contents of *L. stoechas* are presented in Table 1. The results of the analyses are established to give nutrient values per 100 g of the used portion of the dried weight.

Table 1. *Minerals content of Lavandula steochas L.*

Minerals	**Content [mg / 100 g dw]**
Macro-elements	
Calcium (Ca)	nd
Sodium (Na)	75.891 ± 0.719
Magnesium (Mg)	368.929 ± 10.414
Potassium (K)	1451.169 ± 7.817
Micro-elements	
Iron (Fe)	159.213 ± 6.226
Zinc (Zn)	5.73 ± 0.0737
Copper (Cu)	0.655 ± 0.001
Manganese (Mn)	6.363 ± 0.190

Except for the calcium that is not detected, the studied species contain large quantities of many essential minerals including K, Mg, Na and trace elements, such as Fe, Zn, Cu and Mn.

Among macro-elements, potassium is the main mineral element, showing the highest values (1451.169 mg / 100 g), followed by magnesium (368.929 mg / 100 g) and sodium (75.891 mg / 100 g.).

Regarding the micro-elements, as can be seen from Table 1, the highest values are reached by iron (159.213 mg / 100 g). Zinc and manganese were recorded in the present study with values of 5.73 mg / 100 g and 6.363 mg / 100 g, respectively. The content of copper is not high, presents the lowest values (0.655 mg / 100 g).
To our knowledge, this is the first reports of mineral contents of *L. stoechas* leaves, its results have been compared to those of other species of the same family such as *Lavandula dentata* from Morocco [20] and *Lavandula officinalis* L. from Turkey [21] or Romania [22]. In all scientific references, potassium and magnesium have an important level as a macro-elements followed by iron that predominated as micro-elements in all samples from the different countries. Potassium content was high in most cases and ranged from 893 mg / 100g in Romanian *Lavandula officinalis* to 27047 mg / 100 g in Moroccan *Lavandula dentata*. Magnesium contents of spices were found in similarly high percentages in all references species varied from 289 mg / 100g in Romanian *L. officinalis* to 4976.5 mg / 100 g in Moroccan *L. dentata*. Iron content ranged from 61 mg / 100 g in Romanian *Lavandula officinalis* to 844 mg / 100 g in Moroccan *L. dentata*. Minerals content in Turkish *L. officinalis* is not far from our result its content was 1752 mg of potassium / 100 g, 459 mg of magnesium / 100 g and 122 mg of iron /100 g. Others minerals contents (Zn, Mn, Cu) of spices were found in small percentages in all reference species.
From this comparison, *Lavandula stoechas* has a moderate mineral content compared to other species. The traditional use of this medicinal plant can be attributed to the presence of an adequate concentration of these macro- and micro-elements, given their positive roles in maintaining certain critical physical and chemical processes, current results can contribute significantly to the mineral content of the human body [23].

Volatile compounds

The results obtained by GC-MS analysis of essential oil (OE) of *L. stoechas* are presented in Tables 2.
Forty-four volatile components were identified. The major components of *L. stoechas* essential oils were α-fenchone (39.0 %), camphor (18.5 %), bornyl acetate (7.79 %), viridiflorol (4.55 %), myrtenyl acetate (3.32 %). Other compounds were presented at lower levels such as: *m*-cymen-8-ol (1.91 %), *p*-cymen-8-ol (1.73 %), borneol (1.71 %).
The chemical composition of *L. stoechas* essential oils, determined in this work, is in accordance to some previously reported works and differs from some others. For example in Italy it was found that the major constituents of *L. stoechas* essential oils were fenchone (37.0 %), camphor (27.3 %), bornyl acetate (6.2 %),1,8-cineole (6.0 %), but also thymol (3.1 %), carvacrol (3.4 %), viridiflorol (2.6 %), myrtenyl acetate (1.7 %), borneol (0.8 %) and *p*-cymen-8-ol (0.4 %) are in low quantity [4]. In Greece, the major compounds were: fenchone (45.2 %), 1,8-cineole (16.3 %), and camphor with 9.9 %. Other predominant compounds were: *p*-cymene (4.9 %), *α*-cadinol (4.2 %), lavandulyl acetate (3.2 %), *α*-pinene (2.5 %), myrtenyl (1.10 %) and *p*-cymene-8-ol (0.2 %) [5]. However, a remarkable difference was found in Turkish essential oil, the chemical analysis being characterized by high contents of pulegone (40.37 %), menthol (18.09 %), menthone (12.57 %) and low contents of 1,8-cineol (3.9 %), *β*-pinene (3.2 %), 2,6,6-trimethyl-1-cyclohexene (3.2 %) and *β*-terpineol (2.3 %) [19].

