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Minerals analysis, antioxidant and chemical composition of extracts of *Laurus nobilis* from southern Algeria

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Abstract
Oxidative stress is a major factor related to the development of diseases, which have traditionally been treated with medicinal plants. The aim of this work is the valorization of *Laurus nobilis* by chemical characterization, study of the antioxidant and the mineral analysis of the extracts and essential oils. The content of total phenols was determined using Folin-Ciocalteu reagent, whereas aluminum chloride colorimetric method was used for flavonoid determination. The total antioxidant capacity was estimated by metal chelating activity, the scavenging of free radicals $DPPH^{\bullet}$ and $ABTS^{\bullet+}$,the phosphomolybdenum assay and by the FRAP (Ferric reducing /antioxidant power), expressed as IC_{50}, TCEAC and VEAC. The essential oil composition of the leaf of *Laurus nobilis* was investigated by Gas chromatography-Mass spectrometry (GC-MS). Thirty four constituents were identified corresponding to 99.97 % of the total oil. The major components are 1,8-cineole (44.13 %), α-Terpinyl acetate (17.33 %), Methyl eugenol (6.53%) and Sabinene (5.25 %) .Mineral and heavy metal concentration of *Laurus nobilis* possessing health promoting effects and used in indigenous medicine (as medicinal food) were determined using Atomic Absorption Spectroscopy , A total of 7 elements Ca , K, Mg, Fe, Mn , Cu and Zn have been measured. Therefore, this plant is rich in some essential minerals, especially Ca, K, Fe and Mg .

Keywords : FRAP, ABTS, DPPH, Phosphomolybdenum, minerals analysis .

1. Introduction
Laurus nobilis L. (Lauraceae), commonly known as bay are shrubs or small trees with slender twigs, is represented by two species: *Laurus azorica* and *Laurus nobilis* [1]. *Laurus nobilis* is an evergreen shrub up to 2.15 m height and commonly named bay laurel [2]. This tree belongs to the Lauraceae family and is native to the southern Mediterranean region and widely cultivated in Europe and the USA as an ornamental plant. It is grown commercially for its aromatic leaves in Turkey, Algeria, Morocco, Portugal, Spain, Italy, France and Mexico. As the pharmacological properties of *L.nobilis*, it has been reported to have antiulcer (seeds) and antidiabetic (leaves) effects [3] and to enhance in liver glutathione S-transferase (GST) activity [4].According to Amin et al. (2007) [2], the yield of essential oil (EO) from leaves varies between 0.8 % to 1.5% (v/w). This oil is used as a cure for rheumatic pain, dermatitis, antimicrobial and food preservative [5].

Polyphenol compounds consist of different phenolic rings, out of which one of the major subgroups of these secondary metabolites are flavonoids. As human consumption aspects, flavonoids are one of the major groups of phytochemicals with high antioxidant activity; they have been interesting subjects for general studies in recent years.

Essential oils are secondary metabolites produced by aromatic plants. The organoleptic properties and biological activities of essential oils are distinguished by their respective compositions [6-7].They have been found to have antioxidant properties, which are attributed to the presence of terpenoid and phenolic compounds. The main compounds are made up of terpenes and terpenoids and also aromatic and aliphatic components [8].

Antioxidants play a very important role in the body defense system against reactive oxygen species (ROS). The ROS are the harmful byproducts generated during normal cell aerobic respiration [9] . Since ROS are considered responsible for a number of diseases such as cardiovascular disease, some forms of cancer, cataract, age-related muscular degeneration, and rheumatoid arthritis [10]. Therefore, numerous studies have been conducted in order to evaluate the antioxidant capacity of certain compounds or plant materials. A wide variety of methods have been developed for the investigation of antioxidant capacity [11] including the total radical trapping parameter (TRAP) assay [12] , the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonate) radical cation (ABTS•+) assay [13] , the ferric reducing antioxidant power (FRAP) assay [14] , the oxygen radical absorbance capacity (ORAC) assay [15], the cupric reducing antioxidant capacity (CUPRAC) assay[16] , the electrochemical estimation of total reducing capacity [17], the 2,2- diphenyl-1-picrylhydrazyl radical (DPPH•) assay [18] , the chelating on ferrous ions assay and the phosphomolybdenum assay [19,20].

