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Research Article

Study of Tunisian Nettle Leaves (*Urtica dioica* L.): Mineral Composition and Antioxidant Capacity of their Extracts Obtained by Maceration and Supercritical Fluid Extraction

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ABSTRACT
This work was undertaken to explore the potential of extract of leaves Nettle (*Urtica dioica* L.) of Tunisia as sources of minerals and natural antioxidants. Two extraction methods were used and compared, the supercritical fluid extraction (SFE) with carbon dioxide (CO_2) and traditional maceration extraction using ethanol (EtOH) as solvent. The SFE was explored at various operating conditions of pression (15 and 18 MPa) and temperature (40 and 60°C) (with or without glass beads). The phenolic and flavonoid contents were determined respectively by Folin-Ciocalteu and Aluminium chloride colorimetric method. The ABTS assay was used for determining the antioxidant activity. The protein assay was performed by the Kjeldahl method and the multielemental analysis by atomic emission spectrometry with inductively coupled plasma. The results show some variations between both extracts in terms of extraction yield and antioxidant activity. The SFE, with carbon dioxide (SC-CO^2) is an interesting alternative to the conventional extraction using organic solvents. However, higher values of phenolic content (11.62 mg GAE.g^{-1} DW), flavonoid content (7.10 mgCE.g^{-1}DW) and antioxidant activity TEAC (8.11 µM) correspond to extracts obtained by maceration. In all SFE extract assays, the highest yield was reached at 60°C - 15MPa with glass beads (2.5%). The ICP analysis shows the abundance of *Urtica dioica* L. in calcium (1116 mg.$100g^{-1}$DW) and magnesium (544). The protein assay showed high content (15.75 %.). The present study indicates that the leaves of *Urtica dioica* L. of Tunisia are a potential source of minerals, proteins and antioxidants.

Keywords: *Urtica dioica, Total phenolic, Flavonoid, ABTS, Antioxidant activity, Supercritical fluid extraction.*

INTRODUCTION

The Stinging Nettle (*Urtica dioica* L.) belongs to the urticaceae family which gathers about thirty species[1,2]. Nettle leaf has a long history as an herbal remedy and as nutritious additif to the human diet. The stinging nettles present many medicinal properties since antiquity. The archaeologists found strips, surrounding the mummies of old Egypt, consisting of fibers whose analysis showed that the latter are fibers of nettles[3]. The stinging nettle leaf contains chlorophyll, carotenoids, vitamins C, K, B group vitamins (B1, B2 and B5), tannins, essential oil, proteins, and minerals[4] (Fe, Cu, Mn and Ni), while the stem and root contain flavonoids[5,6]. The leaves are edible, they can be eaten raw, in quiches or in soup, but they are mostly eaten cooked (like spinach). Young plants were harvested by Native Americans and used as a cooked plant in spring when other food plants were scarce[7]. Besides culinary uses, this plant was harvested commercially for extraction of the chlorophyll, which is used as a green coloring agent (E140) in foods and medicines[8]. It was also used as forages, textile industry and paper. It is also known that nettle has an antioxidant, antimicrobial, anti-ulcer and analgesic properties[9]. Tests carried out in the United States, in Germany and in Japan showed the efficacy of the nettle in the treatment of the prostate hypertrophy. At the first century after J.C Dioscoride described already several uses of the nettle, its fresh leaves used for the infected wounds and its juice against the nose bleed[10]. Several researchers declared that the use of the *Urtica dioïca* L. seeds with or without other plants has several effects on certain diseases like, diabetes, eczema, ignition of the liver, anemia, rheumatism[1]. The objective of this research was on one hand the valorization of the Tunisian nettle in minerals and protein, because they are among the main ingredients of the nettle, on the other hand the study of the antioxidant capacity of extracts obtained by two extraction methods: the supercritical fluid extraction (SFE) and the maceration extraction (ME).