Table 2.*Chemical composition of Lavandula steochas essential oil*

	Compound	**RT**	**RI (exp)**	**[%]**
1	***α-Fenchone***	9.23	1424	***39.0***
2	(Z)-Linalool oxide	10.10	1451	0.23
3	1-Octen-3-ol	10.21	1454	0.3
4	(Z)-Limonene oxide	10.36	1459	0.17
5	(E)-Limonene oxide	10.48	1462	0.16
6	(E)-Sabinene hydrate	10.70	1469	0.39
7	Camphenilone	10.77	1471	0.23
8	Linalool oxide	11.01	1478	0.22
9	α-Fenchyl acetate	11.03	1479	1.03
10	α-Ylangene	11.47	1492	0.43
11	α-Campholenal	11.70	1499	0.36
12	α-Copaene	11.82	1503	0.31
13	***Camphor***	12.71	1529	***18.5***
14	Linalool	13.46	1550	0.97
15	Isopulegol	13.54	1553	0,34
16	1-Terpineol + β-pinene	13.98	1566	0,17
17	Pinocarvone	14.27	1574	0.21
18	***Bornyl acetate***	14.77	1589	***7.79***
19	Terpinen-4-ol	15.39	1606	1.06
20	Myrtenal	16.34	1632	1.09
21	(E)-2-Decenal	17.26	1658	0.57
22	trans-Pinocarveol	17.57	1667	0.26
23	α-Terpineol	17.88	1675	0.63
24	Verbenol	18.09	1681	1.09
25	***Myretenyl acetate***	18.58	1694	***3.32***
26	α-Terpineol	18.83	1701	0.74
27	***Borneol***	18.92	1704	***1.71***
28	Verbenone	19.09	1708	0.29
29	(Z)-*p*-Mentha-1,5-dien-8-ol	19.58	1722	0.91
30	(E)-*p*-Mentha-1,5-dien-8-ol	19.88	1730	0.69
31	δ-Carvone	20.13	1737	0.88
32	*n*-Methylacetophenone	20.86	1757	0.18
33	δ-Cadinene	21.05	1762	0.77
34	Neryl acetate	21,13	1764	0.25
35	*p*-Methylacetophenone	21.58	1776	0.34
36	Myrtenol	22.27	1795	1.47
37	(E)-Carveol	23.85	1839	0.81
38	***m-Cymen-8-ol***	24.24	1850	***1.91***
39	***p-Cymen-8-ol***	24.39	1854	***1.73***
40	Palustrol	27.10	1930	0.21
41	Caryophyllene oxide	28.98	1983	1.02
42	***Viridiflorol (unidentified isomer)***	32.55	2088	***4.55***
43	Eugenol	35.38	2174	0.34
44	Viridiflorol (unidentified isomer)	35.71	2184	2.35

The chemical composition from our *L. stoechas* essential oil compared to Italy and Greece works is more or less similar, with a few degrees of difference. Fenchone and camphor are the major monoterpenes in *L. stoechas* oils or a significant amount is observed in our plant studied. There is a difference especially for thymol (3.1 %) and carvacrol (3.4 %), these two phenols only found in the *L. stoechas* essential oil from Italy or Turkey. The compounds presented in our extract at minor amounts such as *p*-cymene-8-ol (monoterpene), viridiflorol (sesquiterpenols), borneol and linalool (terpene) are very low or absent in *L. stoechas* from Italy, Greece, and Turkey. It was also reported that the oil yield from *L. steochas* leaves was 0.7 %, 1.46 %, and 1.33 % w/w dw in Italy [4], Greece [5] and Turkey [24] respectively whereas in our study the oil yield was 1.36 % w/w dw. These quantitative and qualitative differences in yield or in chemicals composition with other countries depend mainly on the growing environment of plants, climatic conditions, region as well as harvesting season and extraction method [19, 25].