Throughout the world, there is increasing interest in the importance of dietary minerals in the prevention of several diseases. Minerals are of critical importance in the diet, even though they comprise only 4–6% of the human body. Major minerals are those required in amounts greater than 100 mg per day and they represent 1% or less of bodyweight. These include calcium, phosphorus, magnesium, sulphur, potassium, chloride and sodium. Trace minerals are essential in much smaller amounts, less than 100 mg per day, and make up less than 0.01% of bodyweight. Essential trace elements are zinc, iron, silicon, manganese, copper, fluoride, iodine and chromium [21].

The aim of the present study was to characterize *L.nobilis* from Algeria. For that the analysis were: i) *functional properties of plant extracts*: the total phenolic, total flavonoid content and total antioxidant potential ($DPPH^{\bullet}$ and $ABTS^{+}$, among others), and ii) *quality parameters*: the mineral analysis and essential oils.

2. Materials and methods

2.1. Plant material

The leaves of *L.nobilis* were collected from Laghouat (400 Km southern Algiers). Briefly, *L. nobilis* was milled and sieved, fine powder was kept in a sealed plastic bag and stored at room temperature, under darkness to avoid possible oxidation of compounds.

2.2. Preparation of the extracts

One gram of the air dried plant material is crushed and extracted for 48 h with 100 ml of 80% (v/v) aqueous ethanol at room temperature. Another successive extraction with 50 ml of the same hydroalcoholic solution was carried out at room temperature for 24 h. After removal of ethanol under reduced pressure in a rotary evaporator at 40 °C, the remaining aqueous solution of the extraction is defatted twice with petroleum ether to remove lipids. Then, the lyophilized solution is extracted with ethyl acetate in presence of aqueous solution with 20% ammonium sulphate, and 2% of ortho-phosphoric acid solution. The ethyl acetate fraction is dried by addition of a sufficient amount of anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The precipitate is dried, dissolved in 10 ml of absolute ethanol and kept at 10°C [22].

2.3. Determination of total phenols (Folin-Ciocalteau)

Total phenolics were determined using Folin-Ciocalteu reagent as described by Slinkard and Singleton. (1977)[23]. The extract (250 µL) was mixed with 1.25 mL of Folin-Ciocalteu reagent (previously diluted 10 times with distilled water). 1 mL sodium bicarbonate solution (75 g/L) was added to the mixture and after incubation for 30 min at room temperature, the absorbance level was measured at 765 nm using a UV-Visible spectrophotometer (shimadzuuv mini 1240). Total phenolic were quantified by calibration curve obtained from measuring the absorbance of the known concentrations of Gallic acid standard solutions. The results were calculated as Gallic acid equivalent (GAE) per one gram dry extract and reported as mean value ± SD.

2.4. Flavone and flavonol content

Flavone and flavonol content was identified as described by Ahnet al. (2007)[24]. Briefly, 1 mL of 2% $AlCl_3$ solution was added to 1 mL of sample or standard. After 1 h at room temperature, the absorbance was measured at 420 nm. Quercetin was used as standard for the construction of calibration curve.

2.5. Extraction of essential oils

100 g of plant material and 500 mL water were placed in a Clevenger type apparatus. The essential oil was isolated by hydrodistillation for three hours, then separated, dried overanhydrous sodium sulphate and stored in a sealed vial, at –5 °C, before use.

2.6. Chromatographic analysis

The chromatographic analysis of the plant extracts (isolation and identification of the volatile compounds) were performed on a Shimadzu GC-17A gas chromatograph-mass spectrometer (GC–MS) (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector QP-5050A. The GC–MS system was equipped with a TRACSIL Meta.X5 column, 95% polydimethylsiloxane and 5% polydiphenylsiloxane (Teknokroma S. Coop. C. Ltd., Barcelona, Spain; 60 m × 0.25 mm × 0.25 µm film thickness). Analyses were carried out using He as carrier gas at a column flow of 0.3 mL min^{-1} at a split ratio of 1:11. The oven program was: (a) 80 °C, 0 min; (b) rate of 3.0 °C $min^{-1}$from 80 to 210°C and hold for 1 min; (c) rate of 25 °C min^{-1} from 210 to 300 °C and hold for 6 min. The temperatures of the injector and detector were 230 and 300 °C, respectively.

