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Determination of Total Phenolics Contents, Antioxidant Capacity of *Thymus vulgaris* Extracts using Electrochemical and Spectrophotometric methods

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The present study aims to investigate the antioxidant activity of *Thymus vulgaris* extracts evaluated by DPPH, ABTS, chelating activity on ferrous ions tests, and evaluation of antioxidant capacity by electrochemical techniques (Cyclic Voltammetry Techniques CVT). Total polyphenols and flavonoids contents were quantified by spectrophotometric using Folin-Ciocalteu and $FeCl_3$ reagents, respectively. The results showed that the total polyphenols contents and flavonoids contents were in the order MetE > EAE > ChE > BolE >AqE > PEE. The DPPH activity showed that IC_{50} values were ordered as follows: MetE (8.49 ± 0.02 µg/ml), BolE (16.98 ± 0.05 µg/ml), EAE (21.61 ± 0.22 µg/ml), ChE (26.01 ± 0.08 µg/ml), AqE (42,21 ± 0.68 µg/ml and PEE (149.1 ± 0.52 µg/ml). The $ABTS^+$ assay revealed an important antioxidant activity of the extracts. The capacity of the metal chelating of MetE =17 ± 2 µg/ml, EAE =124 ± 1 µg/ml, ChE =142 ± 1 µg/ml, BolE =185 ± 1 µg/ml, AqE =250 ± 4 µg/ml and PEE =527 ± 1 µg/ml. Antioxidant activity measured by cyclic voltammetry method present important activity were in the order MetE > EAE > ChE > BolE >AqE > PEE. All methods showed a correlation between antioxidant activity and phenolics and/or flavonoids content. Finally these results clearly show that *Thymus vulgaris* possesses important antioxidant activity and can serve as potential source of bioactive healthy compounds that consumption could be useful in the prevention and/or treatment of diseases where reactive oxygen species and free radicals are involved.

Keywords: Cyclic voltammetry techniques, spectrophotometric methods, polyphenols, *Thymus vulgaris*, antioxidant capacity.

1. INTRODUCTION

Medicinal plants produce large number secondary metabolites that possess biological and pharmacological activities. These compounds are synthesized as a response to different stress of environmental factors. The main bioactive phytochemicals are terpenes, phenolics and alkaloids [1]. The most studied biological and pharmacological activities of medicinal plants were as antimicrobial, antioxidant, antitumor, anti-inflammatory and hypoglycemic effects [1, 2]. Epidemiological studies have shown that a diet rich in fruits and vegetables is associated with a decreased risk of cardiovascular diseases [3] and certain cancers [4]. These beneficial health effects have been attributed in part to the antioxidant properties of phenolic present in these plants [5, 6]. Flavonoids are a large group of naturally occurring polyphenols, which have been estimated to be present in human diet with an average of 23 mg/day [7] and have recently been extensively investigated for their antioxidant properties [8]. Therefore, antioxidants are widely used in the food industry as potential inhibitors of lipid peroxidation [9]. Many synthetic antioxidants used in foods, such as butylated hydroxyanisole and butylated hydroxytoluene, may accumulate in the body, resulting in liver damage and carcinogenesis [10]. For this reason, more natural non-toxic antioxidants were studied and evaluated for their ability to protect the human body from oxidative stress and retard the progress of many chronic diseases. Several medicinal plants contain large amount of antioxidants such as phenolic acids, flavonoids, terpenes, tocopherols and vitamin C (ascorbic acid), and a group of carotenoids (β-carotene, etc.), which can play an important role in adsorbing and neutralizing free radicals [11, 12]. The genus *Thymus* is an aromatic herb that is extensively used to add a distinctive aroma and flavor to food. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics. Traditionally, *Thymus* also judged to possess various beneficial effects: antiseptic, carminative, antimicrobial and antioxidative properties [13, 14]. For this reason, the aims of the present work were: (**1**) study of the relationship among different methods, spectrophotometric methods including DPPH, $ABTS^{+}$ and chelating activity on ferrous ions, and electrochemical method by cyclic voltammetry (CV) for measuring antioxidant activity (2) study of the relationship between the antioxidant properties and polyphenols and flavonoids contents.

2. EXPERIMENTAL

2.1 Material and Reagents

Chemicals reagents: Folin-Ciocalteu, aluminium chloride, 2,2'-diphenyl-1- picrylhydrazyl, (DPPH), ferrozine, 2,2_-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), gallic acid, quercétine, rutin, dragendorrf's reagent, stiasny's reagent, Ethylene diamine tetraacetic acid (EDTA), butanol, chloroform, ethyl acetate, methanol and petroleum ether.

Plant material and extraction of phenolics compounds: *Thymus vulgaris* leaves were collected from Setif region in east of Algeria during 2014. The plants were identified by Prof H. Laouer, Department of Vegetal Biology and Ecology, Faculty of Nature and Life Sciences, University Ferhat

Abbas Setif 1, Algeria. The extractions were carried out using various polar and non-polar solvents according to Markham [15].