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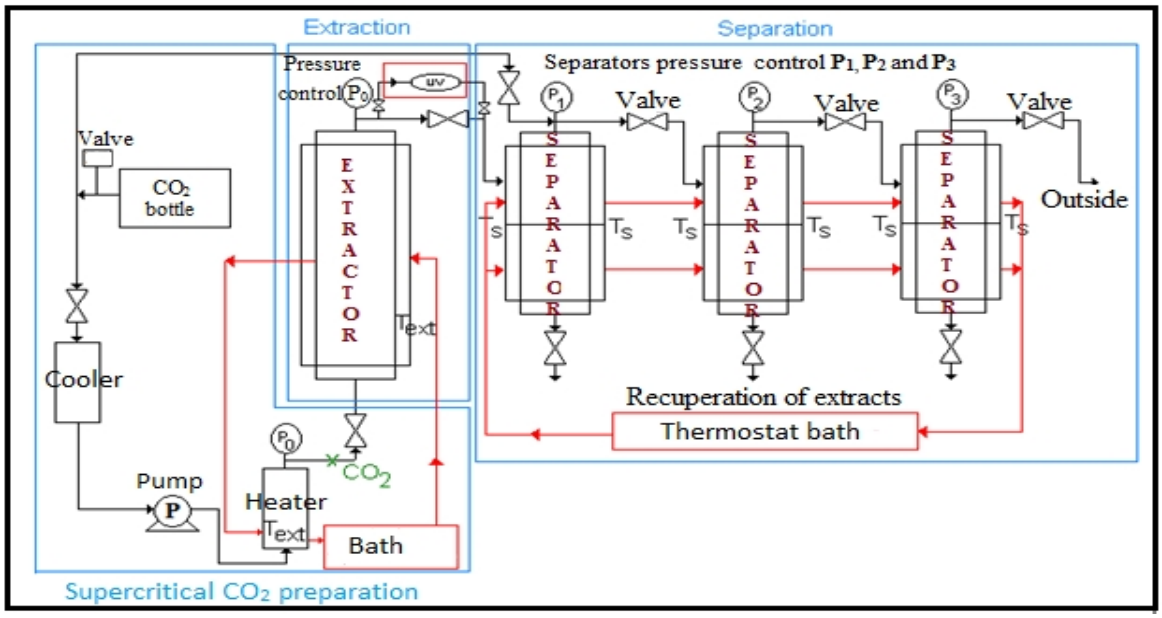

Figure 1: Diagram of dynamic extraction of carbon dioxide

Table 1: Contents of heavy metals and major elements mg.100g^{-1} DW of nettle

Minerals	Ca	Mg	P	Al	Zn	Fe	Cu	Cr	Ni	Co	Cd	Pd
Values	1116	544	81	2,8	1,1	0,37	0,21	0,21	0,2	0,09	0,055	< 0,005

Lower than the limit of detection of the apparatus

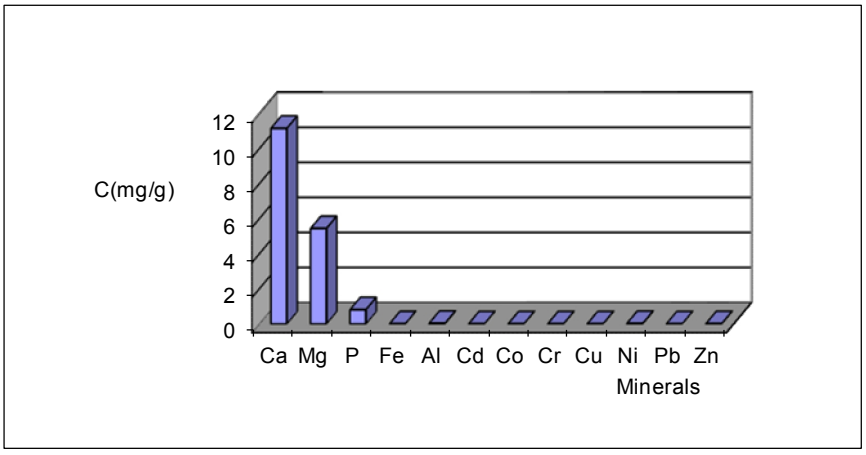

Figure 2: Mineral content of leaves nettle

MATERIALS AND METHODS

Plant material

Nettle leaves (Urtica dioica L.) were collected in June 2013 form Bizerte north coast of Tunisia located at 37° 16′ 27″ N latitude 9° 52′ 26″E longitude and 5 m altitude. Leaves were immediately transported to laboratory (LACReSNE). Plants were dried at room temperature then grounded into fine powder and passed through a sieve. Dried samples were stored at -4° C until analyses.