2.7. Antioxidant activity

2.7.1. Phosphomolybdenum assay

The total antioxidant capacity was evaluated by the method of Prieto et al., (1999) [20]. 0.3 mL of each sample at different concentrations was combined with 3mL of reagent (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Ethanol (0.3 mL) was used as blank in place of the sample. The tubes were capped and incubated in a boiling water bath at 95 ◦C for 90 min, and then cooled at room temperature. The absorbance of each solution was then measured at 695 nm. Results were expressed as equivalents of ascorbic acid (VCEAC) (mmol/g) .

2.7.2. Ferric-reducing antioxidant power assay (FRAP)

The reducing power was determined according to the method of Oyaizu. (1986)[25].Each sample at different concentrations was mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionized water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm. Results were expressed in mmol ascorbic acid equivalents /g dry weight of three replicates.

2.7.3. ABTS radical cation scavenging activity

The $ABTS^{\bullet+}$ method was based on the procedure described by Dorman and Hiltunen. (2004) [26]. Briefly, ABTS was diluted in potassium persulfate solution (2.45 mM) and final concentration of $ABTS^{\bullet+}$ was 7mM. The mixture was left to stand in dark at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ was diluted to the absorbance of 0.70 ± 0.02 and stocked for off line and on line assays. 15 µl of each sample at different concentrations was added with 1.485 ml of $ABTS^{\bullet+}$ solution and stand in dark at room temperature for 60 min . The absorbance was measured at 734 nm .The percentage inhibition was plotted as a function of concentration and the Trolox equivalent antioxidant capacity (TEAC) was calculated against a Trolox calibration curve.

2.7.4. DPPH radical-scavenging capacity

Free radical scavenging activity of different plant fractions against stable DPPH was determined spectrophotometric ally by the slightly modified method of Brand-Williams et al. (1995) [27]. When DPPH reacts with an antioxidant, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light yellow) were measured at 517 nm. 50 µL of each sample at different concentrations was added to 2 mL of DPPH ethanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity was expressed as IC_{50} (mg/mL) .Ascorbic acid was used as a positive control.

2.7.5. Chelating effect on ferrous ions

The ferrous ion chelating activity of extract was assessed as described by Wang et al.(2004)[19] . Each sample at different concentrations was added to 0.05 mL of $FeCl_2$,$4H_2O$ solution (2 mM) and left for incubation at room temperature for 5 min. Then, the reaction was initiated by adding 0.2 mL of ferrozine (5 mM), shaken vigorously and

left standing at room temperature for 10 min. Absorbance of the solution was then measured at 562 nm. Results were expressed as IC_{50} (mg/mL) . EDTA was used as a positive control.

2.8. Minerals analysis

Dried samples (0.5 g) were digested for 2 h at a temperature below 130 °C in a multi-place digestion block (Block Digest 20, Selecta, Barcelona, Spain) using 5mL of 65% HNO_3[28]**.**Samples were left to cool to room temperature and then transferred to volumetric flasks. The dilutions were prepared using ultrahigh-purity deionized water. Samples were stored at 4 ◦C until analysis. Determination of Ca, Mg, K, Na, Cu, Fe, Mn and Zn in previously mineralized samples was performed using a Solaar 969 atomic absorption–emission spectrometer (Unicam Ltd, Cambridge, UK). K and Na were analyzed by atomic emission, while the other elements were analyzed by atomic absorption . Instruments were calibrated using certified standards. In each analytical batch, at least two reagent blanks were included to assess precision and accuracy for chemical analysis. Calibration curves were used for the quantification of minerals and showed good linearity ($R^2 \geq 0.997$). Analyses were run in triplicate.