Material: UV, Potentionstat Galvanostat (AUTOLAB AUT7219), Glassy carbon electrode (GCE), Pt, Hg/Hg_2Cl_2 (saturated with KCl) and Rotary Evaporator.

2.2 Methods

2.2.1 Phytochemical analysis

The phytochemical tests to detect the presence different phytochemicals were performed according to the method described by Bruneton [16]. The tests were based on the visual observation of color changes or formation of a precipitate after the addition of specific reagents. The plates were sprayed with Dragendorrf's reagent, $AlCl_3$ (10%) or Stiasny's reagent for the detection of alkaloids, Tanins and flavonoids, respectively. Detection of anthraquinones, sterols/triterpenes and mucilages is carried out using NH_4OH (10%), acetic anhydride/H_2SO_4 (95%) and ethanol (100%) reagents, respectively [16].

2.2.2 Determination of total polyphenol and flavonoids contents

Total polyphenol content was estimated by the Folin–Ciocalteu method [17]. Briefly, 0.2 ml of extracts were mixed with 0.5 mL of Folin-Ciocalteu reagent (diluted 1/10). After 4 min, 0.8 mL of sodium carbonate (75 g/L) solution were added. The mixture was shaken and incubated for 2 h at room temperature in the dark. The absorbance was measured at 760 nm. Polyphenol contents were expressed as µg of gallic acid equivalent / g of dried extract (µg GA-Eq/mg).

Flavonoids contents were quantified using aluminum chloride reagent ($AlCl_3$) [18]. To 1 mL of extracts dissolved in methanol, 1 mL of $AlCl_3$ (2% in methanol) was added. After incubation of 10 min, absorbance was measured at 430 nm and the results were expressed as mg of rutin and quercetin equivalent / mg dried extract (µg R-Eq/mg and µg Q-Eq/mg).

2.2.3 Antioxidant Capacity Measured by Spectrophotometric methods

2.2.3.1 DPPH radical scavenging activity

The free radical scavenging activities of extracts were measured using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) test as we have previously described [19]. Scavenging effect was expressed as the inhibition percentage: $I\ \% = (A_{blank} - A_{sample} / A_{blank}) \times 100$. Where A_{sample}: absorbance of the sample and A_{blank}: absorbance of the blank.

2.2.3.2 $ABTS^+$ radical scavenging activity

The ABTS method was carried out according to Samarth et al [20] and Re et al [21]. A mixture of ABTS (7 mM) and potassium persulfate (2.45 mM) was incubated at room temperature for 16 h to obtain $ABTS^+$. $ABTS^+$ was freshly diluted with H2O2 to reach an A_{734} around 0.8. A photometric assay was conducted on a mixture of 300 µl extract and 2500 µl of $ABTS^+$ solution. After an uncubation of 30 min. the absorbance was read at 734 nm.

2.2.3.3 Chelating activity of ferrous ions

The chelating activities of the extracts were assayed following the method reported by Dinis *et al.* [22], with some modifications. Briefly, the solutions of samples (250 µl) were initially mixed with 50 µl $FeCl_2$ (11.9 mg in 100 ml of distilled water) and 450 µl of methanol. After 5 min, 100 µl of Ferrozine (12.3 mg in 5 ml methanol) were added, the mixture was agitated then left to react during 5 min at room temperature thus allowing the formation of complex with a purple color (Fe^{2+} - Ferrozine) having a maximum of absorption at 562 nm. The EDTA was used as a positive control. The chelating activity of the test compound on Fe^{2+} was calculated as follows: Chelating activity (%) = [1- (A_{simple} - $A_{control}$)] x 100, where A_{sample}: absorbance of the sample, $A_{control}$: absorbance of the control [22].

2.2.4 Evaluation of antioxidant capacity by electrochemical technique

A two-compartment Pyrex cell using a conventional three electrode configuration was used to perform Cyclic voltammograms experiments, a glassy carbon disk working electrode (GCE), 2 mm in diameter, polished before each measurement, a platinum wire counter electrode, and an Ag/AgCl saturated KCl reference electrode were used. Glassy carbon is thus an excellent electrode material to study the electrochemistry of natural antioxidants

The electrochemical cyclic voltammetry technique was used in order to evaluate the antioxidant capacity of the studied samples. For this, a potentionstat galvanostat apparatus of the type (Autolab Aut 7219) was used. The measurements were carried out in a 50 ml volume electrochemical cell equipped with three electrodes. The first electrode is the working one (a glassy carbon electrode GCE), the second is a counter electrode which consists of a Pt wire and the third is a KCl saturated Hg/Hg_2Cl_2 as a reference. The potential was varied in an inverse scanning mode in the range (-200 to +800 mV) at a rate of 25 mV/s. Before each test, the sample is placed in the electrochemical cell which is de-aerated by passing through it high purity nitrogen gas and its flow is maintained during the electrochemical measurement. The antioxidant capacity of the studied samples was obtained using calibration curves where ascorbic and gallic acids were used as standards [23].