Chemical reagents

Folin-Ciocalteu reagent, ABTS ($C_{18}H_{18}O_6S_4.2NH_3$, 98 %), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium nitrite (≥ 99.0 % purity), gallic acid and catechin reagent were purchased from Sigma Aldrich (St. Louis, MO, USA). β-carotene, Sodium carbonate were from Fluka Biochemika (Switzerland), concentrated hydrochloric acid (37%) and absolute methanol were purchased from Panereac Quimica Sa (Barcelone).

Determination of soluble proteins

Crude protein was determined using Kjeldahl apparatus (KjeltecTM 2200, FOSS). This method is based on the principle of conversion of nitrogen into ammoniac in food product. Briefly, 1 g of freeze-dried sample was digested in sulfuric acid, NH_3 was distilled over H_2SO_4 (0,1N) and the excess of sulfuric acid was titrated against NaOH (0,1N). Total nitrogen content was converted to protein content by using the conversion factor 6.25. Percentages of crude protein were calculated using the following equation.

$$\% \text{ protein} = \left(\frac{N \times 100}{S}\right) \times 6.25$$

N: weight of nitrogen in the sample, S: weight of the dry sample, 6.25 is conversion factor

Mineral composition

The preparation of samples for Inductively Coupled Plasma (ICP OPTIMA 33000 Perkin Elmer) analyses were developed using the experimental modified protocol described by NF.EN.1482 standard (March 2003). 5g of the obtained ash after incineration in a muffle (Muffle Furnace mls1200) for 24h at 500°C were extracted with 5 mL of HNO_3 65% and 50 mL of distilled water. The mixture is heated for few minutes in a boiling water bath to dissolve the ash. After cooling the solution was filtered and then placed in a 50 mL volumetric flask, the volume is adjusted by adding distilled water, where mineral composition was directly measured at the suitable wavelength for each element, using standard solutions for calibration purposes. The concentration of each analyte in the different samples is expressed as $mg.100g^{-1}$ of dry material from their calibration curves taking into account the dilution factor.

Extraction methods

Maceration extraction (ME)

The ground plant material (7.5 g) and the 150 mL of aqueous ethanol solution (50: 50, V/V) were put in erlenmeyer flask. The extraction was performed at 25°C in 24 h with sporadic agitation. The extract was filtered through Whatman N° 2 filter paper and the solvent was evaporated in a vacuum evaporator (Buchi Rotavapor R-205) at 40°C and 17.5 MPa. The obtained extracts were stored in a freeze for subsequent analysis.

Supercritical fluid extraction (SFE)

The use of supercritical fluid extraction (SFE) is a novel approach to the extraction of volatile compounds and possesses significant advantages over the more traditional processes such as maceration extraction or soxhlet extraction. Carbon dioxide is generally the most desirable solvent for supercritical fluid extraction (SFE). Its low critical temperature (31°C) and pressure (7.38 MPa) make it more effective for the extraction of heat sensitive compounds.