Total phenolic and total flavonoids contents

The polarity of solvent is one of interest in the processing of phenolic and flavonoids extraction. Usually, the more polar solvents are considered to be suitable for the extraction of phenolic and flavonoid contents, methanol, and ethanol, in combination with water have usually been used [26]. There is no a lot of works that study the phenolic compounds in *L. stoechas*, most of them being interested in the biological activity of the essential oil. Our results of total phenolic content (TPC) and flavonoids contents (FC) in hydroalcoholic maceration extract are presented in Table 3. The estimation of the polyphenol content was carried out according to the Folin method and the total content was expressed in gallic acid equivalent / g of dry plant.

Table 3. *Total phenolic content and flavonoid content of Lavandula stochas L.*

	TPC [mg GAE / g]	**FC [mg RE / g]**
Lavandula stochas L.	9.475 ± 0.48	4.615 ± 0.18

According to the results obtained, the *L. stoechas* contains a significant amount of polyphenols, which is of the order of 9.74 mg ± 0.48 GAE / gram of plant. The content of flavonoid compounds ($mg \cdot g^{-1}$) in *L. stoechas* was determined using the regression equation of rutin calibration curve. The content of total flavonoids in *L. stoechas* was 4.615 mg ± 0.18 RE / gram of plant.

Antioxidant capacity

The essential oil (EO) and phenolic compounds (PhC) extracted from dry leaves of *L. steochas* has been tested with different assays of antioxidant activity with cyclic voltammetry DPPH, ABTS, and FRAP.

In cyclic voltammetry, the potential is applied to the compounds in solution in order to extract electrons from the oxidizable molecules. The working electrode picks up the electrons, used in conjunction with the platinum counter-electrode. These electrons produce a positive (anodic) current measured against an Ag/AgCl reference electrode. We obtain the voltammogram represented in Figures 1.