The antioxidant capacity was calculated using the following relation: AC (g/mg) = Ceq /C, where AC: antioxidant capacity, Ceq: equivalent concentration of gallic acid or ascorbic acid and C: initial concentration of the extracts.

2.2.5 Statistical Analysis

All results are expressed as mean ± standard deviation (SD) of three determinations. Student's *t* test is used to evaluate the significance and analysis of variance (SAS System) is employed for the multiple comparison of the effect of different extracts. Significance is considered at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

As an initial step of the phytochemical screening, the main groups of chemical constituents were qualitatively determined. The results of *Thymus vulgaris* plant showed the presence of polyphenols, tannins, flavonoids and sterols/triterpènes. The results of aqueous (AqE), butanol (BolE), chloroform (ChE), ethyl acetate (EAE), methanolic (MetE) and petroleum ether (PEE) extract showed the presence of tannins and flavonoids.

3.2 Total polyphenols and flavonoids contents

The total phenolic and flavonoids contents of samples were estimated by Folin-Ciocalteu reagent and $FeCl_3$, respectively. The total phenolic contents were expressed as µg gallic acid equivalents per mg extract (µg GA-Eq/mg) and total flavonoids contents as µg quercetin and rutin equivalents per mg extract (µg Q-Eq/mg and µg R-Eq/mg). The results showed that the methanolic extract contained the highest amount of phenolics compounds and of flavonoids compared to the other extracts of *Thymus vulgaris*.

Table 1. Total polyphenols and flavonoids contents of *Thymus vulgaris* extracts.

	total phenols and flavonoids content					
	MetE	**PEE**	**ChE**	**EAE**	**BolE**	**AqE**
µgGA Eq/mg	270.9 ± 1.19	77.27± 4.71	166.71 ±1.19	221.48 ±0.71	135.38 ±1.67	97.11 ± 2.83
µg Q-Eq/mg	21.92 ± 1.14	03.20 ± 0.23	14.38 ± 0.21	17.11 ± 0.14	12.6 ± 0.56	07.63 ± 0.08
µg R-Eq/mg	36.10 ± 1.84	05.26 ± 0.31	23.63 ± 0.43	28.14 ± 0.25	20.73 ± 1.24	12.55 ± 0.22

3.3 Antioxidant potentials by spectrophotometric techniques

3.3.1 Free radical scavenging activity using DPPH and $ABTS^+$ radical

All extracts scavenge the $DPPH^•$ radical (shown as Fig.1). The inhibition of the extracts depends on the nature of the phytochemical molecules present in the sample [24]. The antioxidant activity is caused by the interaction of extracts to chelate free radicals. All extracts do not have the

same capacity to produce oxidative free radicals due to the differences in polyphenols and/or flavonoids contents in addition to their composition in bioactive compounds. The following order was found: methanol > ethyl acetate > chloroform > butanol > water> petroleum ether.

The total antioxidant capacity of extracts using DPPH$^{\bullet}$ (deep violet) which give becomes yellow when non radical form is produced [25, 26]. The scavenger effect on the free radicals depends on the presence of free OH groups in particular 3-OH on ring C of flavonoids, with a configuration 3', 4'-dihydroxyl on the B ring in the polyphenols [27, 28]. Plant extracts contains several bioactive molecules such as flavonoids, phenols, saponins, tannins and aromatic compounds responsible of such activities [24, 29].

The results showed that all extracts of *Thymus vulgaris* have hydrogen donating capabilities with different intensities. In fact, the difference of inhibition percentages are possibly due to the quality of the extracted compounds which depend on various parameters such as the region of the collection of the plant and the conditions of the extraction (temperature, time of extraction and solvent polarity) [29].

The total antioxidant properties of extracts were evaluated using a spectrophotometer by the improved $ABTS^{+}$ radical cation method [30, 31]. All extracts show antioxidant activities proving their capacity to scavenge the $ABTS^{+}$ radical (shown as Fig.1). The results of $ABTS^{+}$ method showed similar trend as DPPH assay. In fact, the methanolic extract showed the highest antioxidant activity with a concentration of the extract (or the compound) that inhibits (scavenge) 50% of $ABTS^{+}$ radical (IC_{50}) = 16.02 ± 0.89 µmol Trolox-Eq/g. The scavenger effect on the free radicals depends on the presence of free OH groups in particular 3-OH on ring C, with a configuration 3',4'-dihydroxyl on ring B in the polyphenols [27]. The difference in antioxidant activities of extracts might be attributed to a difference in total polyphenols and/or flavonoids contents. Several studies showed a correlation between antioxidant activity and phenolics and flavonoids content [32, 33, 34].