A flow sheet of the supercritical fluid extraction process is shown on the figure 1. SFE unit used has been conceived and assembled in the Reactions and Process Engineering Laboratory (LRGP, Nancy, France). The CO_2 used was 99.95% of purity (Messer France). The installation is composed of an extraction column (300 x 23 mm), with a stainless steel frit, followed up by three cyclonic separators. 10 g of plant powder was introduced in the extraction reactor. When using glass beads which allow increasing the contact surface between the fluid and the solid matrix and promotes a uniform distribution, the sample was placed between two layers of these. The CO_2 is liquefied at 3.2 °C using a pumped cold exchange (Dosapro Milton Roy - MILROYALD) and heated with another exchanger to obtain the supercritical state. At the beginning of SFE the plant material was kept 30 mn under supercritical CO_2 (without flow of supercritical CO_2) at the same pressure and temperature as that used during SFE (static mode). After the period of time the continuous flow of supercritical CO_2 was established. The extract is separated from CO_2 by successive decompression. The fall of pressure is accompanied by a significant cooling. The dried leaves of nettle were extracted at 15MPa and 18MPa for pressure, 40°C and 60 °C for temperature. The time of the extraction is between 2 and 3h. The temperature in the separators was 20°C, and the pressures were respectively 4, 2 and 1 MPa. The supercritical CO_2 flow rate was comprised between 7.5 and 13.5 $g.min^{-1}$.

Antioxidant contents

The dry residue of the ME and SFE extracts were dissolved in methanol and the obtained solution was used to determine the content of total phenols, flavonoids, and antioxidant activity.

Total polyphenol content

The total polyphenol content was quantified in the leaves using the Folin–Ciocalteu reagent, according to the method of Singleton and Rossi[11]. 1 mL of diluted extract was transferred to a 25 mL volumetric flask containing 9 mL of ultra pure water, the Folin–Ciocalteu reagent (1mL) was added and mixed, then 1 mL of sodium carbonate (15 %) was added. After 30 min of incubation at 40 °C in the dark, the absorbance was measured at 700 nm using the Shimadzu UV-Vis spectrophotometer. The results are expressed as equivalents of gallic acid (mg $GAE.g^{-1}$ DW).

Total flavonoid content

The total flavonoid content was measured according to the modified colorimetric method of Zhishen et al[12]. Briefly, 125 µL of the methanol extract of leaves were added to 75 µL of $NaNO_2$ (5%). The mixture was incubated for 6 minutes. 150 µL of $AlCl_3$, 6 H_2O (10%) freshly prepared are added, after 5 minutes of incubation, 500 µL of NaOH solution (1M) were added to the mixture. The final volume was adjusted to 2500 µL with distilled water. The absorbance was measured at 510 nm with a spectrophotometer (Shimadzu UV-Vis spectrophotometer). The blank was prepared using the same procedure with ultra pure water. A series of methanolic dilutions of catechin were prepared and assayed; flavonoid amounts in extract were expressed in mg catechin equivalent flavonoid / g dry matter (mg $CE.g^{-1}$ of DM).

Each measure was made in triplicate

Antioxidant capacity

Antioxidant activity of nettle extract was analyzed by investigating their ability to scavenge the $ABTS^{\bullet+}$ free radical using the modified method previously reported by Ozgenet al.[13]. The stock solutions included 7 mM ABTS solution and 4.9 potassium persulfate solutions. The working solution was prepared by mixing the two stock solutions in equal proportions and allowing them to react for 16 hours before use in order to produce ABTS radical ($ABTS^{\bullet+}$). This solution was stored in a dark place at room temperature. Before use, the solution was diluted with ethanol to obtain absorbance between 700 nm and 800 nm. This solution was mixed with sample (5 to 40 $\mu g.mL^{-1}$). A control containing methanol and $ABTS^{\bullet+}$

solution was also realized. The absorbance was read at 734 nm after 30 min incubation at 30°C. As unpaired

Table 2: Yield obtained in different extraction conditions.

	Extraction methods conditions			
Extraction	P(MPa)	T (°C)	t(min)	Yield % (W/W)
SFE 1	15	40	30	0
			120	0.103
			135	0.103
SFE 2	15	60	30	0.32
			120	0.84
			150	1.06
SFE 3	18	60	30	0.35
			120	1.48
			150	1.48
SFE 4*	15	60	30	0.49
			120	1.74
			150	2.59
ME	1(atm)	25	24(h)	7,5