3. Results and discussion

3.1. Total phenolic, Flavones and flavonols contents

The phenolic extracts of *L.nobilis* known for their therapeutic properties in traditional Arab medicine were tested for their antioxidant status. The total phenol content of extract estimated by the Folin–Ciocalteu procedureis presented in Table 1.The total phenols, and flavonols and fllavones content was observed in extract from *L.nobilis* (25.70 ± 0.86 mg GAE g^{-1}dw and 12.11 ± 0.43 mg CE g^{-1}dw respectively), which were higher than the previously found in this plant, that showed 20.94 ± 0.97mg GAE g^{-1}dw of Total phenolic and 8.2 ± 0.21mg CE g^{-1}dw of flavonoids[29].

The plant phenolic constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts. Flavones and flavonols as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolic[30]. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties . Many studies have demonstrated a positive correlation between flavonoid amounts and antioxidant activity [31].

Table 1: Total phenolic, Flavones and flavonols contents (mg g^{-1} dry weight, dw) found in plant extract.

Plant	**Total phenol (Folin) (mg GAE g^{-1}dw)**	**Flavonols and flavones (mg CE g^{-1}dw)**
L.nobilis extract	25.70 ± 0.86[†]	12.11 ± 0.43

[†]*Data are presented as mean ± standard deviation (S.D.) of n = 3.*

3.2. Chromatographic analyses

GC/MS analysis of the essential oil of *L.nobilis* leaves led to the identification and quantificationof 34 components. The table 2 shows the retention indices (RIs) and percentages of the detected compounds. Retention indices of the sample components were calculated on the basis of homologous n-alkane hydrocarbons (C_8-C_{16}) under the same conditions . The identities of the separated volatile components of essential oil were determined using their recorded mass spectra matched by library searching with the databank spectra provided by the instrument software and comparing their calculated RI with literature values measured on columns with identical polarities [32] .The composition of the essential oil isreported in Table 2 , where the components are listed in order of their elution from the TRACSIL Meta.X5 column . Altogetthirty four compounds have been identified, accounting from 99.97 % of the whole oil. 1,8-cineole (44.13 %) , α-Terpinyl acetate (17.33%) , Methyl eugenol (6.53%), Sabinene (5.25%) , Terpinen-4-ol (4.56 %) , Linalool (4.18 %) , β-Pinene (3.26 %) were the most abundant components of the oil . Studies from Mediouni et al. (2012) described 1,8-cineole (34.62%) , Linalool (12.57%) , Isovaleraldehyde (8.82%) , β-phellandrene (5.71 %) as the major components of the plants collected in Algeria[33]. These essential oils have very different compositions from the essential oil extracted from the aerial parts of *L.nobilis* leaves presented in this study .Quantitative variations were observed of compounds due to the environmental factors, growing conditions, ontogeny, extraction method, and GC-MS conditions carried out in the

experiments [5-34]. As in the present study, 1,8-cineole has been consistently detected as the main component of *L.nobilis* oil .

Table 1: Chemical composition of *Laurus nobilis* essential oil from Laghouat (Algeria).

Compound[†]	**Retention index**		**Concentration (% total area)**
	Experimental	**Literature**	
t-2-Hexenal	858	860	0.06
α-Thujene	917	933	0.30
α-Pinene	930	939	2.92
Camphene	950	954	0.20
Sabinene	973	975	5.25
β-Pinene	982	979	3.26
t-β-Ocimene	1006	1023	0.17
α-Terpinene	1015	1017	0.35
Limonene	1031	1029	1.06
1,8-Cineole	1047	1044	44.13
γ-Terpinene	1057	1060	0.84
cis-Sabinene hydrate	1076	1070	0.06
α-Terpinolene	1084	1089	0.28
Linalool	1100	1097	4.18
t-Sabinene hydrate	1110	1098	0.09
1-Terpineol	1130	1147	0.12
Isopulegol	1149	1150	0.12
Pinocarveol	1153	1152	0.10
Camphor	1167	1156	0.07
Terpinen-4-ol	1192	1191	4.56
α-Terpineol	1206	1200	2.58
Nerol	1227	1233	0.19
Linalool acetate	1241	1257	0.12
Borneol acetate	1268	1283	0.14
2-Undecanone	1286	1295	0.25
Sabinylacetate	1289	1298	0.35
Ocimenylacetate[‡]	1317	nd	0.92
α-Terpinylacetate	1359	1350	17.33
Eugenol	1370	1362	2.43
β-Elemene	1392	1404	0.35
Methyleugenol	1412	1406	6.53
β-Caryophyllene	1431	1418	0.20
α-Caryophyllene	1479	1452	0.25
γ-Cadinene[‡]	1523	1515	0.21