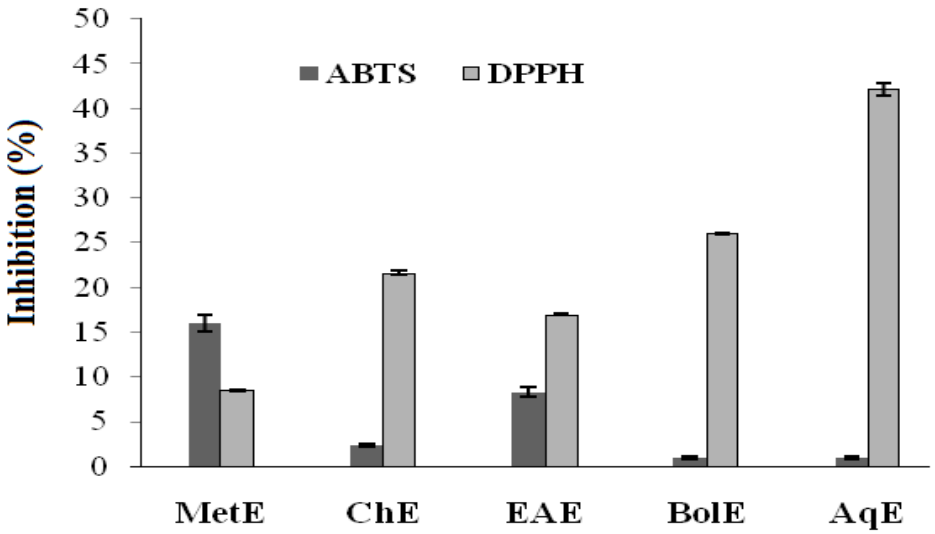

Figure 1. Radical scavenging activity of *T.vulgaris* measured by DPPH and $ABTS^{+}$ method of various extracts. **MetE**: methanol extract, **ChE**: Chloroform extract, **EAE**: ethyl acetate extract, **BolE**: butanol extract, **AqE**: aqueous extract, expressed as IC_{50} means ± SD.

3.3.2 Chelating activity of ferrous ions

In complex systems, different mechanisms may contribute to oxidative stress where reactive oxygen species are produced and participate in lipids, proteins and ADN damage [35]. Additionally, ferrous ions are thought to be effective pro-oxidants [36]. High iron levels may act to produce reactive oxygen species such as hydroxyl radicals which decompose lipid hydroperoxides into highly reactive lipid alkoxyl and peroxyl radicals, which perpetuate the chain reaction of lipid peroxidation [37, 38].

Ferrous ion chelating activities of samples are show in Fig.2. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, complex formation is disrupted, resulting in a decrease in the colour of the complex. In this assay, the samples interfered with the formation of ferrous and Ferrozine complexes, suggesting that they have chelating activity and are able to capture ferrous ion before Ferrozine. Because EDTA is a strong metal chelator, it was used as a standard in this study. Extracts or compounds with chelating activity are believed to inhibit lipid peroxydation by stabilizing transition metals (because they reduce the redox potential), and form σ-bonds with metals [36, 39]. The absorbance of Fe^{2+}- Ferrozine complex decreased by extracts in a dose dependent manner. As in the above techniques, PEE (used as defating agent) showed the lowest potential (527 ± 1 µg/ml).

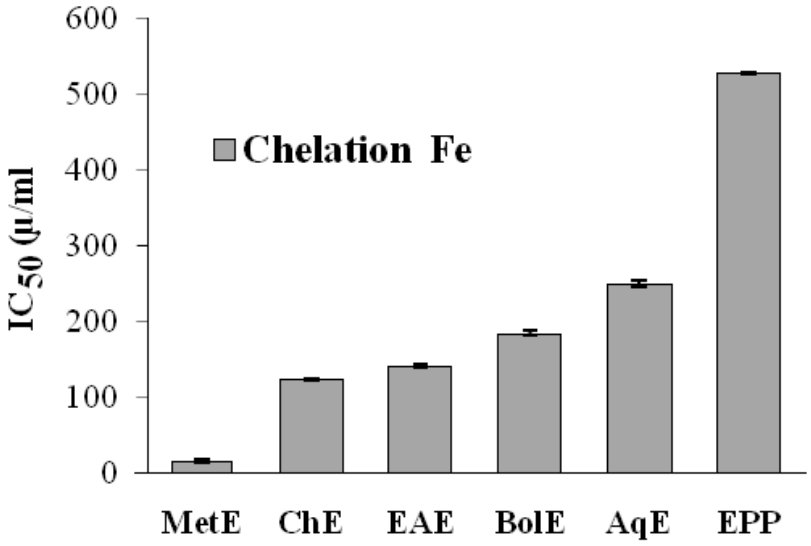

Figure 2. Iron chelating capacity expressed as IC_{50} of ethyl acetate extract (**EAE**), chloroform extract (**ChE**), butan-1-ol extract (**BolE**), methanolic extract (**MetE**) and aqueous extract (**AqE**) of *Thymus vulgaris,* expressed as IC_{50} means ± SD

3.4 Evaluation of antioxidant capacity by electrochemical techniques

The major limitation of spectrophotometric methods used in the measurement of antioxidant activity is the interference of biomolecules that absorb in the same wavelength [40, 41]. Therefore, search of other methods based on different principle is necessary. Voltammetric method is one of these

novel tools as speed, cheaper, simple, consuming fewer reagents and depending mostly on the electrochemical properties of antioxidants [42,43, 44].