**Assay with glass beads*

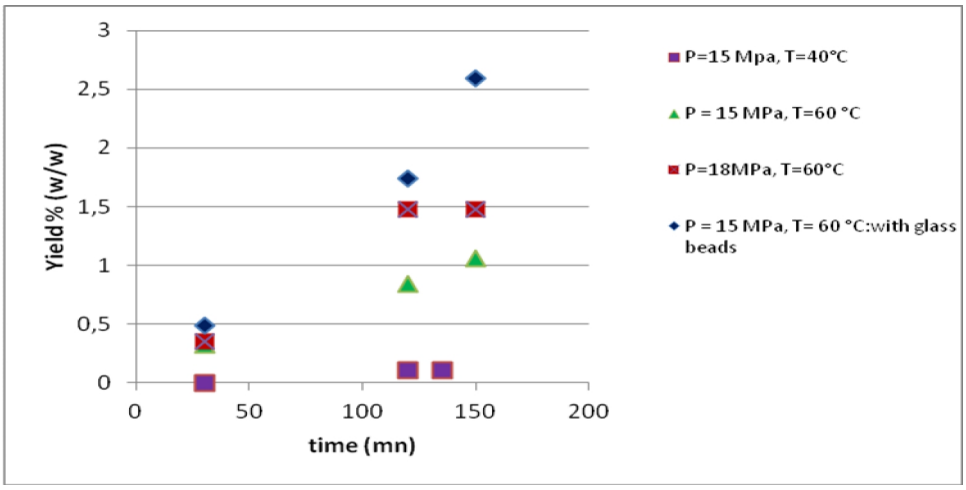

Figure 3: Kenetic CO_2 extraction

electrons are sequestered by antioxidants in the sample the test solution turns colorless and the absorbance at 734 nm is reduced. The percentage of inhibition (%I) of free radical ABTS by extract samples was calculated using the formula given below:

$$\%I = ((Abs\,Control - Abs\,test) / Abs\,Control) \times 100$$

The results are expressed in terms of Trolox equivalent antioxidant capacity (TEAC), or on the bases of IC50 values, defined as the concentration of the sample or the reference compound to decrease the absorbance at 515 nm (or concentration) of ABTS solution to half of its initial value. The IC50 was compared with the reference values, BHT and vitamin C.

Statistical analysis

All assays were performed in triplicate for each extracting condition. An analysis of variances (ANOVA) for each experiment (yield and quality evaluation) was carried out. The results are reported as standard deviation ±SD (standard deviation) obtained from the three measurements.

Mineral composition and total proteins

The ICP analysis shows the richness of this plant in minerals, especially calcium (1116 mg.100g^{-1}DW) and magnesium (544 mg.100 g^{-1}DW). The results are summarized in table 3. *Urtica dioica* studied presents higher content in calcium than values mentionned by Aksu and Kaya[1] for the Turkish nettle (873 mg.100 g^{-1}DW). Regarding the microelements, the highest values were reached by aluminum, zinc and iron (2,85 ; 1,1 and 0,37 mg.100g^{-1} respectively). Metals such as nickel, chrome, cadmium, lead and cobalt present lower contents than the limit of detection of the apparatus that allows the consumption of this plant without risk of intoxication. The high contents in calcium, magnesium and phosphorus of the nettle allow to classify this plant among food able to bring supplements in these elements. Nevertheless the mineral composition is generally influenced by the type of soils and by the fertilizers used. The determination of total nitrogen by the Kjeldahl method shows that nettle contains 25.2 mg.g^{-1}, this result is similar to that cited by Khan et al[14] (21.4 mg.g^{-1}). The percentage of protein deduced from the nitrogen content was 15.75 %.