[†]*Compounds are listed in order of their elution time from TRACSIL Meta.X5,*
and nd= not detected or below the detection limit of the equipment. [‡] *tentatively identified.*

3.3.Antioxidant activity

3.3.1.Phosphomolybdenum assay

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate / Mo (V) complex with a maximal absorption at 695 nm.

Increase of the absorbance indicated the increase of the total antioxidant capacity. The phosphomolybdenum method is quantitative since the antioxidant capacity is expressed as the number of equivalents of ascorbic acid .The antioxidant capacities of phenolic extracts from *L.nobilis* were measured spectrophotometrically through this method .The results indicated that The VCEAC (vitamin C equivalents mmol of vitamin C/ g dry weight) value for the phenolic extract and the oil essential of *L.nobilis* had comparatively low total antioxidant activity (0.133 ± 0.015mmol/g , 0,211± 0.016 mmol/g respectively) , are shown in Table 3 . The differential response of the extracts antioxidant tests may be explained by the fact that the transfer of electrons/hydrogen from antioxidants occur at different redox potential in various assay systems and the transfer also depends on the structure of the antioxidants[35].

3.3.2.Ferric-reducing antioxidant power assay (FRAP)

Antioxidants can be explained as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species is reduced at the expense of the oxidation of the other. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [36]. Reducing power will increase accordingly to the increase in absorbance. The antioxidant capacity of the *L.nobilis* under study determined as vitamin C equivalents (VCEAC) (mmol of vitamin C/ g DW) using reducing power . The results obtained are summarized in (Table 3),the VCEAC value for the phenolic extracts of *L.nobilis* was 0.116 ± 0.011 mmol /g were lower than those of the standards BHT (VCEAC of 0.243±0.0003 mmol/g) , but higher than that of *L.nobilis* oil (VCEAC of 0.076 ± 0.002mmol/g) . The reducing power was observed in this test of the phenolic extracts of *L.nobilis* , *L.nobilis* oil and standard compound exhibited the following order : BHT > phenolic extracts of *L.nobilis*>*L.nobilis* oil.The results on reducing power demonstrate the electron donor properties of extracts of *L.nobilis* thereby neutralizing free radicals by forming stable products. Accordingly, *L.nobilis* might contain amounts of reductone , which could react with free radicals to stabilise and block radical chain reactions . As more Fe^{3+} are reduced to the ferrous form or when more electrons are donated by antioxidant components.

3.3.3.ABTS radical cation scavenging activity

The ABTS radical scavenging method is based on the reduction of preformed radical cation $ABTS^{\bullet+}$ by the addition of antioxidant. The extent of decolorization of the $ABTS^{\bullet+}$chromophore measured spectrophotometrically at 734 nm gives the measure of the antioxidant activity of the sample[37]. The extent of inhibition of $ABTS^{\bullet+}$ was plotted as a function of concentration , through measuring the reduction of the cation radical as the percentage inhibition [38].

Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC). This index is defined as the millimolar concentration of a Trolox solution whose antioxidant capacity is equivalent to a 1.0 mM solution of the substance under study[39] , This values are shown in table 3 , were 0.222 ±0,0.018 mmol/g of the extracts of *L.nobilis* is higher than TEAC value of the *L.nobilis* oil (0.071 ± 0,0019 mmol/g) . This assay is frequently used for assessing antioxidant activity and developing structure–activity relationships (SARs) of flavonoids.