In addition, it is well established that electrochemical methods are considered as simple methods for the right evaluation of the total amounts and the types of polyphenols in vegetables and plants [45-46]. Furthermore, these techniques have been capable to give the global amount of different types of polyphenols in the same time and to characterise new compounds containing polyphenols which could play important role in food and pharmaceutical industry [47].

Different concentrations of the standards ascorbic acid and gallic acids were plotted verses the anodic current density obtained from different cyclic voltammograms at pH 7 in 0.1 M phosphate buffer solution as a supporting electrolyte using a 3 mm-diameter glassy carbon electrode present typical irreversible oxidation processes with the existence of an irreversible one oxidation peak. As can be seen there is an increase in peak current with the increase in ascorbic acid and gallic acid concentrations which leads to a linear relation between these two parameters, y=36.86 x+2.595 and y=49.35 x+12.63 respectively, with a correlation coefficient of R^2 = 0.99 for both equations.

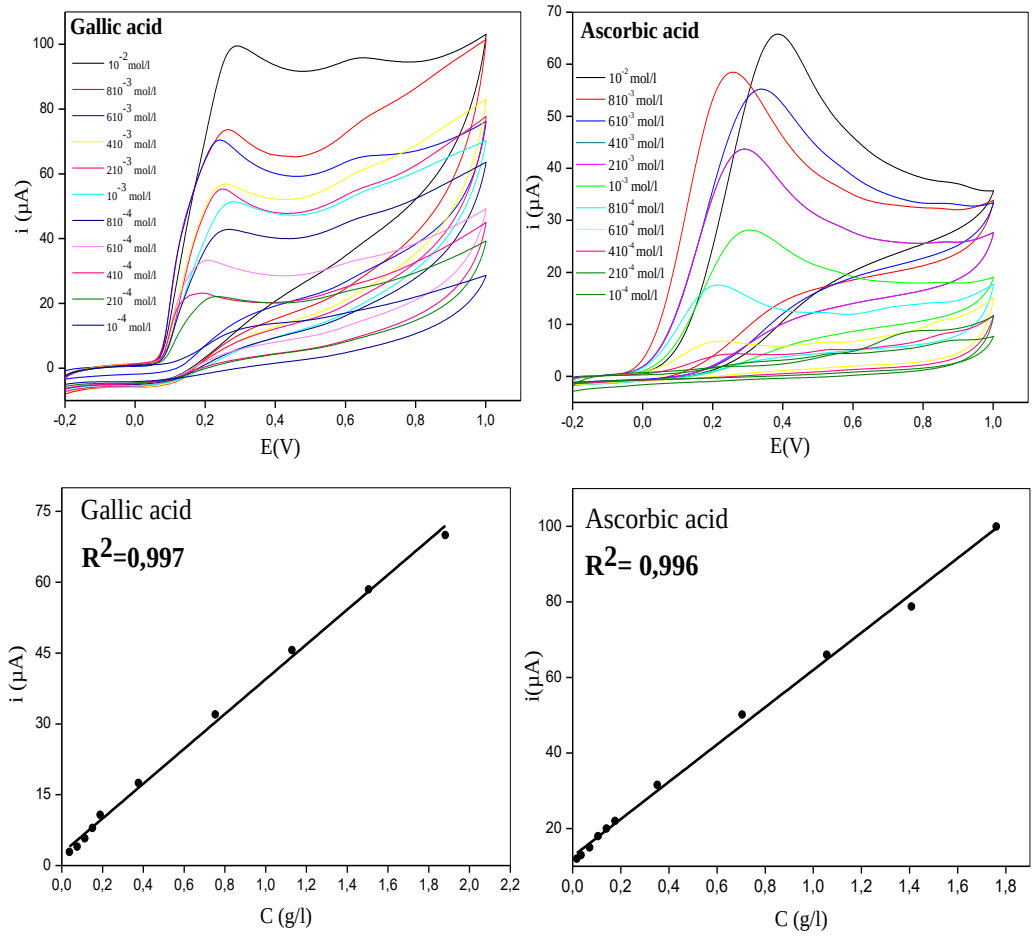

Figure 3. Cyclic voltammograms and linear curves referring to different gallic and ascorbic acids concentrations in pH = 7, 0.1 mol/l phosphate buffer solution containing 0.1 mol/l NaCl at scan rate 25 mV/s.