RESULTS AND DISCUSSION

Table 3: Polyphenols, flavonoids contents and antioxidant activity of nettle extracts by maceration and supercritical fluid extraction;

Extraction method	Polyphenols (mg GAE.g^{-1} DW)	Flavonoid (mg CE.g^{-1} DW)	TEAC (mM /g)	IC50 (mg.mL^{-1})
SFE3	6.13 ± 1.80	3.68± 0.10	4.314	0.106
SFE4	8.12 ± 2.30	4.01± 0.70	6.114	0.078
ME	11.62 ± 1.75	7.10± 1.10	8.11	0.053

SFE3 : SFE without glass beads
SFE4 : SFE with glass beads
ME : maceration extraction

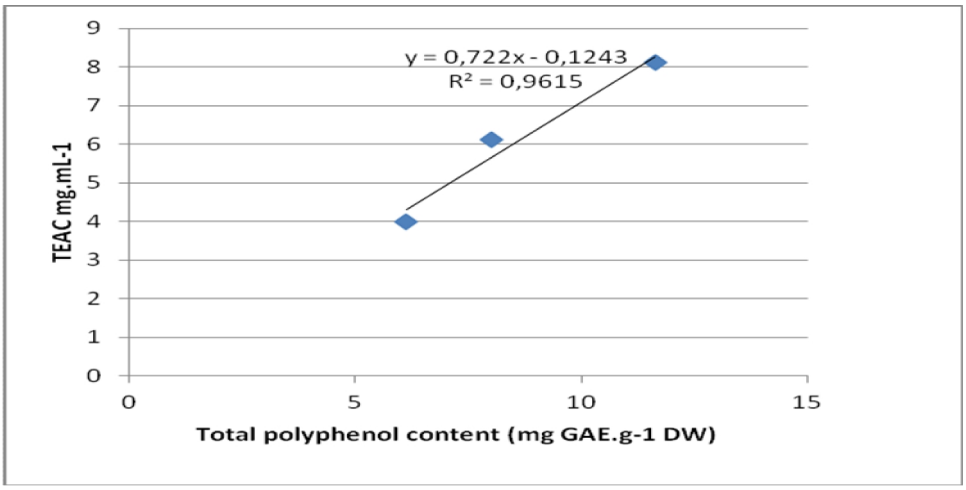

Figure 4: Relationship between total polyphenol content and TEAC.

Extraction yield

The results of extraction yield obtained by ME and by the four assays SFE at different temperatures and pressures, with and without glass beads, are presented in table 2. As revealed by the results, the highest yield of leaves extracts obtained by maceration. In SFE the yield results increase directly with pressure and temperature. The highest extraction yield (2.59%, w/w) was obtained at 60°C and 15MPa with glass beads.

The effect of extraction conditions on the yield is presented in the figure 3. The obtained yields are influenced by the experimental conditions such as pressure, temperature and time of extraction. At constant temperature, the increasing of pressure increases the extraction yield. Indeed at 60°C after 120 minutes, the yield increases from 0.84% at 15MPa, to 1.48% at 18 MPa (Fig 3). This behavior is due to the increased density of the supercritical CO_2 under pressure, which leads to an increase in the solvent power and the solubility, therefore a greater extraction yield. It is observed that the addition of glass beads significantly increased yields (from 1.06 to 2.59 %), there is evidence that presence of glass beads increases the contact surface between solvent and the solute, which increases the solubility.

Antioxydants contents

Total phenolic contents of nettle leaves, expressed in mg gallic acid equivalent.g^{-1}DW, are present in table 3. The results showed the presence of phenolic compounds in all extracts but with a predominance in organic solvent extract ME (11.62 ± 1,75 mg GAE.g^{-1} DW). Indeed ethanol is the best solvent for the extraction of polyphenol. Previous studies conducted by Kornélia Kőszegi et al.[15] in 2014 had shown that the SFE extraction is not the best method to extract polyphenols from roots of the nettle. This behavior is explained by the polar characteristic of the ethanol. Using a polar co-solvent with the CO_2 is therefore necessary to extract polar compounds. Other works conducted by Josef Hudec et al.[16] using ethanol extraction of leaves nettle reported similar value (7.62 mg GAE.g^{-1}). However the values were lower than those found in methanol extract of Iran nettles[17] (24.1 mg GAE.g^{-1}).