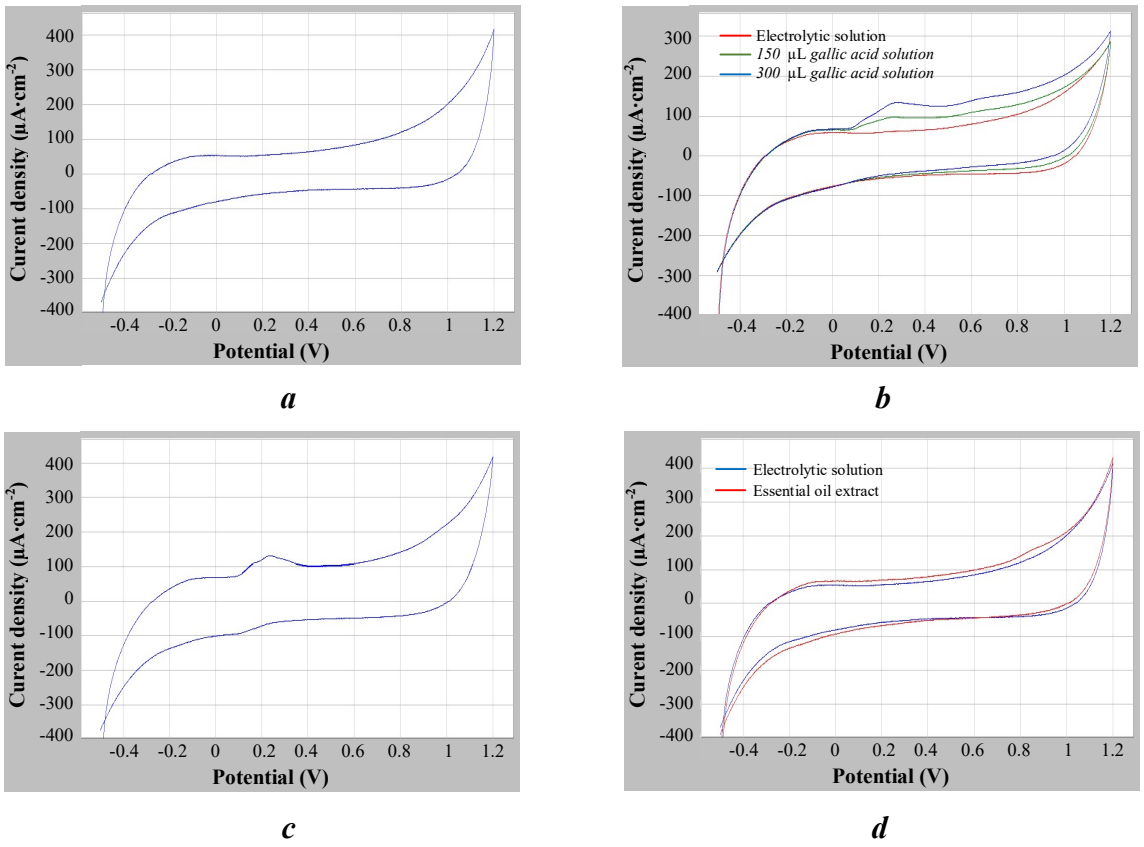

Figure 1. *Voltammogram of electrolyte, standard and studied extracts: a) electrolytic solution, b) gallic acid solution, c) phenolic extract, d) essential oil extract*

Figure 1a shows the behavior of the electrolytic solution alone, which transports only the current and has no effect on the sample solutions. Figures 1b, 1c, 1d, show the voltammograms of the standard and studied extracts. The peaks observed on the graph of the current as a function of the potential are the currents produced by the oxidation of molecules. The height of the peak depends on the number of electrons captured at a given potential. The injection of different concentrations of gallic acid gives peak height proportional to the concentration (Figure 1b). The two oxidation and reduction peaks observed in the voltammogram of the phenolic extract (Figure 1c) are explained according to Filipiak [27], that when the potential is reversed, the previously oxidized forms of the phenolic compounds are reduced to their initial state, which creates a negative current (cathode). A downward peak on the voltammogram represents this reduction. In order to increase the contact between the essential oil and the solution of the electrolyte, the oil was solubilized in methanol. However, the extract of the essential oil does not reveal any remarkable change in their voltammogram (Figure 1d), which indicates the difficulty of the separation of electrons from the oxidizable molecules by the applied potential.
Table 4 presents the electrochemical parameters of the studied extracts and the standard.

Table 4. *Electrochemical parameters of the studied extracts and the standard*

Sample	Injected volume [µL]	Ip.a [µA·cm^{-2}]	E,a [V]	Ip.c [µA·cm^{-2}]	Ep,c [V]
Gallic acid	150	96.1	0.2442	-	-
	300	130.1	0.2705		
Phenolic extract	150	126.2	0.2272	-98.2	0.0910
Essential oil	150	0.4	0.2330	-	-

Ip,a - anodic current density, Ip,c - cathodic current density, E – potential.

Founded on DPPH assay, IC_{50} is the concentration of antioxidant needed to decrease the initial DPPH concentration by 50 % from the radical to non-radical form [26]. This parameter is an index to compare and to express the scavenger effect of our extracts. The lower IC_{50} value indicates a high antioxidant capacity [13]. The values of IC_{50} determined for *L. stoechas* extracts, as well as for the reference compounds (vitamin C and BHA) are presented in Table 5.

Table 5. *Antioxidant capacities determined by DPPH, ABTS and FRAP assays*

Antioxidant activity	*Lavandula stoechas* L.		Standards	
	PhC	EO	Vitamin C	BHA
DPPH [µg·mL^{-1}]	0.023 ± 0.0015	15.269 ± 0.248	0.002 ± 3.08E^{-05}	5.951 ± 0.09
ABTS [TEAC]	0.284 ± 0.002	0.0025 ± 0.0005	1.68 ± 0.02	3.47 ± 0.26
FRAP [TEAC]	0.368 ± 0.001	0.001 ± 0.0001	2.04 ± 0.36	1.06 ± 0.02

Phenolic compounds (PhC) of *L. stoechas* showed a scavenging ability on DPPH radical (IC_{50} = 0.02395 mg·mL^{-1}) which was more active than the essential oils (EO) that could reduce the stable free radical (DPPH) with IC_{50}'s of 15.269 mg·mL^{-1}. However, when comparing this antioxidant activity with that of the reference substances (vitamin C and BHA), *L. stoechas* is less effective than the reference standard. Vitamin C and BHA have very low IC_{50}s of 0.002 mg·mL^{-1} ± 3.08E^{-05} and 0.005 mg·mL^{-1} ± 9.5E^{-05}, respectively.