The same irreversible electrochemical (no cathodic peak was observed on inverting the scan direction, indicating the irreversibility of oxidation) behavior is observed for *Thymus vulgaris* extracts (shown as Fig.4). The methanolic fraction (MetE) has a high antioxidant capacity (62.196±1.994 mg gallic acid Eq /g and 94.612 ± 4.157 mg ascorbic acid Eq /g) that the other extracts. This capacity is dependant mainly on their phenolic composition.

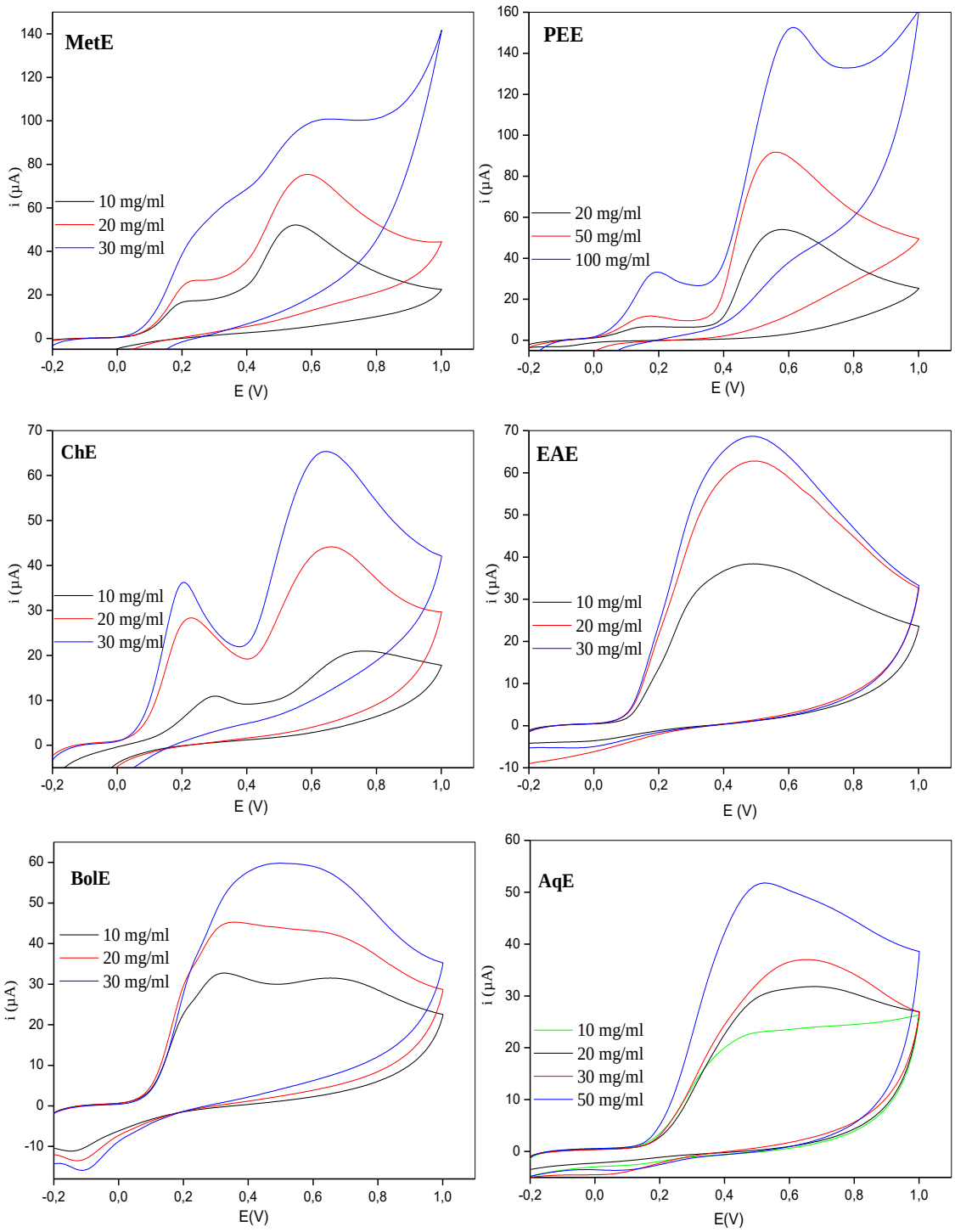

Figure 4. Cyclic voltammograms of *MetE*, *PEE*, *ChE*, *EAE*, *BolE* and *AqE* extracts at pH=7, 0.1 M phosphate buffer solution containing 0.1 M NaCl at scan rate 25 mV/s.