The flavonoid contents (TF) of the extracts expressed by quercetin equivalent ranged from 3.68 ± 0.10 to 7.1 ± 1.1 mg CE.g^{-1} DW (Table 3). We find that flavonoids were present in large quantities in both of the SE extract and SFE extracts. However, the content of total flavonoids is relatively low compared to the values given by Zoran et al.[18] for Srpska nettle (20.29± 0.48 mg QE.g^{-1}DW), and Pourmorad et al.[17] for Iran nettle (43.3±0.37 mg QE.g^{-1}DW).

In summary the supercritical fluid extraction (SFE) is not an appropriate method for the extraction of polyphenols. Indeed the Supercritical carbon dioxide behaves as a lipophilic solvent, this low polarity makes it difficult for the extraction of polar compounds, and therefore the adding of polar co-solvent with CO_2 is required for the extraction of polyphenols.

Assessment of radical scavenging activity ($ABTS^{\cdot+}$ assays)

Free radicals are involved in many diseases like atherosclerosis, diabetes, cancer and AIDS. Antioxidants

through their scavenging power are useful for the management of those pathologies. ABTS method is a common method for determination of antioxidant activity of herbal extracts. The results were shown in table 3, the ME extract which contains the highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity TEAC (8.11 mM /g of dry extract) but this value was lower than those cited by Ozkan et al.[19] for methanol extracts of nettle leaves from Turkey (40.59 mM TE /g of dry extract) and by Biesiada et al.[20] in Poland (17.3 µM TE/g of dry extract) (TE-trolox equivalent). This high scavenging property may be due to hydroxyl groups existing in the phenolic compounds whose the chemical structure can provide the necessary component as a radical scavenger. The results show that all extract of nettle leaves had low antioxidant activity (IC50 = 0.053 mg.mL^{-1}) compared to the standard control antioxidants such as vitamin C (1.84 µg mL^{-1}) and BHT (6.75 µg mL^{-1}). The IC50 observed was also higher than the one obtained for methanol extracts of nettle leaves from Srpska[18] (IC50 = 23.55 µg mL^{-1}). This result is predictable: the nettles in Serbia contain more polyphenols and flavonoids, that which gives them a higher antioxydante activity.

Correlation between total polyphenol content and trolox equivalent antioxidant capacity

Results revealed that the total polyphenols of ME extract were higher than SFE extracts and exhibits also higher antioxidant activity. Phenolic compounds are a class of antioxidant agents which act as free radical terminators[21]. Therefore a high correlation was found between trolox equivalent antioxidant capacity (TEAC) and the total polyphenol.

The polyphenols in extracts of *Urtica dioica* are probably responsible for the anti-radical activity of these extracts. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. However, antioxidant activity of plant extracts is not limited to phenolics content but also at the presence of other antioxidant secondary metabolites. On the other hand several factors can be considered to understand the action and the capacity of antioxidants: the capacity for scavenging free radicals, the localization of antioxidant, but also the interaction with other antioxidants, and the mobility of antioxidant at the microenvironment[22].

CONCLUSION

This study has showed the richness of nettle leaves from Tunisia in minerals (Ca, Mg), in protein and in antioxidants (phenolic compounds and flavonoids), the leaves extracts were found to possess strong antioxidant activity. The phenolic compounds appear to be responsible for the antioxidant activity of extracts; a linear correlation of trolox equivalent antioxidant capacity (TEAC) versus the total phenolic content of *Urtica dioïca*. L was established. A comparative study of two extraction methods SFE and ME was applied. In all SFE extract assays, the highest yield value was observed for SFE extraction at 15 MPa, 60°C. However the maceration extraction (ME) presented the best results, combining extraction yield and product quality (antioxidant activity, TP, TF). On the basis of the obtained results, the leaves nettle from Tunisia can be used for a variety of beneficial chemo-preventive effects.