The PhC and EO from *L. stoechas* were also evaluated by ABTS assay it is a decolorization assay applicable to both lipophilic and hydrophilic antioxidants [28]. In reactions process, $ABTS^{\bullet+}$ radical involves an electron transfer whereas the reactions with DPPH radical involve H atom transfer. The radical cation $ABTS^{\bullet+}$ is produced by its oxidation with potassium persulphate and is reduced in presence of antioxidants [18]. The antioxidant activity with this test is expressed in TEAC that is defined as the concentration of a standard Trolox solution with the same antioxidant capacity as a 1 mg·mL^{-1} of the tested extract. It should be noted that the higher TEAC value, more the molecule is active [16]. As can be seen in Table 5, all extracts from *L. steochas* have shown a scavenging activity of the radical cation $ABTS^{\bullet+}$, the best activity of *L. steochas* is found in PhC with TEAC value 0.284 mg·mL^{-1} more than 0.0025 mg·mL^{-1} of EO extract. Parallel to the DPPH results, the capacity of *L. steochas* to scavenging the radical cations $ABTS^{\bullet+}$, is lower than standard antioxidants (vitamin C and BHA).

FRAP method measures the ability of a sample to reduce metals, the antioxidant activity with FRAP assay is also expressed in TEAC. All extracts from *L. steochas* have a ferric reducing potential, which increased with an increase in concentration. However, the best

activity of *L. steochas* is found in PhC its values of TEAC = 0.368 mg·mL^{-1}. EO have a very low capacity TEAC = 0.001 mg·mL^{-1}. *L. stoechas*'s ability to reduce metal is generally lower than that of standard antioxidants, namely vitamin C and BHA.
From the all methods used, it seems that *L. stoechas* has important antioxidant activity; this capacity is mainly owed to their content of phenolic compounds that are widely found as secondary metabolites in plants [29]. It had been reported that the content of phenolic compounds played a very important role in antioxidant activity [30 – 32], because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen extinguishers and metal chelators [33]. The efficiency of the phenolic compounds is influenced by the presence of functional groups of increasing polarities such as carbonyl and hydroxyl groups. Karimi *et al.* [29] have shown that the structure of flavonoids has been found to be very effective at removing most oxidative molecules, including singlet oxygen and various free radicals. Zulueta *et al.* [34] have also shown in their research that the relative capacities of carotenoids to trap the radical cation $ABTS^{\bullet+}$ are influenced by the presence of carbonyl and hydroxyl functional groups. Aidi Wannes *et al*. [35], in their comparison research between methanolic extract and essential oil of myrtle leaf, found that methanolic extract showed better antioxidative capacity than the essential oil, suggesting that the myrtle extracts have a potent antioxidant activity mainly due to their richness on phenolic compounds.
The low capacity of the EO in *L. steochas* can be explained by the fact that our EO are low effect to give a hydrogen atom or singular electron, and also the low solubility of their constituents in the reaction medium of the test. Moreover, it has been observed that the influence of the methodology on the antioxidant activity presented by the different essential oils and compounds evaluated, demonstrates the importance of the methodology for the determination of the activity [36]. Benabdelkader *et al.* [37] and Barkat and Laib [38] have been shown that there is no significant correlation between the levels of the major components (fenchone, camphor, bornyl acetate, borneol) and the antioxidant activities. The antioxidant activity is related to the presence of phenolic compounds in the essential oil. Several compounds that we have detected in our
L. stoechas EO, even with a trace state, have already been treated as antioxidants, for example, camphor, eugenol, linalool, terpinen-4-ol can explain the trapping activity of DPPH radical. Ruberto and Baratta [39] have reported that thymol and carvacrol, from oxygenated monoterpenes, are the most active components, these two phenols are in fact responsible for the antioxidant activity of many essential oils which contain them. However, our GC-MS analysis reveals the presence of other oxygenated monoterpenes or phenolic constituents that are probably responsible for the activity. It appears that the antioxidant activity of *L. stoechas* EO may be due to the presence of different molecules.

CONCLUSION

Lavandula stoechas contained large quantities of many essential minerals and trace element with a significantly high level of phenolic and flavonoid content. GC-MS analyses of essential oils showed a very diverse composition with 44 constituents. Our comparison indicated that phenolic extract has much higher antioxidant activity than the essential oils.

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