3.5 Correlation between total phenolics and flavonoids content and antioxidant activities

The correlation coefficient between antioxidant activity methods (DPPH, $ABTS^+$, chelating antioxidant capacity and cyclic voltammetry assay) and total phenolics and flavonoids contents of Algerian plants *T. vulgaris* is varied between (0.730 and 0.989), (shown in Table 2). This result suggests that the antioxidant capacity of *T. vulgaris* is due to the contribution of phenolics and flavonoids compound with individual activity of bioactive molecules.

Table 2. Antioxidant power of *T. vulgaris* extracts (average ± *SD*) (*n* = 3).

	DPPH	**$ABTS^+$**	**Iron chelation**	**Cyclic Voltammetry**	
	µg/ml	µmol Trolox Eq/g	µg/ml	Gallic acid Eq	Ascorbic acid Eq
MetE	8.49 ± 0.02	16.02 ± 0.90	17 ± 2	62.20 ± 1.99	94.61 ± 4.16
EAE	16.98 ± 0.05	8.272 ± 0.54	124 ± 1	51.46 ± 1.01	89.32 ± 1.98
ChE	21.61 ± 0.22	2.335 ± 0.23	142 ± 1	32.44 ± 0.50	54.66 ± 4.12
BolE	26.01 ± 0.08	0.920 ± 0.18	185 ± 4	29.83 ± 1.17	44.05 ± 2.47
AqE	42.21 ± 0.68	0.890 ± 0.14	250 ± 4	15.53 ± 0.92	26.87 ± 1.76
PEE	149.11 ± 0.52	/	527 ± 1	16.86 ± 0.98	34.34 ± 4.43
			Correlation		
R^2/ Phenolics	0.901	0.899	0.730	0.964	0.989
R^2 /Flavonoids	0.966	0.826	0.886	0.859	0.920

The synergism, additive and antagonist effects between the compounds in the extracts result in the differences in the antioxidant activity depending not only on the concentration, but also on the nature of the compounds. These results suggest that polyphenols and flavonoids are responsible of a great part of the antioxidant ability [47].

4. CONCLUSION

In this study, it was concluded that the antioxidant activity of *Thymus vulgaris* extracts collected from Setif was investigated. Different employed assays, cyclic voltammetry and DPPH, $ABTS^+$, chelating activity on ferrous ions methods showed that the antioxidant activity is mainly dependant on the phenolics composition. The obtained results suggest that *Thymus vulgaris* can serve as potential source of bioactive healthy compounds and their consumption could be useful in the protection of food peroxidation and the prevention of diseases where antioxidant properties are required.