REFERENCES

1. Aksu M. I., Kaya M. Effect of usage *Urtica dioica* L. or microbiological properties of sucuk, a Turkish dry-fermented sausage. Food control 2004; 15: 591-595.
2. Alibas I. Energy Consumption and Colour Characteristics of Nettle Leaves during Microwave, Vacuum and convective Drying. Biosystems Engineering 2007; 96: 495-502.
3. Bertrand A. J., Bertrand B. Les secrets de l'ortie. Ed 10, 1, Collection Le compagnon, France 2008, p.127.
4. Stanković M.: Čajne mešavine posebne namene, Tehnološki fakultet u Leskovcu, Leskovac 1995. p.39.
5. Akbay P., Basaran A.A., Undeger U., Basaran N. In vitro Immunomoudulatory Activity of Flavonoid Glycosides from *Urtica dioica* L. Phytother Res. 2003; 17 (1) : 34-37.
6. Chaturvedi S.K. A new Flavone from Urtica dioica Roots. Acta Cienc Indica Chem. 2001; 27 (1): 7.
7. Gregory L. Tilford. Edible and Medicinal Plants of the West. Mountain Press Publishing, Canada 1997.
8. Brown D. Encyclopedia of Herbs and Their Uses. Dorling Kindersley, London, 1995. p. 106
9. Gulcin I., Kufrevioglu O. I., Oktay M., Buyukokuroglu M. E.,. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L). Journal of Ethnopharmacology 2004*;* 90: 205–215.
10. Hirano T., Homma M., Oka K., Effects of stinding nettle root extracts and their steroidal components on the Na^+, K^+-ATPase of the benign prostatic hyperphusia. Planta Medica 1994; 60**:** 30-33.
11. Singleton V. L., Rossi J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic 1965; 16: 144 -158.
12. Zhishen J., Mengcheng T., Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 1999; 64: 555–559.
13. Ozgen M., Reese R. N., Tulio A. Z., Scheerens J. C., Miller A. R. Modified 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) methods. Journal of Agricultural and Food Chemistry 2006; 54(4): 1151-1157.
14. Khan K.S., Joergensen R.G. Decomposition of heavy metal contaminated nettles (*Urtica dioica.* L) in soils subjected to heavy metal pollution by river sediments. Chemosphere 2006, 65: 981-987.
15. Kőszegi K., Vatai G., Békássy-Molnár E. Comparison the Soxhlet and Supercritical Fluid

Extraction of Nettle Root (*Urtica dioica* L.). Periodica Polytechnica Chemical Engineering. Online 2015; paper 7582. DOI: 10.3311

16. Hudec J., Burdova M., Kobida L., Komora L., Macho V., Kodan G., Turianica I., Kochanova R., Lozek O., Haban M., Chlebo P. Antioxidant Capacity Changes and Phenolic Profile of *Echinacea purpurea*, Nettle (*Urtica dioïca* L.), and Dandelion (*Taraxancum officinale*) after Application of Polyamine and Phenolic Biosynthesis Regulators. J. Agric. Food Chem. 2007; 55: 5689-5696.
17. Pourmorad F., Hosseinimehr S. J., Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology 2006; 5 (11): 1142-1145.
18. Zoran Z. Kukrić Ljiljana N. Topalić-Trivunović, Biljana M. Kukavica, Snježana B. Matoš, Svetlana S. Pavičić Mirela M. Boroja , Aleksandar V. Savić. Characterization of Antioxidant and Antimicrobial Activities of Nettle leaves (*Urtica dioica* L). Acta Periodica Technologica. 2012; 43: 257-272.
19. Ozkan A., Yumrutas O., Saygideger S.D., Kulak M.: Evaluation of Antioxidant Activities and Phenolic Contents of Some Edible and Medicinal Plants from Turkey's Flora. Adv. Envir. Biol 2011; 5 (2): 231-236.
20. Biesiada A., Kucharska A., Sokó£-£êtowska A., Kuoe A.: Effect of the Age of Plantation and Harvest Term on Chemical Composition and Antioxidant Activity of Stinging Nettle (*Urtica dioica* L.). Ecological Chemistry and Engineering 2010; 17 (9): 1061-1066
21. Shahidi F., Wanasundara P. K. J. P. D. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition 1992; 32: 67-103.
22. Niki E. Assessment of antioxidant capacity in vitro and in vivo. Free Radic. Biol. Med. 2010; 49: 503-51