3.3.4.DPPH radical-scavenging capacity

DPPH is a free radical and accepts an electron or hydrogen radical to become a stable molecule[40]. The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidant activities in a relatively short time. The absorbance decreases as a result of a colour change from purple to yellow as the radical is scavenged by antioxidants. Acid ascorbic , BHT and were used as positive control .The results of IC_{50} the phenolic extract and oil essential of *L.nobilis* were presented in Table 3 . IC_{50} is a parameter widely used to measure antioxidant activity. As the IC_{50} value of the extract decreases, the free radical scavenging activity increases , The phenolic extracts from *L.nobilis* were characterized by a higher free radical scavenging property (IC_{50} = 0.024±0.003 mg/mL) showed the highest activity, while the sample of oil essential of *L.nobilis* showed the lowest activity (IC_{50} = 0,494±0.030 mg/mL) with comparison to that of synthetic antioxidant the standard Ascorbic acid (IC_{50} = 0.014±0.001mg/mL) and BHT (IC_{50} =0.080±0.0015 mg/mL) (Table 3) . The results were showed good correlations between the total polyphenols, flavonoids contents and IC_{50} theDPPH of extracts . The obtained results for IC_{50} values for tested extracts from

L.nobilis (Table 3) have higher IC_{50} value than those in literature (IC_{50}= 0.15mg/mL) [41]. In addition, antioxidative activities observed in extracts from *L.nobilis* could be the synergistic effect of more than compounds that may be present in the plant.

3.3.5.Chelating effect on ferrous ions

Transition metals such as ion can stimulate lipid peroxidation by generating hydroxyl radicals through Fenton reaction and accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, therefore drive the chain reaction of lipid peroxidation. The chelating activities for ferrous ion of the extracts were assayed by the inhibition of formation of red-colored ferrozine and ferrous complex. One measurement of the metal-chelating activity of an antioxidant is based on the absorbance measurement of Fe^{2+}–ferrozine complex after prior treatment of a ferrous ion solution with test material. The formation of the red-colored complex was inhibited in the presence of extracts of plants, indicating chelating activity. However, the color did not change much in the presence of extracts of *L.nobilis*, suggesting weak chelating activity with IC_{50} values of 0,954±0.010 mg/ml . EDTA is a strong metal chelator ; hence, it was used as standard metal chelator agent in this study (IC_{50} = 0.062±0.0026 mg/ml) EDTA demonstrated excellent chelating ability . These results suggested that extracts of *L.nobilis* are very weak Fe^{2+}chelators (Table 3) .

Table 3:Antioxidant activity of hydro-alcoholic extracts and essential oils of *L.nobilis* plant, expressed in IC_{50} (mg/ mL) for DPPH and Chelating assay, Trolox equivalent TEAC (mmol/g) for ABTS assay , Ascorbic acid equivalent VCEAC for Reducing power and Phosphomolybdenum.

Sample	Phosphomolybdenum	Reducing power	ABTS	DPPH	Chelating metalions
L. nobilis extracts	0,211± 0.016[†]	0.116± 0.011	0.222 ± 0.018	0.024 ± 0.003	0.954 ± 0.010
*L.nobilis*essential oil	0.133± 0.015	0.076± 0.002	0.071 ± 0.002	0.494 ± 0.030	NA[‡]
Ascorbic acid	-	-	ND	0.014 ± 0.001	ND
EDTA	ND[‡]	ND	ND	ND	0.062 ± 0.003
BHT	ND	0.243±0.0003	ND	0.080±0.0015	ND

[†] *Data are presented as mean ± standard deviation (S.D.) of n = 3.*
[‡] *ND = not determined; NA = not active (interferences).*

3.4.Mineral analysis

Knowledge of the elemental content in medicinal plants is very important since many trace elements play significant roles in the formation of active constituents responsible for the curative properties. Moreover, some of these elements are vitally important for various metabolic processes in the human body. They are closely linked to human growth and general health [42]. In this study, a four microelements (Fe , Mn , Zn, Cu) and three macro elements (K ,Ca, Mg) measured in *L.nobilis* samples . The concentrations of various metals were shown in Table 4 .this analysis has shown that this plant is rich sources of this elements. the concentration of Ca is 7958,92±247,94 mg/Kg , Calcium imparts strength and rigidity to bones and teeth . Calcium ions are also needed in neuromuscular transmission, in excitability of nerves for normal excitability of heart, in clotting of blood and promoting muscular contraction.