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References

1. R. Gyawali, A. I. Salam, *Food Control*, 46 (2014) 412.
2. S. Kumar, B.S. Bajwa, K. Singh, A.N. Kalia, *Int. J. Adv. Pharm. Biol. Chem.*, 2 (2013) 272.
3. L. A. Bazzano, J. He, L.G. Ogden, C. M. Loria, S. Vupputuri, L. Myers, P. K. Whelton, *Am. J. Clin. Nutr.*, 76 (2002) 93.
4. G. Block, B. Patterson, A. Subar, *Nutr. Cancer*, 18 (1992) 1.
5. M. G. Hertog, E. J. Fesken, P. C. Hollman, M. B. Katan, D. Kromhout, *Lancet*, 342 (1993) 1007.
6. P. Knekt, R. Jarvinen, R. Seppanen, M. Hellovaara, L. Teppo, E. Pukkala, A. Aroma, *Am. J. Epidemiol*., 146 (1997) 223.
7. M. G. Hertog, P. C. Hollman, M.B. Katan, D. Kromhout, *Nutr. Cancer*, 20 (1993) 21.
8. P. G. Pietta, *J. Nat. Prod.*, 63 (2000) 1035.
9. R. Scherer, H. T. Godoy, *Food Chem*., 112 (2009) 654.
10. D. H. Luo, B.S. Fang, *Carbohyd. Polym*., 72 (2008) 376.
11. K. J. Anderson, S. S. Teuber, A. Gobeille, P. Cremin, A. L. Waterhouse, F. M. Steinberg, *J. Nutr*., 131 (2001) 2837.
12. D. Marquardt, J. A. Williams, N. Kucerka, J. Atkinson, S. R. Wassall, J. Katsaras, T. A. Harroun, *J. Am. Chem. Soc*., 135 (2013) 7523.
13. M. Gavahian, A. Farahnaky, K. Javidnia, M. Majzoobi, *Innov. Food Sci. Emerg. Technol*., 14 (2012) 85.
14. A. G. Pirbalouti, M. Hashemi, F. T. Ghahfarokhi, *Ind. Prod*., 48 (2013) 43.
15. K. R. Markham, Techniques of flavonoid identifica-tion. Academic Press, (1982), London.
16. J. Bruneton, Pharmacognosy, phytochemistry: medicinal plants (in French), (2009), Paris.
17. S. Boumerfeg, A. Baghiani, D. Messaoudi, S. Khennouf, L. Arrar., *Phytother. Res*., 23 (2009) 283.
18. T. Bahorun, B. Gressier, F. Trotin, C. Brunete, T. Dine, J. Vasseur, J. C. Gazin, M. Pinkas, M. Luycky, M. Gazin, *Drug Res.*, (1996) 1.
19. A. Baghiani, D. Ameni, S. Boumerfeg, M. Adjadj, M. Djarmoun, N. Charef, S. Khennouf, L. Arrar, *Am. J. Med. Sci.*, 2 (2012) 25.
20. R. Samarth, M. Panwar, M. Kumar, A. Soni, *Food. Chem.*, 106 (2008) 868.
21. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, *Free Radic. Biol. Med*., 26 (1999) 1231.
22. T. C. P. Dinis, V. M. C. Madeira, L. M. Almeida, *Arch. Biochem. Biophys*., 315 (1994) 161.
23. A. Rebiai, T. Lanez, M. L. Belfar, *Int. J. Pharmaceut. Sci*., 6 (2013) 975.
24. N. Wu, K. Fu, Y. J. Fu, Y. G. Zu, F. R. Chang, Y. H. Chen, *Molecules*, 14 (2009) 1032.
25. M. Hidalgo, C. Sánchez-Moreno, S. Pascual-Teresa, *Food Chem*., 121 (2010) 691.
26. J. Taira, E. Tsuchida, M. C. Katoh, M. Uehara, T. Ogi, *Food Chem*., 166 (2015) 531.
27. M. R. Saha, A. Alam, R. Akter, R. Juhangir, *Bangladesh J. Pharmacol*., 3 (2008) 90.
28. R. Q. Ferreira, S. J. Greco, M. Delarmelina, K. C. Weber, *Electrochim. Acta*, 163 (2015)161.
29. M. A. Hossain, Z. H. Al-Mijizy, K. K. Al-Rashdi, A. M. Weli, Q. Al-Riyami, *J. Coast. Life Med*., 1 (2013) 130.
30. J. S. Bao, Y. Cai, M. Sun, G. Y. Wang, H. Corke, *J. Agr. Food Chem*., 53 (2005) 2327.
31. Y. Z. Cai, Q. Luo, M. Sun, H. Corke, *Life Sci*., 74 (2004) 2157.
32. A. Luximon-Ramma, T. Bahorun, M. A. Soobrattee, O. I. Aruoma, *J. Agr. Food Chem*., 50 (2002) 5042.
33. A. Lamien-Meda, C. E. Lamien, M. M. Y. Compaore, R. N. T. Meda, M. Kiendrebeogo, B. Zeba, J. F. Millogo, O. G. Nacoulma, *Molecules*, 13 (2008) 581.
34. E. Suhartono, E. Vian, M. A. Rahmadhan, I. S. Gultom, M. F. Rakhman, D. Indrawardhana, *APCBEE Procedia*, 4 (2012) 235.
35. Halliwell, B. *Crit. Rev. Food Sci. Nutr*., 35 (1997) 7.

36. T. Yamaguchi, H. Takamura, T. Matoba, J. Terao, *Biosci. Biotech. Biochem*., 62 (1998) 1201. 37. R. S. Britton, K. L. Leicester, B. R. Bacon. Iron toxicity and chelation therapy. *Int. J. Hematol.*, 76 (2002) 219.
37. G.Minotti, S. D. Aust, *Lipids*, 27 (1992) 219.
38. E. Suhartono, E. Viani, M. A. Rahmadhan, I. S. Gultom, M. F. Rakhman, D. Indrawardhana, *APCBEE Procedia*, 4 (2012) 235.
39. A.M. Jordão, A.C. Correia, *S. Afr. J. Enol. Vitic*., 33 (2012) 214.
40. S. Mannino, O. Brenna, S. Buratti, M.S. Cosi, *Electroanal.*, 10 (1998) 908.
41. F.M.A. Lino., L.Z.de Sá, I.M.S.Torres, M.L. Rocha, T.C.P. Dinis, P.C. Ghedini, V.S. Somerset, E.S. Gil, *Electrochim. Acta*, 12 (2014) 25.
42. E. Balogh, A. Hegedüs, B. E. Stefanovits, *Sci. Hortic*., 125 (2010) 332.
43. N. S. Reis, S. H. P. R. Serrano, M . Meneghatti, E. S. Gil, *Am. J. Pharm*., 28 (2009) 949.
44. A Rebiai, T. Lanez, M. L. Belfar, *Int. J. Pharmacol*., 7 (2011) 113.
45. R. Keyrouz, M. L. Abasq, C. L. Bourvellec, N. Blanc, L. Audibert, E. Argall, *Food Chem*., 126 (2011) 831.
46. H. Zieliński, D. Zielińska, H. Kostyra, *Food Chem*., 130 (2012) 1098.
47. A. Djeridane, M. Yousf, B. Nadjemi, D. Boutassouna, P. Stocker, N. Vidal, *Food Chem.*, 97 (2006) 654.