Potassium concentration of 6665,92± 495,36 mg/Kg , The high concentration of potassium in plants is needed for many essential processes including enzyme activation, photosynthesis, water use efficiency, starch formation and protein synthesis.

Magnesium is the fourth most abundant cation in the body. Much of Magnesium is present in bones in association with Calcium and Phosphate and the rest in soft tissues and body fluids. In muscles and other tissues .Magnesium is highest in *L.nobilis* 1605,97 ± 33,70 mg/Kg .

Iron concentration is high in *L.nobilis* 162,12± 17,19 mg/Kg (1,323±0,140 ppm) , however it is within normal background level for the element in plants under the critical concentration of 300-500 ppm .The results suggest that high amount of Fe in plants may also be due to the soil nature . Low Fe content

causes gastrointestinal infection, nose bleeding and myocardial infarction . Iron deficiency is the most prevalent nutritional deficiency in humans and is commonly caused by insufficient dietary intake .

Copper is an essential enzymatic element for normal plant growth and development but can be toxic at excessive levels. Phytotoxicity can occur if its concentration in plants is higher than 20- 100 ppm DW (dry weight).As can be seen from the data (Table 4) The concentration of Copper was found in *L.nobilis* 20,37± 1,84 mg/Kg (0,831±0,075 ppm).

As evident from Table 4, high concentration of Zn was found in *L.nobilis* 32,88 ± 1,80 mg/Kg (1,342±0,073 ppm) . Zinc is an essential trace element for plant growth and also plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing. The dietary limit of Zn is 100 ppm [43].

Manganese concentration of *L.nobilis* 10,95 ± 0,30 mg/Kg , Manganese is an important modulator of cells functions and play vital role in the control of diabetes mellitus [44] .

In this work, our result is comparable with other findings , this elements (Ca , K, Fe, Mg, Mn , Zn, Cu) of *L.nobilis* are higher than those obtained by Ujowundu et al .(2011) [45]. Therefore, these medicinal plant *L.nobilis* is rich in some essential minerals are essential for human health. Results presented here clearly show that the examined medicinal plant *L.nobilis* play a meaningful role in human nutrition as micro-nutrients sources. The minerals and heavy metals found to be below the recommended maximum acceptable levels proposed by the Joint FAO/WHO Expert Committee on Food Additives.

Table 4 : Concentrations of mineral elements (mg kg^{-1}dw) in *L.nobilis* leaves.

Mineral	***L.nobilis*leaves**
Macro-elements (mg kg^{-1}dw)	
Calcium (Ca)	7959 ± 248[†]
Magnesium (Mg)	1606 ± 34
Potassium (K)	6666 ± 495
Micro-elements (mg kg^{-1}dw)	
Iron (Fe)	162 ± 17
Zinc (Zn)	32.9 ± 1.8
Copper (Cu)	20.4 ± 1.8
Manganese (Mn)	11.0 ± 0.3

[†] *Data are presented as mean ± standard deviation (S.D.) of n = 3.*

Conclusion

The results of this study indicated that *Laurus nobilis* has a high antioxidant activity determined by ABTS , DPPH and low antioxidant activity determined by FRAP, phosphomolybdenum and chelating metal ions. There was a positive relationship between antioxidant activity and total phenols and thus can be used for the evaluation of antioxidant active compounds as well as for their assessment according to IC_{50} ,TEAC,VEAC . *L.nobilis* may be a good source of minerals (Ca , K, Fe, Mg, Mn , Zn, Cu) to treat number of diseases that are mainly caused due to the deficiency of those minerals .

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